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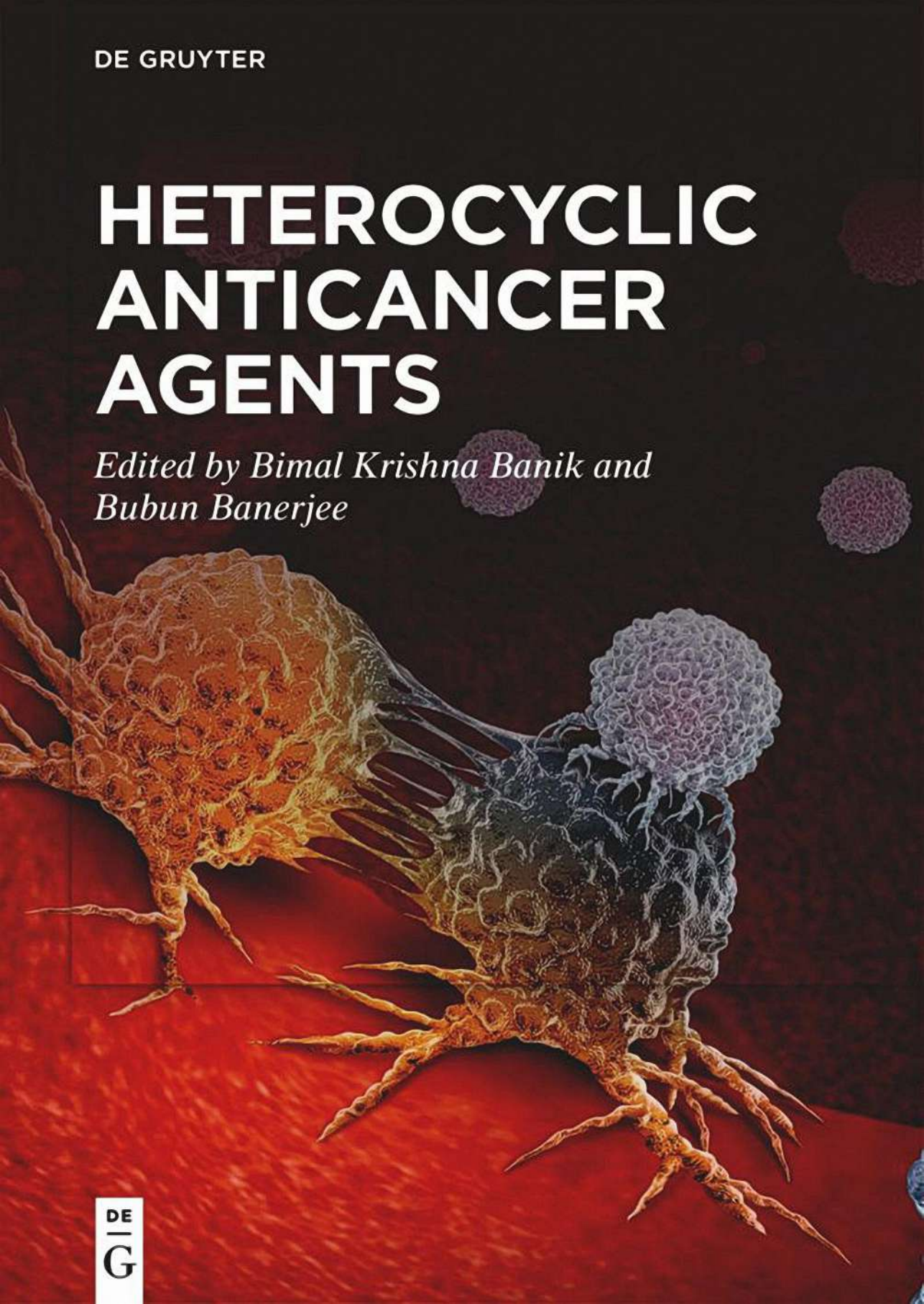

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Heterocyclic Anticancer Agents

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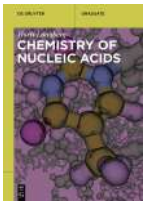

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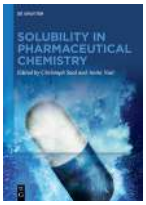

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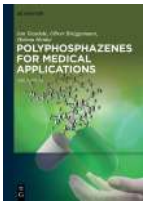

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Dedicated to

Professor Usha Ranjan Ghatak (1931–2005)
for his remarkable contribution to Science.

Preface

Heterocyclic anticancer agents are most widely known than carbocyclic compounds. Many of these compounds are natural products that have nitrogen, oxygen and sulfur in the ring systems. The mechanism of action of some of these molecules is addressed. In contrast, the mechanism of many of these compounds has not been identified. An involvement of multiple mechanisms seems to be involved. Nevertheless, heterocyclic organic compounds have demonstrated significant promise to fight against diverse cancers.

This book entitled '*Heterocyclic anticancer agents*' has 14 chapters written by established scientists of high calibers.

Banerjee *et al.* have explored numerous commercially available natural and synthetic heterocyclic compounds with anticancer activities in chapter 1.

In chapter 2, Misra and Pathak have described condensed heterocyclic anticancer compounds that act as antimetabolite, antioxidant, and antiproliferative. These compounds work through targeting different signaling pathways, over expressive receptors and physiological enzymes.

In chapter 3, Banerjee and his group have explored the applications of lawsone as a starting compound for the preparation of various organic scaffolds that have potential activities against several cancer cell lines.

Das and Banik have investigated small heterocyclic organic compounds that target DNA of cancer cells through intercalation in chapter 4. Ten crucial organic drugs belong to this categories are discussed.

In Chapter 5, Wahan and Chawla have discussed anticancer activities of thiazole, pyrazole, oxazole, and triazole heterocycles that become available over the last five years.

Danac *et al.* have described the synthesis and anticancer properties of condensed pyrrolo-pyridine, indolizine, azaindole, and pyrrolo-quinoline derivatives in chapter 6.

Matada *et al.* have explored and updated quinoline heterocycles as antitumor compounds in chapter 7.

In chapter 8, Kumar and Goel, have discussed the synthesis, pharmacological property and scope of imidazole-based compounds as anticancer molecules. It is claimed that some of these compounds have improved pharmacodynamic and pharmacokinetic properties.

Majhi has presented the synthesis and anticancer activity of acridine and xanthine-based compounds in chapter 9.

In chapter 10, Karmakar and Mukhopadhyay have explored benzothiazoles as anticancer agents.

Rao *et al.* have investigated triazine derivatives that have anticancer activity in chapter 11. The mechanism of action of the triazines is identified.

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Fátima *et al*. have discussed the antineoplastic activity of promising aryl-chromene and aryl-chroman derivatives in chapter 12.

Pathak *et al*. have explored anticancer activity of natural and synthetic indoles that target a few receptors and enzymes in chapter 13.

In chapter 14, Salunke and his group have discussed diverse indoles that specifically work against prostate cancer.

The editors of this book express their sincerest gratitude to all the authors for contributing significant chapters on anticancer agents. Ms. Stella Muller and Ms. Christene Smith have extended their generous support to us. This book is possible because of the timely support from the authors, Ms. Muller and Ms. Smith. It is our expectation that this book on heterocyclic anticancer agents will be given serious consideration by the scientific community. Thank you ALL.

Prof. Bimal Krishna Banik
and
Dr. Bubun Banerjee

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1 Naturally occurring, natural product inspired and synthetic heterocyclic anti-cancer drugs

Abstract: This chapter describes the importance and activity of a huge number of commercially available naturally occurring, natural product derived or synthetic heterocyclic anti-cancer drugs.

Keywords: heterocyclic; marketed anti-cancer drugs; naturally occurring anticancer drugs; semi synthesis of anticancer drugs; synthetic anticancer drugs.

1.1 Introduction

Worldwide cancer is a great threat for mankind. It is caused by the uncontrolled cell divisions which lead to abnormal growth of the tissues. It was characterized that cancer is a disease associated with unsuppressed growth and spread of anomalistic cells [1]. Among many others, lung cancer and breast cancer are the most common types. Cancer treatments do not have any specific potent medicine but a large number of drugs have been used for the treatment of various cancers. Various drugs are being used as potential chemotherapeutic agents. In some instances, these available drugs are causing side effects. Scientists are effortlessly trying to design various drug molecules or modify the existing drugs to reduce the side effects of these drugs [2]. Under this purview, during the last three decades, naturally occurring and natural product inspired drug development for the prevention of cancer has received immense attention. As a result, many approved commercially available anti-cancer drugs are of natural origin. Moreover, several other naturally occurring compounds and its semi-synthetic derivatives are presently in clinical or preclinical anticancer trials [3]. In generally, the costs of the natural product based drugs are comparatively high due to the difficulties in their isolation from natural sources. After involving a huge amount of natural resources and tedious work up, sometimes it was found difficult to isolate the targeted compound in the pure form even in milligram scale. Therefore it is very difficult to depend on the natural sources solely for the preparation of drugs. That is why various

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scientific groups have been trying to synthesize the same naturally isolated drugs in their laboratory. A huge numbers of synthetic compounds were also evaluated for the anti-cancer efficacies. Among them many such compounds showed significant anti-tumour activities and thus they are available in the market as promising drugs.

On the other hand, heterocyclic scaffolds, in general, are found to possess a wide range of biological activities [4–9]. More than half of the commercially available various drugs are consist of structurally diverse heterocyclic scaffolds [10]. Interestingly, it has been observed that among the commercially available anti-cancer drugs, majority of them possess heterocyclic moiety either as the main structural unit or as an important subunit. In this chapter we have highlighted a large number of potent commercially available heterocyclic anti-cancer drugs either isolated from the natural sources or prepared in the laboratory.

1.2 Naturally occurring heterocyclic anti-cancer drugs

1.2.1 Actinomycin D

In 1940, actinomycin D (Figure 1.1) was first isolated from *Streptomyces sp* [11]. It is an antineoplastic agent. It has been used to treat Wilms' tumour, choriocarcinoma, rhabdomyosarcoma, testicular carcinoma, and Ewing's sarcoma in humans [12–15].

1.2.2 Arglabin

In 1982, Adekenov et al. [16] first time isolated arglabin (Figure 1.2) from *Artemisia glabella*, a species of wormwood, endemic to the Karaganda region of Kazakhstan.

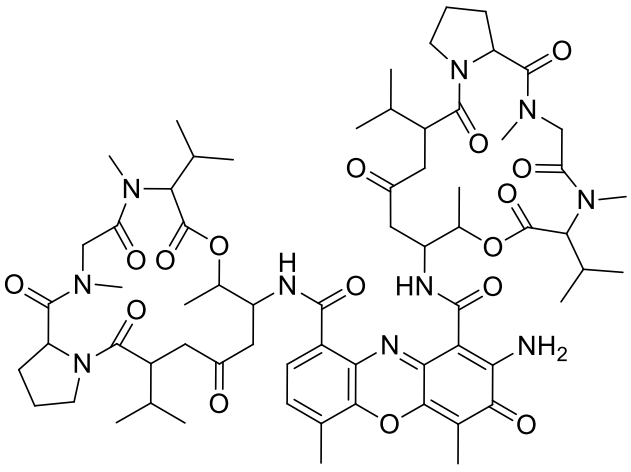


Figure 1.1: Structure of actinomycin D.

Arglabin belongs to guaianolide class of sesquiterpene lactones. It shows promising anti-tumour activity against various tumour cell lines through its inhibition of farnesyl transferase (FTase) which leads to the activation of RAS proto-oncogene, a process that play a vital role in majority of human tumours [17, 18]. It was reported that arglabin inhibits the incorporation of farnesyl pyrophosphate into human H-ras proteins by the enzyme farnesyl transferase [17].

1.2.3 Bleomycin

In 1966, Umezawa et al. [19] first isolated bleomycin from *Streptomyces verticillus*. Bleomycin belongs to the family of glycopeptides (Figure 1.3). Bleomycin has been extensively used for the treatment of testicular cancer [20, 21] and certain types of lymphoma [22, 23]. It has also been used as an anti-tumour agent for the treatment of various kinds of malignancy. However, Bleomycin reported to cause a number of side effects [24–26].

1.2.4 Carzinophilin

In 1954, carzinophilin (Figure 1.4), also known as azinomycin B, was isolated from *Streptomyces sahachiroi* [27]. Carzinophilin exhibited significant anti-tumour activity

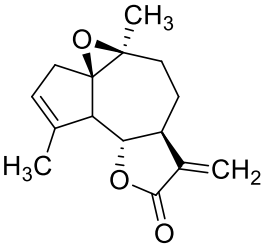


Figure 1.2: Structure of arglabin.

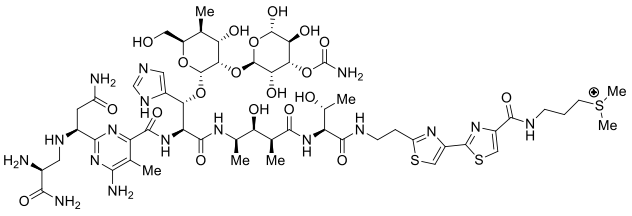


Figure 1.3: Structure of bleomycin.

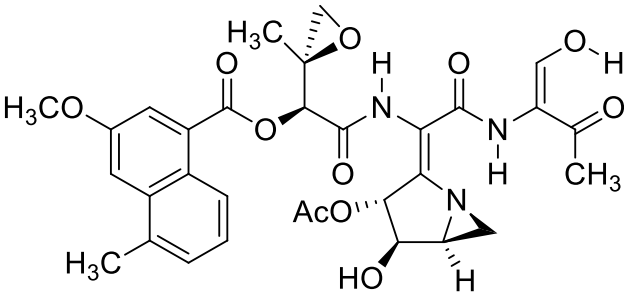


Figure 1.4: Structure of carzinophilin.

against several tumour cell lines [28–30]. Both *in vitro* and *in vivo* studies revealed that carzinophilin caused significant DNA damage which result cross-link formation [31].

1.2.5 Daunorubicin

Daunorubicin (Figure 1.5), also known as daunomycin, rubidomycin and rubomycin C, has been an effective and promising drug in the treatment of acute leukemia [32, 33]. In 1960, daunorubicin, the first anthracycline antibiotic, was isolated from *Streptomyces peucetius* [34]. This is among the most effective anti-neoplastic agents and has been extensively used for the treatment of acute lymphoblastic or myeloblastic leukemias [35].

1.2.6 Doxorubicin

In 1969, doxorubicin (Figure 1.6) was first time isolated from a culture of chemically mutated *S. peucetius* [36]. Doxorubicin is useful for chemotherapeutics. It was also found effective in the treatment of various solid tumours and hematopoietic

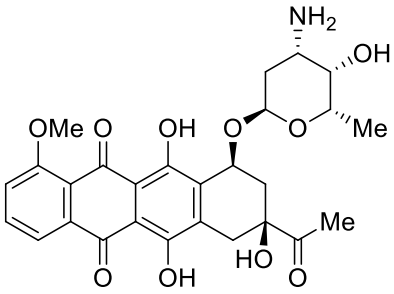


Figure 1.5: Structure of daunorubicin.

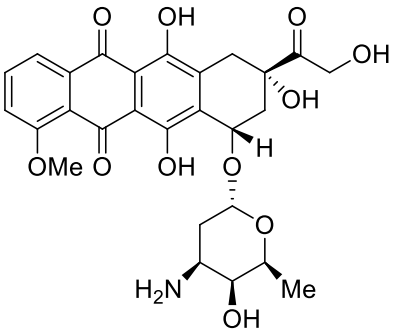


Figure 1.6: Structure of doxorubicin.

malignancies [37]. Immuno-nanoparticles conjugated with doxorubicin were found efficient for localized co-delivery of chemotherapeutics and antibodies [38].

1.2.7 Epirubicin

Madduri et al. [39] isolated epirubicin (Figure 1.7) from the *S. peucetius* strain. Epirubicin was found efficient specifically against breast cancer [40]. It was also found highly effective agent against metastatic disease [41].

1.2.8 Idarubicin

Idarubicin (Figure 1.8) belongs to anthracycline group [42] was isolated from *S. peucetius* bacterium [43]. Idarubicin was found highly effective against human adenocarcinoma cell lines. This is an orally administered drug used against breast cancer and lymphomas [44, 45].

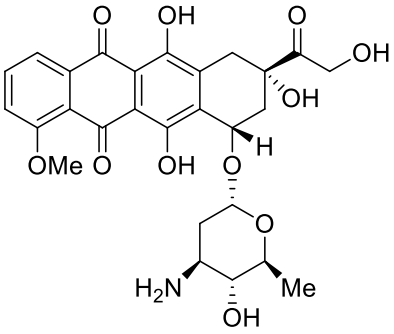


Figure 1.7: Structure of epirubicin.

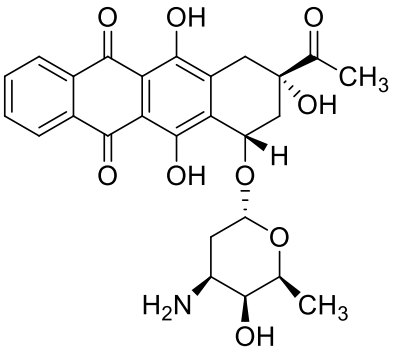


Figure 1.8: Structure of idarubicin.

1.2.9 Mithramycin

Mithramycin (Figure 1.9) is commercially known as plicamycin was isolated from *Streptomyces argillaceus* [46]. This anticancer drug is generally used to treat patients suffering from chronic myeloid leukaemia and acute myeloid leukaemia [47, 48].

1.2.10 Mitomycin C

Mitomycin C (Figure 1.10) is quinone based drug and was isolated from *Streptomyces caespitosus* [49, 50]. In 1989, Fukuyama and Yang [51] reported the total synthesis of

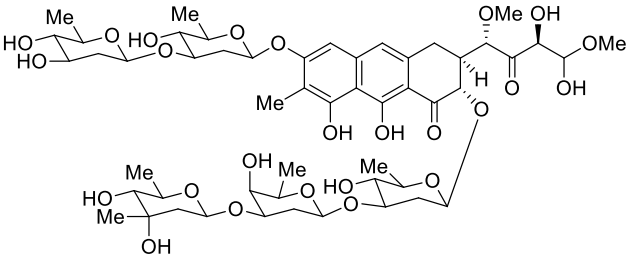


Figure 1.9: Structure of mithramycin.

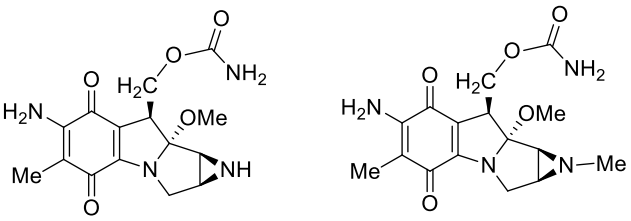


Figure 1.10: Structure of mitomycin C and porfiromycin.

Mitomycin C. It is closely related to porfiromycin. Mitomycin C is mainly used for the treatment against stomach, pancreas, colon and breast cancer. It has also been against chronic myelogenous leukemia [52].

1.2.11 Neocarzinostatin

Neocarzinostatin (Figure 1.11) is a single-chain polypeptide isolated from *Streptomyces carzinostaticus* [53, 54]. In 1998, Myers et al. [55] reported the total synthesis of neocarzinostatin. Anti-tumour activities of neocarzinostatin were found against solid tumours of the stomach, rectum and bladder. This is also fond effective against acute leukemia [54].

1.2.12 Paclitaxel and docetaxel

Paclitaxel (Figure 1.12), also known as taxol, was first isolated from the bark of *Taxus brevifolia* [56]. It has a wide spectrum of anti-neoplastic activity. *In vitro* studies

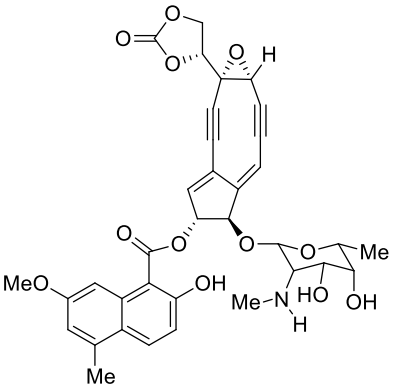
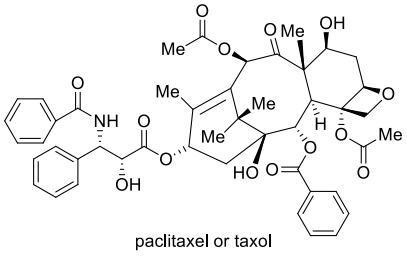


Figure 1.11: Structure of neocarzinostatin.



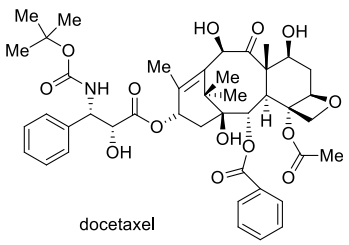


Figure 1.12: Structure of paclitaxel and docetaxel.

revealed the cytotoxic efficiency of Pac1itaxel even at very lower concentrations against various human cancer cells which include ovarian, breast, cervical, pancreas, prostate, head and neck, colon, gastric, bladder, melanoma, lung, hepatoma, leukaemia and CNS cancer cell lines. Activity of Pac1itaxel was found greater than that of cisplatin, tiazofurine, etoposide, doxorubicin or fluorouracil against human tumour cells [57]. However, paclitaxel was found less effective than its semi-synthetic analog docetaxel (Figure 1.12) [58]. Various *in vitro* as well as *in vivo* studies established anti-neoplastic activity of docetaxel against a wide range of cancer cells [59].

1.2.13 Pentostatin

Pentostatin (Figure 1.13) was isolated from the bacterium *Streptomyces antibioticus* [60]. Baker and Putt [61] synthesized pentostatin starting from an available (*E*)-5-nitro-4-styryl-1*H*-imidazole. Pentostatin showed significant activities against hairy-cell leukemia specially prolymphocytic leukaemia, B cell chronic lymphocytic leukaemia, adult T cell leukaemia/lymphoma etc. [62, 63].

1.2.14 Peplomycin

Peplomycin (Figure 1.14), a bleomycin-related cytostatic agent, was isolated from *S. verticillus* [19]. Later on this compound was synthesized by various methods [64]. Since 1981, peplomycin has been used clinically in Japan. Peplomycin is majorly used for the treatment of several neoplastic diseases including testicular carcinomas, squamous cell carcinomas and ovarian cancer [65].

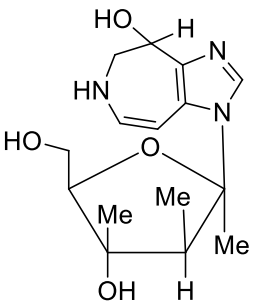


Figure 1.13: Structure of pentostatin.

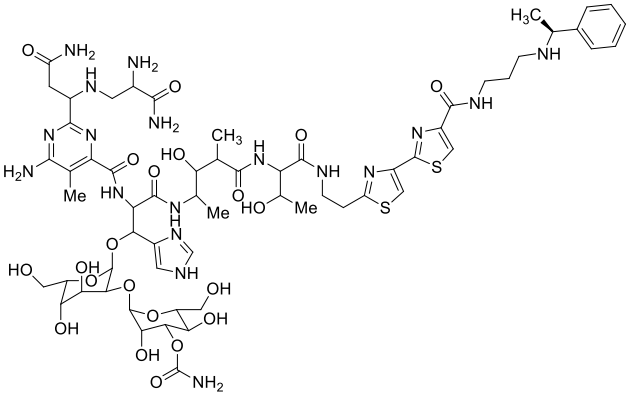


Figure 1.14: Structure of peplomycin.

1.2.15 Trabectedin

Trabectedin (Figure 1.15) was isolated from *Ecteinascidia turbinata*, a marine organism [66, 67]. Being a chemotherapeutic agent it induces rapid apoptosis exclusively in mononuclear phagocytes [68]. Trabectedin was found highly effective against soft tissue sarcoma and ovarian cancer [69]. In 1996, the first total synthesis of trabectedin was reported by Corey et al. [70].

1.2.16 Vinflunine

In 1958, vinflunine (Figure 1.16), a novel bifluorinated *vinca* alkaloid, was discovered from the leaves extract of the *Vinca rosea* plant [71, 72]. It is regarded as an efficient microtubule inhibitor agent [73]. This is the first natural alkaloid with antiproliferative activity [74]. It has been used for the treatment of a range of advanced solid tumours, including advanced transitional cell carcinoma of the urothelium, advanced non-small cell lung carcinoma, metastatic breast cancer and malignant pleural mesothelioma [72].

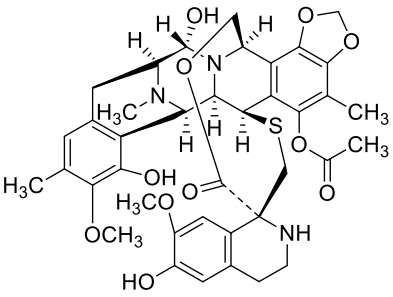


Figure 1.15: Structure of trabectedin.

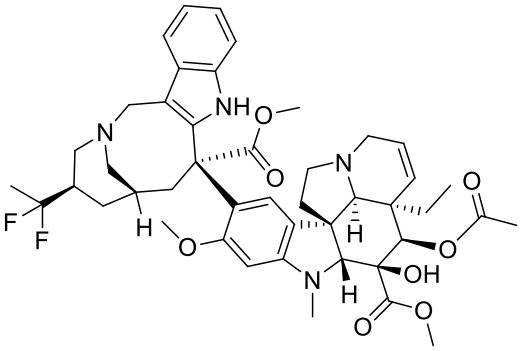


Figure 1.16: Structure of vinflunine.

1.2.17 Zinostatin

In 1965, zinostatin (Figure 1.17), also known as neocarzinostatin, was first isolated from *S. carzinostaticus* [75]. This was found highly effective against pancreatic carcinoma, hepatomas and transitional cell bladder tumours [75]. Zinostatin stimalamer (SMANCS) is used as an intra-arterial chemotherapeutic agent for hepatocellular carcinoma [76].

1.2.18 Cryptophycin F

Cryptophycin F (Figure 1.18) was isolated from *Nostoc* sp. [77]. Cryptophycin F inhibits the polymerization of tubulin thereby destabilize the microtubules which causes metaphasic arrest [78]. This was found more potent than many commercially available anticancer drugs especially for the treatment of breast cancer [79].

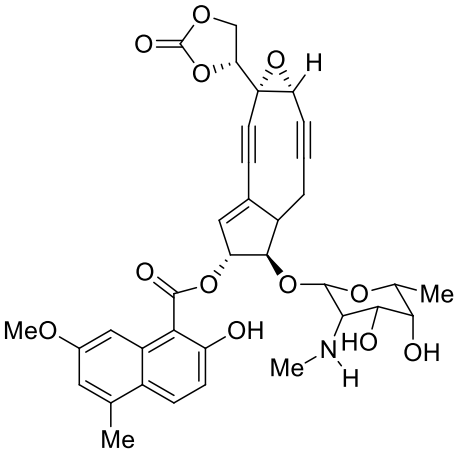


Figure 1.17: Structure of zinostatin.

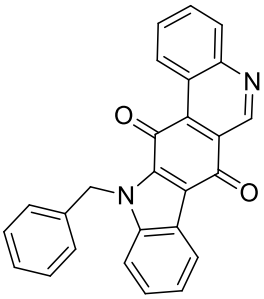


Figure 1.18: Structure of cryptophycin F.

1.2.19 Homoharringtonine

Homoharringtonine (Figure 1.19) is a cephalotaxus alkaloid was isolated from the alcoholic extraction of the evergreen tree *Cephalotaxus harringtonia* [80]. It showed significant anti-tumour activity against several neoplastic diseases [81]. This has been used for the treatment of central nervous system leukemia, nonlymphoblastic leukemia and other nonmalignant conditions [81]. In 1999, Robin et al. [82] first reported the semi synthesis of homoharringtonine.

1.3 Natural product inspired heterocyclic anti-cancer drugs

1.3.1 Belotecan

Belotecan or 7-[2-(*N*-isopropylamine)ethyl]-(20S)-camptothecin, is a water soluble camptothecin analog. This was synthesized by Ahn et al. [83] starting from camptothecin (**1**) by following the two steps shown in Figure 1.20. First, Minisci type reaction was carried out with camptothecin which afforded the corresponding 7-methylcamptothecin (**2**) in 86% yield. Later, reaction of compound **2** with isopropylamine and DMSO produced the

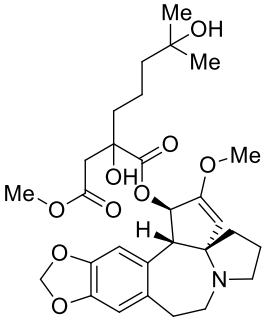


Figure 1.19: Structure of homoharringtonine.

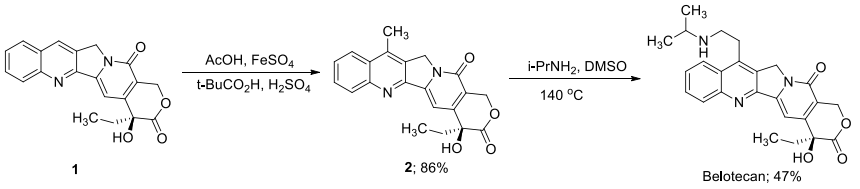


Figure 1.20: Synthesis of belotecan from camptothecin.

desired belotecan in 47% yield. Belotecan showed prominent anti-cancer activities against ovarian [84], gastric [85] and small cell lung cancer cells [86, 87].

1.3.2 Cabazitaxel

Since June 2010, cabazitaxel is an US Food and Drug Administration (US FDA) approved drug. It has been used for the treatment of prostate cancer [88]. It has showed greater anti-tumour activity than docetaxel in xenograft models of CNS disease and pediatric tumours [89]. Zhang and Fang [90] reported the total synthesis of starting from 10-deacetylbaccatin III by followed the pathway shown in Figure 1.21.

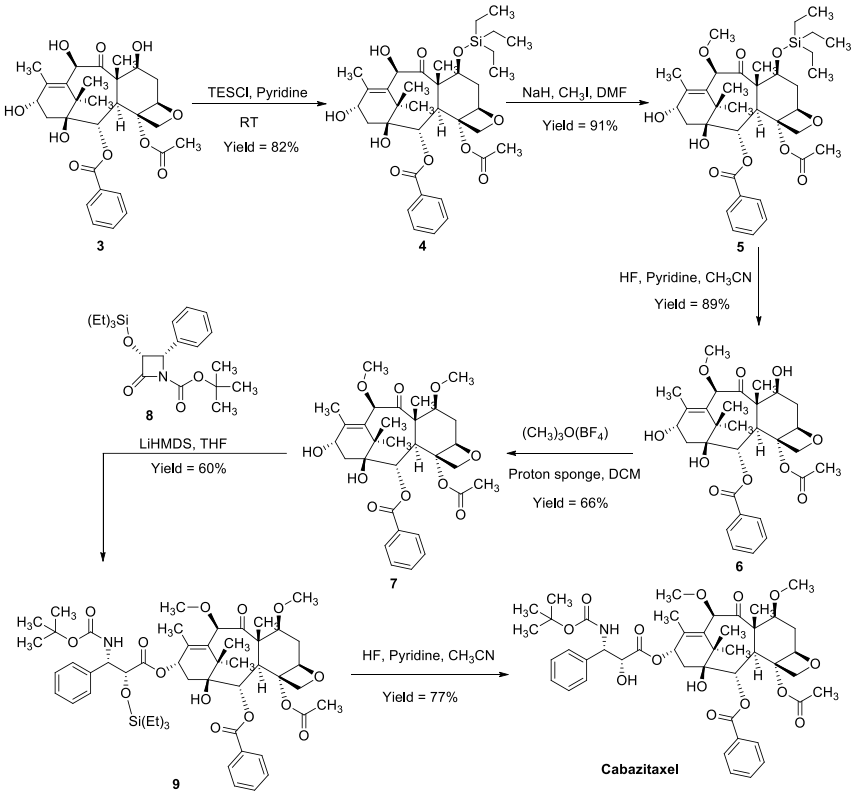


Figure 1.21: Synthesis of cabazitaxel starting from 10-deacetylbaccatin III.

1.3.3 Etoposide

Etoposide belongs to the class of topoisomerase. It is regarded as a potent anti-tumour drug [91]. In 1973, etoposide was first introduced into clinical trials [92]. It has been widely used for the treatment of small cell lung cancer, testicular cancer and lymphomas [93]. The combination of etoposide and cisplatin showed significant antineoplastic activity in the treatment of small or non-small cells lung cancer, lung cancer, testicular cancer, and acute myelogenous leukaemia [94]. Starting from the compounds **10** and **11**, Synthesis of etoposide was achieved *via* the formation of *p*-glucoside (**12**) (Figure 1.22) [95].

1.3.4 Irinotecan

Synthesis of irinotecan was achieved from camptothecin (**13**) which is a naturally occurring alkaloid extracted from stem wood of tree *Camptotheca acuminate* [96] (Figure 1.23). Irinotecan belongs to a new class of antineoplastic agents. Clinical studies revealed its efficacy against solid tumours with tolerable side effects [97, 98]. It has been also used for the treatment of early stage of pancreatic cancer [99].

1.3.5 Ixabepilone

Ixabepilone possess chemotherapy resistance in metastatic breast cancer [100–102]. Synthesis of ixabepilone was achieved starting from epothilone B (**18**) by following the

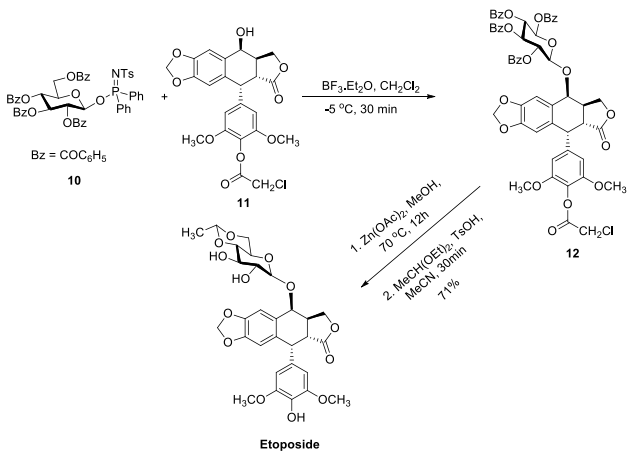


Figure 1.22: Synthesis of etoposide.

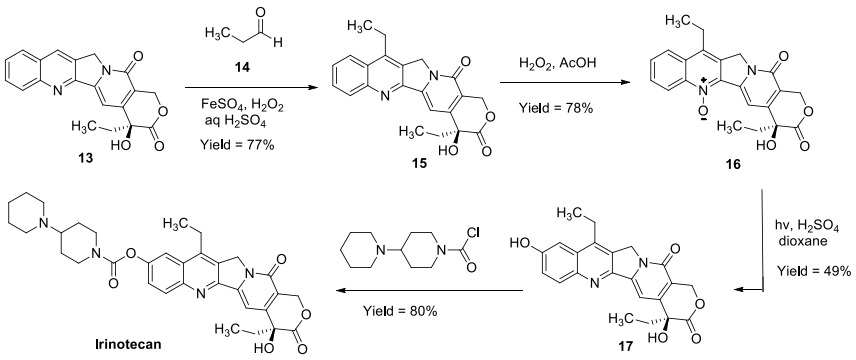


Figure 1.23: Synthesis of irinotecan starting from camptothecin.

pathway shown in Figure 1.24 [103]. Epothilone B was also showed significant anticancer activity and was isolated from the myxobacterium *Sorangium cellulosum* [104, 105].

1.3.6 Teniposide

By following the steps shown in Figure 1.25, teniposide was synthesized starting from epipodophyllotoxin (**22**) which can be isolated from American mandrake *Podophyllum*

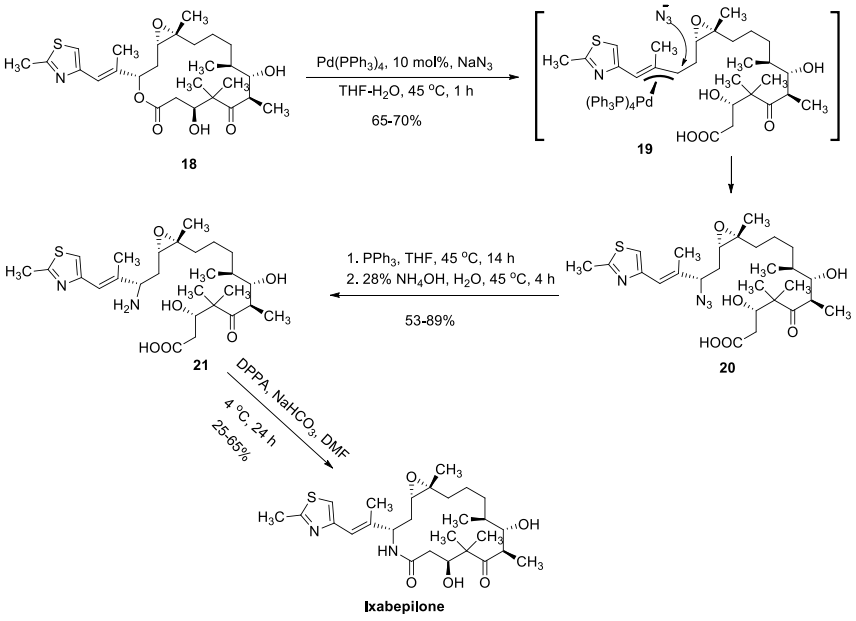


Figure 1.24: Synthesis of ixabepilone starting from epothilone B.

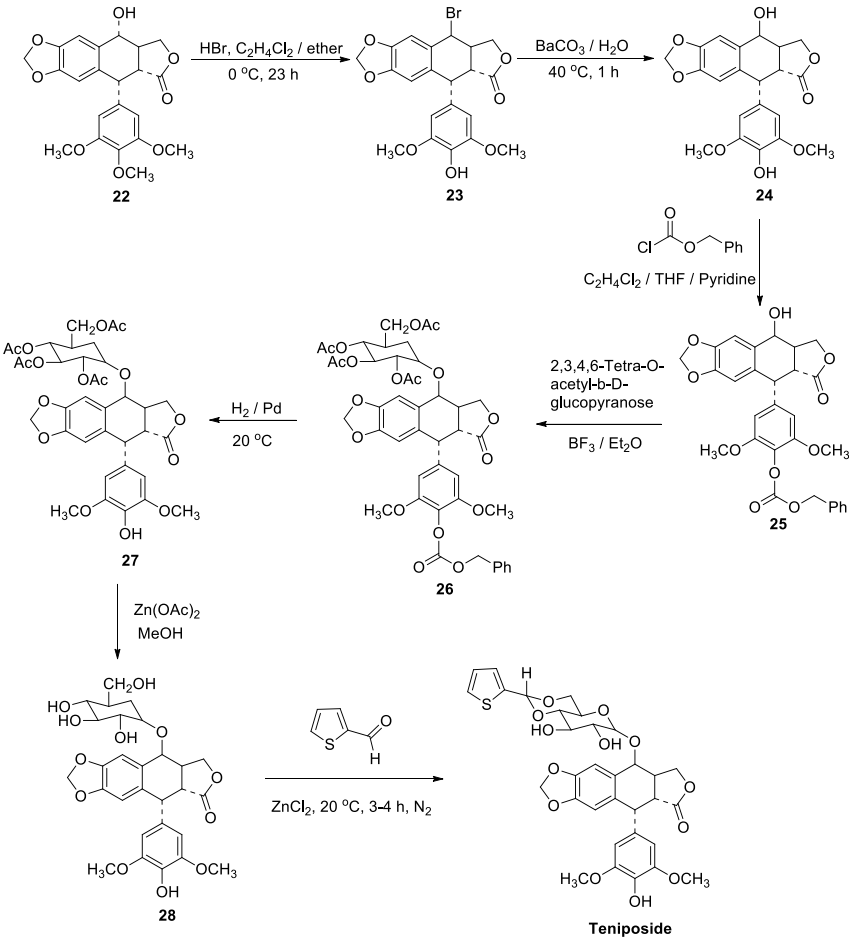


Figure 1.25: Synthesis of teniposide from epipodophyllotoxin.

peltatum and *Podophyllum Linnaeus* [106]. Teniposide has been used as a cytostatic in the treatment of several types of cancer and was also found effective for treating childhood leukemia and brain gliomas [107]. Myelosuppression is the major side effect of this drug which is manageable [108].

1.3.7 Topotecan

Starting from camptothecin (**29**), semi-synthesis of topotecan was achieved by following the steps shown in Figure 1.26 [109]. Camptothecin is a naturally occurring alkaloid which was extracted from the tree *C. acuminate* [110]. In the late 1960s, clinical studies of camptothecin was carried out in the National Cancer Institute, US. Along

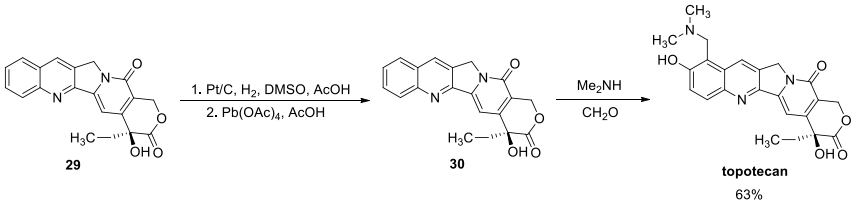


Figure 1.26: Synthesis of topotecan from camptothecin.

with significant anti-cancer efficacies it also showed severe and unpredictable side effects too [111]. Topotecan has been used against variety of tumours, including ovarian cancer and small cell lung cancer [112]. It was also found useful for the treatment of recurrent epithelial ovarian cancer [113]. It possesses inhibitory activity against DNA topoisomerase I [114].

1.3.8 Valrubicin

Figure 1.27 describes the semi-synthesis of valrubicin starting from doxorubicin HCl salt [115]. Doxorubicin is a well reputed anticancer drug was isolated from *Streptomyces* strains [115]. Valrubicin was found potent against chemoresection of superficial bladder cancer [116].

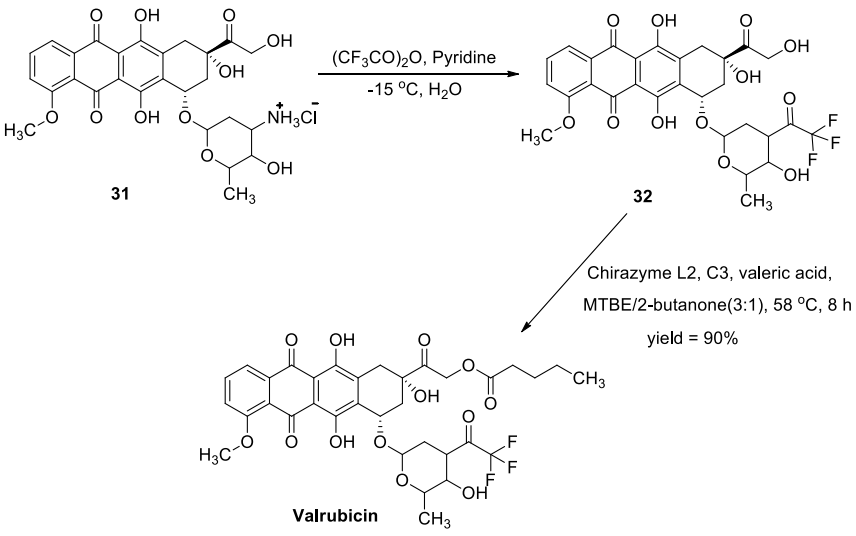


Figure 1.27: Synthesis of valrubicin starting from doxorubicin HCl salt.

1.3.9 Vinorelbine

Catharanthine (**33**) and vindoline (**34**) are the two naturally occurring vinca alkaloids extracted from Catharanthus roseus [117]. Semi-synthesis of vinorelbine was achieved from the reactions of catharanthine (**33**) and vindoline (**34**) (Figure 1.28) [117]. Vinorelbine has been used for the treatment of breast cancer [118, 119]. This was also found effective against inoperable or advanced non-small cell lung cancer [120].

1.4 Synthetic heterocyclic anti-cancer drugs

A huge number of heterocyclic synthetic anti-cancer drugs are also available in the market. They have shown huge potency for the treatment or diagnosis of various cancers. Abarelix act as an antagonist of naturally occurring gonadotropin releasing hormone (GnRH) stimulates the hormonal therapy of prostate cancer (Table 1.1, entry 1) [121, 122]. Abiraterone is used in hormone therapy to treat men with the metastatic-castration resistant prostate cancer and also stops the release of testosterone (Table 1.1, entry 2) [123–128]. Afatinib is inhibitor of protein tyrosine kinase it mainly used in the treatment of non-small-cell-lung cancer (NSCLC) (Table 1.1, entry 3) [129, 130]. Axitinib is indazole derivative, and a type of tyrosine kinase receptor selective towards the treatment of renal cell carcinoma (RCC) and it is also a type of antiangiogenesis (Table 1.1, entry 4) [131–134]. Azacitidine reversibly inhibits DNA methylation by

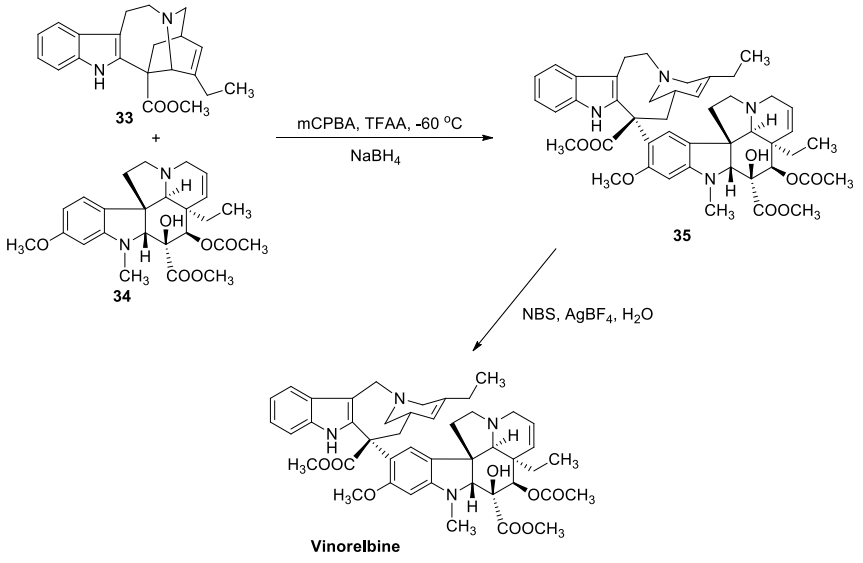


Figure 1.28: Synthesis of vinorelbine starting from catharanthine and vindolin.

Table 1.1: Commercially available synthetic heterocyclic anti-cancer drugs.

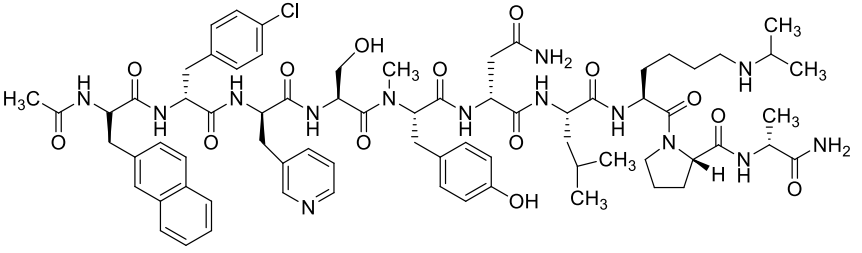
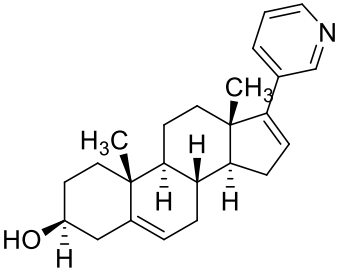
Entry	Structure	Mostly used for the treatment of	Reference
1	 Abarelix	Prostate cancer	[121, 122]
2	 Abiraterone	Prostate cancer	[123–128]

Table 1.1: (continued)

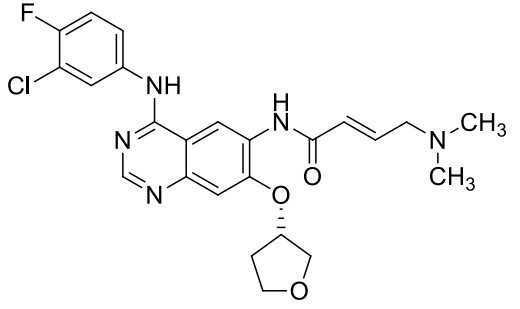
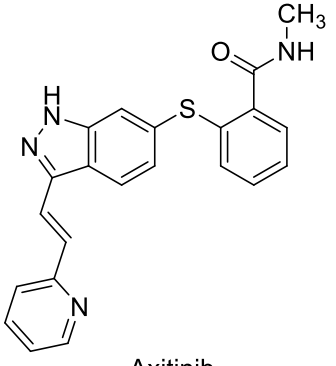
Entry	Structure	Mostly used for the treatment of	Reference
3	 Afatinib	Lung cancer	[129, 130]
4	 Axitinib	Renal cell carcinoma	[131–134]

Table 1.1: (continued)

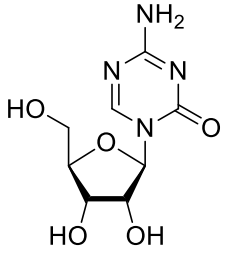
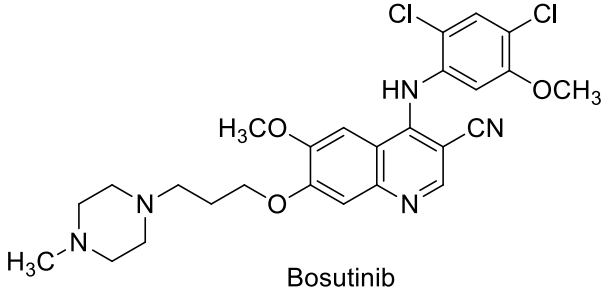
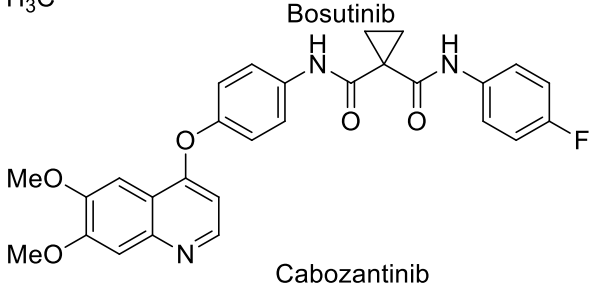
Entry	Structure	Mostly used for the treatment of	Reference
5	 Azacitidine	Myelodysplastic syndromes	[135–137]
6	 Bosutinib	Chronic myelogenous leukemia	[138–140]
7	 Cabozantinib	Medullary thyroid cancer, renal cell carcinoma and hepatocellular carcinoma	[141–144]

Table 1.1: (continued)

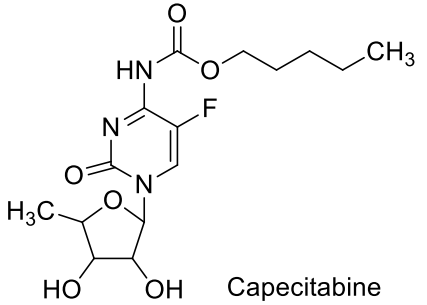
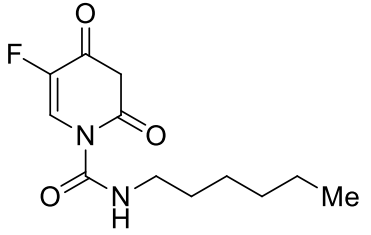
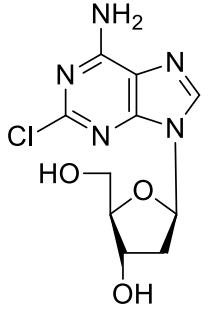
Entry	Structure	Mostly used for the treatment of	Reference
8	 Capecitabine	Breast, colon, prostate, pancreatic, ovarian and rectal cancer	[145, 146]
9	 Carmofur	Gastrointestinal cancer, melanoma and glioblastoma tumours	[147–149]
10	 Cladribine	Chronic lymphocytic leukemia	[150–152]

Table 1.1: (continued)

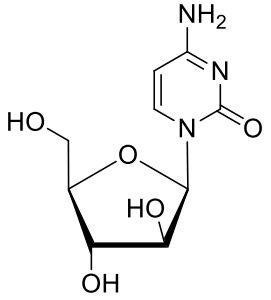
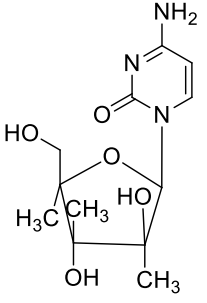
Entry	Structure	Mostly used for the treatment of	Reference
11	 Cytarabine	Acute myeloid leukemia	[153, 154]
12	 Cytosine arabinoside	Acute leukemia	[155–159]

Table 1.1: (continued)

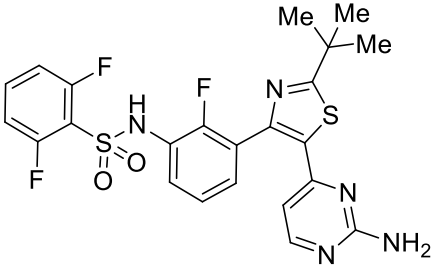
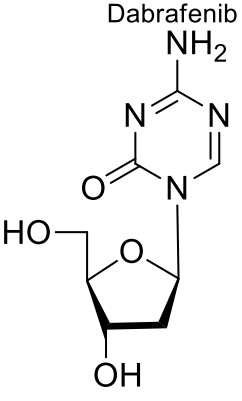
Entry	Structure	Mostly used for the treatment of	Reference
13	 Dabrafenib	Metastatic melanoma	[160–162]
14	 Decitabine	Chronic myelomonocytic leukemia, acute myeloid leukemia	[163–167]

Table 1.1: (continued)

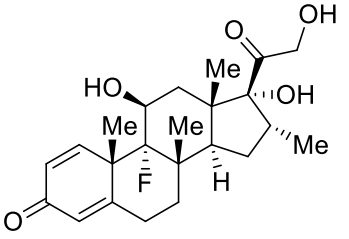
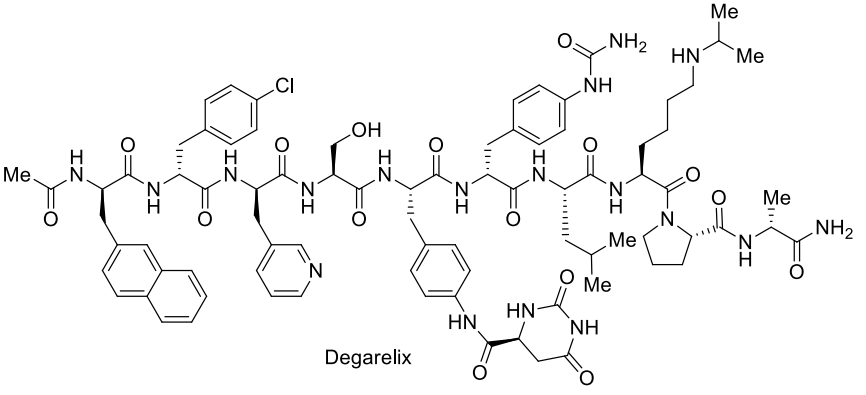
Entry	Structure	Mostly used for the treatment of	Reference
15	 Dexamethasone	Antiemetic agent in cancer therapy	[168–170]
16	 Degarelix	Prostate cancer	[171, 172]

Table 1.1: (continued)

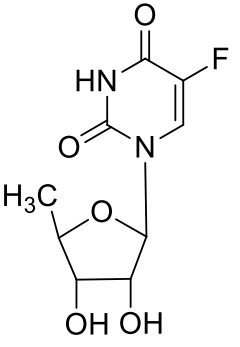
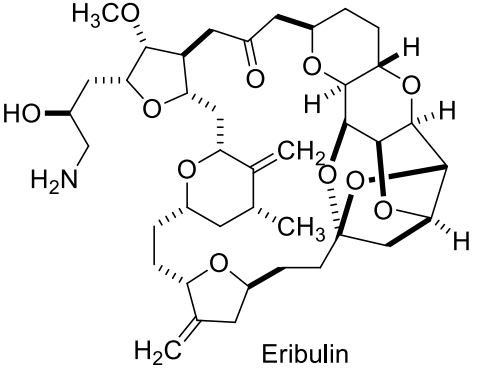
Entry	Structure	Mostly used for the treatment of	Reference
17	 Doxifluridine	Lung carcinoma, skin cancer	[173, 174]
18	 Eribulin	Breast cancer	[175–179]

Table 1.1: (continued)

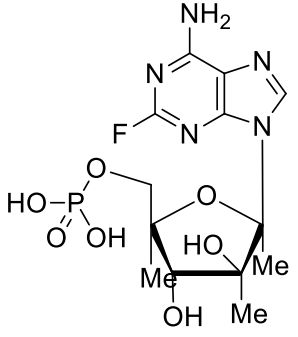
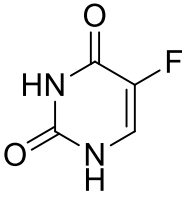
Entry	Structure	Mostly used for the treatment of	Reference
19	 Fludarabine	Lympho-proliferative malignancies	[180–182]
20	 5-Fluorouracil	Advanced colorectal cancer	[183–185]

Table 1.1: (continued)

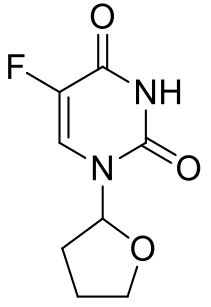
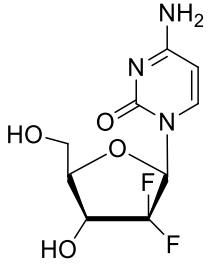
Entry	Structure	Mostly used for the treatment of	Reference
21	 Ftorafur	Colorectal cancer	[186]
22	 Gemcitabine	Lung cancer and advanced pancreatic cancer	[187–189]

Table 1.1: (continued)

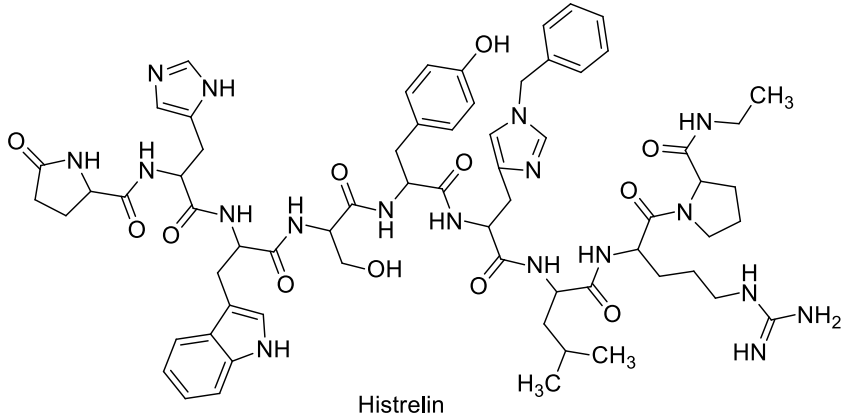
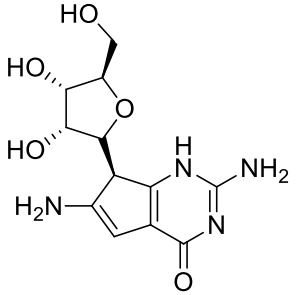
Entry	Structure	Mostly used for the treatment of	Reference
23	 Histrelin	Prostate cancer	[190–194]
24	 Trastuzumab	Chronic lymphocytic leukemia	[195, 196]

Table 1.1: (continued)

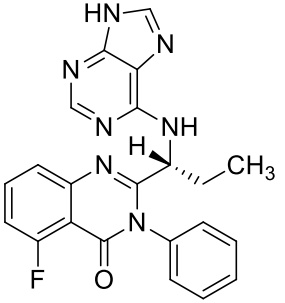
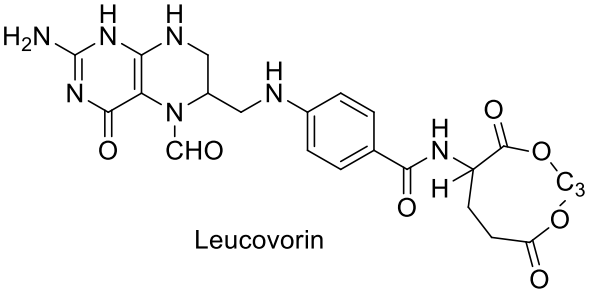
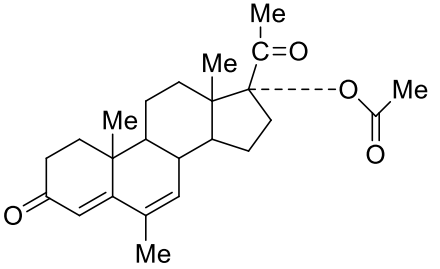
Entry	Structure	Mostly used for the treatment of	Reference
25	 Idelalisib	Chronic lymphocytic leukemia, follicular lymphoma	[197–200]
26	 Leucovorin	Acute lymphoblastic leukemia	[201, 202]
27	 Megestrol acetate.	Advanced breast cancer	[203–206]

Table 1.1: (continued)

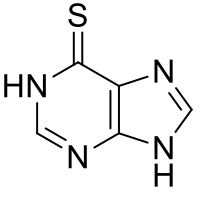
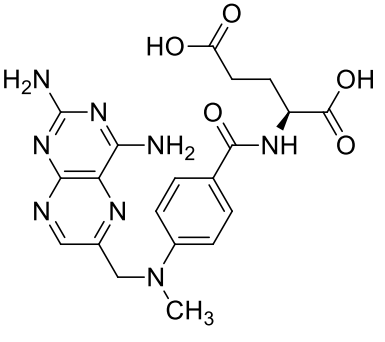
Entry	Structure	Mostly used for the treatment of	Reference
28	 Mercaptopurine	Acute lymphoblastic leukemia	[207–210]
29	 Metothrexate	Brain tumour and breast cancer	[211–213]

Table 1.1: (continued)

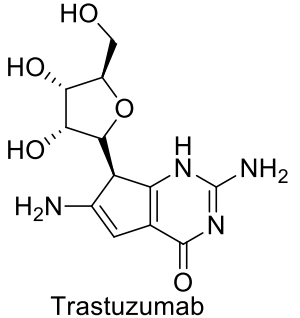
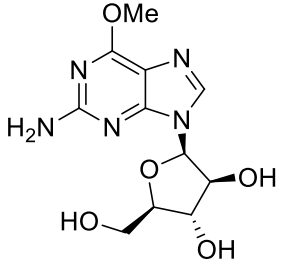
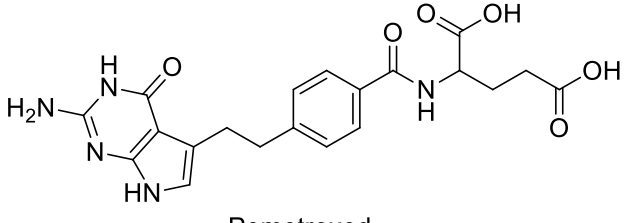
Entry	Structure	Mostly used for the treatment of	Reference
30	 Trastuzumab	Metastatic breast cancer	[214–217]
31	 Nelarabine	T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma	[218–220]
32	 Pemetrexed	Mesothelioma and lung cancer	[221–223]

Table 1.1: (continued)

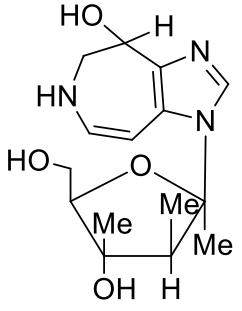
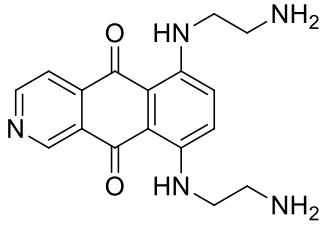
Entry	Structure	Mostly used for the treatment of	Reference
33	 Pentostatin	Lymphoproliferative disorders, cutaneous T cell lymphoma	[61, 63, 224, 225]
34	 Pixantrone dimaleate	Non-Hodgkin lymphoma	[226–229]

Table 1.1: (continued)

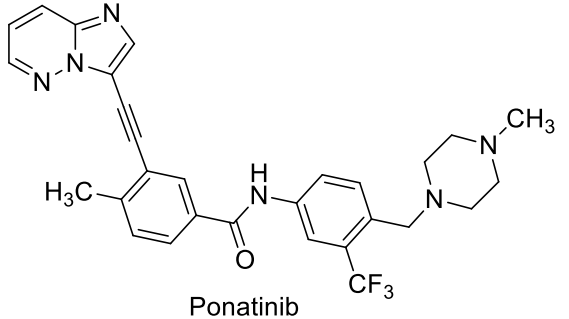
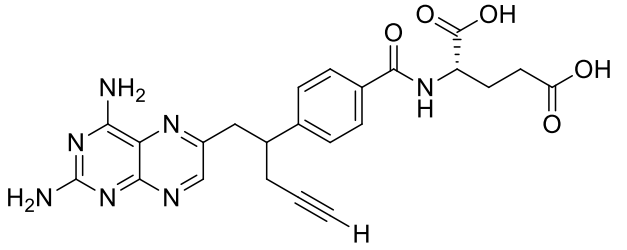
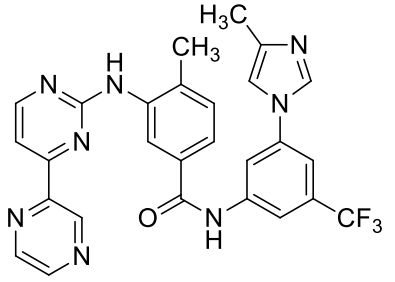
Entry	Structure	Mostly used for the treatment of	Reference
35	 Ponatinib	Chronic myeloid leukemia and lymphoblastic leukemia	[230–233]
36	 Pralatrexate	Peripheral T-cell lymphomas	[234, 235]
37	 Radotinib	Chronic myeloid leukemia	[236–239]

Table 1.1: (continued)

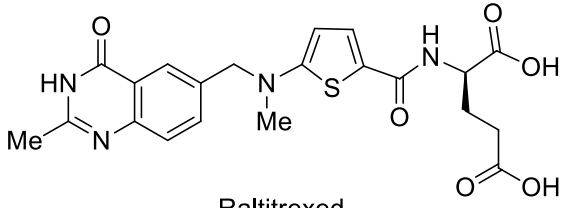
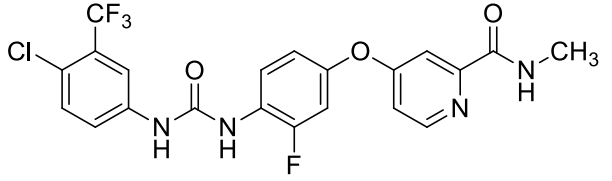
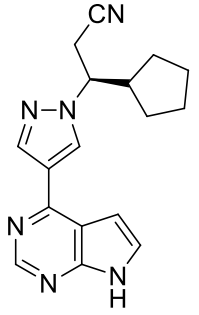
Entry	Structure	Mostly used for the treatment of	Reference
38	 Raltitrexed	Advanced colorectal cancer	[240–242]
39	 Regorafenib	Metastatic colorectal cancer	[243, 244]
40	 Ruxolitinib	Myelofibrosis and polycythemia vera	[245–247]

Table 1.1: (continued)

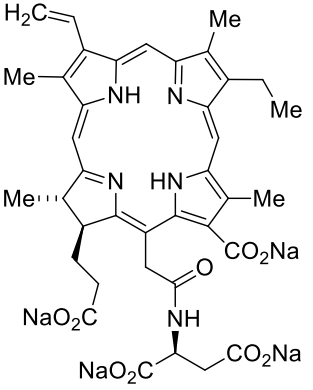
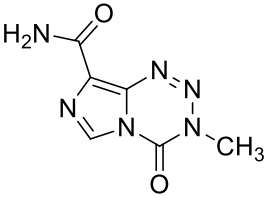
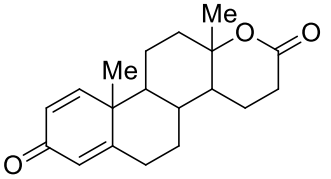
Entry	Structure	Mostly used for the treatment of	Reference
41	 Talaporfin sodium	Esophageal cancer	[248–250]
42	 Temozolomide	Malignant melanoma, malignant glioma and brain tumours	[251, 252]
43	 Testolactone	Breast cancer	[253–255]

Table 1.1: (continued)

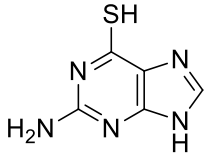
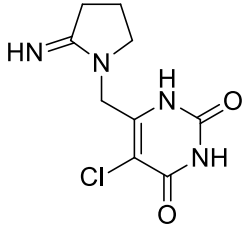
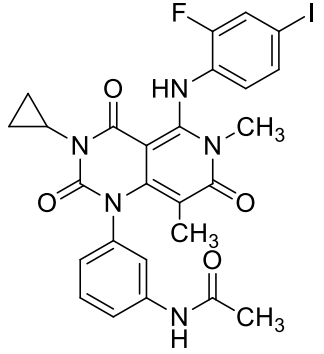
Entry	Structure	Mostly used for the treatment of	Reference
44	 Thioguanine	Acute lymphoblastic and myeloid leukemia	[256, 257]
45	 Tipiracil	Gastroesophageal cancer	[258–260]
46	 Trametinib	Metastatic melanoma	[261, 262]

Table 1.1: (continued)

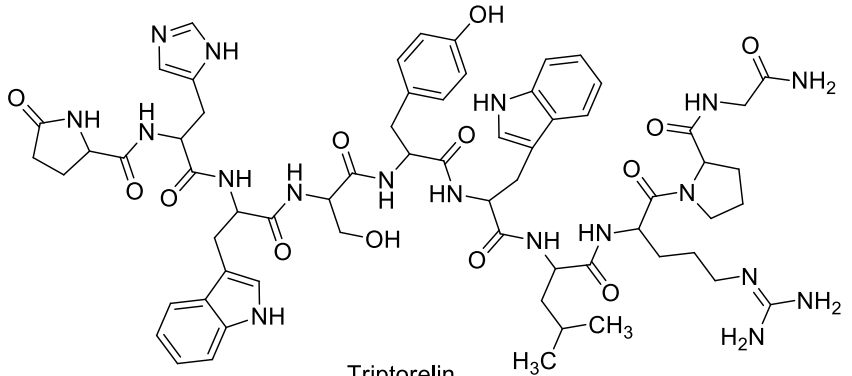
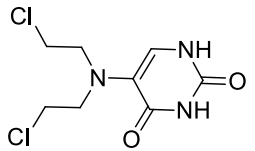
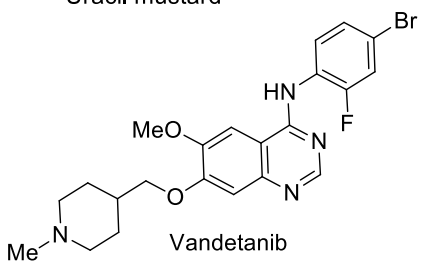
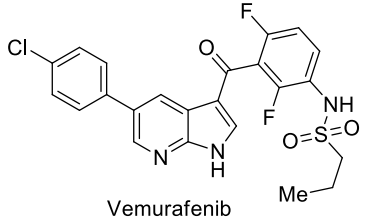
Entry	Structure	Mostly used for the treatment of	Reference
47	 Triptorelin	Metastatic prostate cancer	[263–265]
48	 Uracil mustard	Leukaemias, reticuloses and thrombocythemia	[266–269]
49	 Vandetanib	Medullary thyroid cancer	[270–272]

Table 1.1: (continued)

Entry	Structure	Mostly used for the treatment of	Reference
50	 Vemurafenib	Metastatic melanoma	[273–276]

blocking DNA methyltransferases, resulting in the treatment of myelodysplastic syndromes (MDS) (Table 1.1, entry 5) [135–137]. Bosutinib is a type of tyrosine kinase inhibitor, used for the diagnosis of chronic myelogenous leukemia (Table 1.1, entry 6) [138–140]. Cabozantinib shows clinical activity in patients with medullary thyroid cancer, advanced renal cell carcinoma and advanced hepatocellular carcinoma (Table 1.1, entry 7) [141–144]. Capecitabine is used to treat breast, prostate, pancreatic, colon, ovarian and rectal cancer. It works by slowing or stopping the growth of cancer cells (Table 1.1, entry 8) [145, 146]. Carmofur is an antineoplastic drug. It is effective in the treatment of gastrointestinal cancer and solid tumours such as melanoma and glioblastoma tumours (Table 1.1, entry 9) [147–149]. Cladribine is deaminase-resistant drug, used as a first-line therapy in hairy cell leukemia and B-cell chronic lymphocytic leukemia (Table 1.1, entry 10) [150–152]. Cytarabine is an analogue of nucleoside. It has been used for the treatment of acute myeloid leukemia (Table 1.1, entry 11) [153, 154]. Cytosine arabinoside helps in the inhibition of DNA replication and enzyme DNA polymerase. It is an effective agent for the treatment of acute leukemia (Table 1.1, entry 12) [155–159]. Dabrafenib is kinase inhibitor of mutated BRAF, effective in treatment of metastatic melanoma (Table 1.1, entry 13) [160–162]. Decitabine is a hypomethylating agent and has been used for the patients suffering from a variety of leukemia such as myelodysplastic syndromes, chronic myelomonocytic leukemia, acute myeloid leukemia etc. (Table 1.1, entry 14) [163–167]. Dexamethasone is mainly used as an antiemetic agent in cancer therapy (Table 1.1, entry 15) [168–170]. Degarelix act as an antagonist of gonadotropin releasing hormone (GnRH) stimulates the hormonal therapy of prostate cancer (Table 1.1, entry 16) [171, 172]. Doxifluridine is a derivative of fluoropyrimidine. This has been used for the treatment of lung carcinoma and squamous cell carcinoma of the skin (Table 1.1, entry 17) [173, 174]. Eribulin is a synthetic macrocyclic ketone analogue of Halichondrin B. It has been used as a third line therapy for metastatic breast cancer (Table 1.1, entry 18) [175–179]. Fludarabine is an antineoplastic agent which has been employed for the patients having a variety of lymphoproliferative malignancies (Table 1.1, entry 19) [180–182]. 5-Fluorouracil is a uracil derivative. It can act as an antineoplastic agent and has been used for the treatment of advanced colorectal cancer (Table 1.1, entry 20) [183–185]. Ftorafur is effective in first-line chemotherapy for patients with metastatic colorectal cancer (Table 1.1, entry 21) [186]. Gemcitabine is analogue of deoxycytidine, developed for the treatment of non-small cell lung cancer and advanced pancreatic cancer (Table 1.1, entry 22) [187–189]. Histrelin acts as an antagonist of gonadotropin releasing hormone (GnRH) thereby stimulates the hormonal therapy of advanced prostate cancer (Table 1.1, entry 23) [190–194]. Ibrutinib is regarded as Bruton's tyrosine kinase inhibitor. Additionally, it has been used for the treatment of chronic lymphocytic leukemia (Table 1.1, entry 24) [195, 196]. Idelalisib showed kinase inhibitory potency in many B-cell leukemias and lymphomas such as chronic lymphocytic leukemia and follicular lymphoma (Table 1.1, entry 25) [197–200]. Leucovorin is an antidote to folic acid antagonist methotrexate which has been used to treat childhood acute

lymphoblastic leukemia (Table 1.1, entry 26) [201, 202]. Megestrol acetate is a synthetic derivative of the progesterone, a naturally occurring steroid hormone. This drug has been used for the treatment of advanced breast cancer (Table 1.1, entry 27) [203–206]. Mercaptopurine is an antineoplastic agent. It was found very effective in children with the acute lymphoblastic leukemia (Table 1.1, entry 28) [207–210]. Metothrexate is a folic acid anti-metabolite. This drug has been extensively used for the treatment of brain tumour and breast cancer (Table 1.1, entry 29) [211–213]. Trastuzumab emtansine is an antibody-drug conjugate which has been used for the treatment of human metastatic breast cancer (Table 1.1, entry 30) [214–217]. Nelarabine is a purine nucleoside analogue which has been used for the treatment of T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma (Table 1.1, entry 31) [218–220]. Pemetrexed is regarded as an antifolate agent and has been used to treat variety of solid tumours though maximum potency was observed for the treatment of mesothelioma and non-small cell lung cancer (Table 1.1, entry 32) [221–223]. Pentostatin used in the treatment of various lymphoproliferative disorders, such as B-cell chronic lymphocytic leukaemia, prolymphocytic leukaemia, T-cell leukaemia/lymphoma and cutaneous T cell lymphoma (Table 1.1, entry 33) [61, 63, 224, 225]. Pixantrone dimaleate is an anthracenedione like drug has been used for the treatment of aggressive non-Hodgkin lymphoma (Table 1.1, entry 34) [226–229]. Ponatinib inhibits multi-tyrosine kinase. It has been widely employed for the patients suffering from chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia (Table 1.1, entry 35) [230–233]. Pralatrexate is the only chemotherapeutic agent for the treatment of peripheral T-cell lymphomas (Table 1.1, entry 36) [234, 235]. Radotinib is a tyrosine kinase inhibitor which has been used for the treatment of acute and chronic myeloid leukemia (Table 1.1, entry 37) [236–239]. Raltitrexed is a folate analogue and thymidylate synthase inhibitor. It has been employed to treat patients suffering from advanced colorectal cancer (Table 1.1, entry 38) [240–242]. Regorafenib is a multi-kinase inhibitor. It has been used for the treatment of metastatic colorectal cancer (Table 1.1, entry 39) [243, 244]. Ruxolitinib found to possess myeloproliferative acitivity. It can be used for the treatment of myelofibrosis and polycythemia vera (Table 1.1, entry 40) [245–247]. Talaporfin sodium has been used for the treatment of various types of solid tumours, malignancies and esophageal cancer (Table 1.1, entry 41) [248–250]. Temozolomide is an antineoplastic agent. It has the potency to treat high grade gliomas such as malignant melanoma, malignant glioma and other aggressive brain tumours (Table 1.1, entry 42) [251, 252]. Testolactone has been used in hormonal chemotherapy for women with advanced and metastatic carcinoma of breast (Table 1.1, entry 43) [253–255]. Thioguanine is an important drug for the treatment of acute lymphoblastic and myeloid leukemia both in adults and children (Table 1.1, entry 44) [256, 257]. Tipiracil is an anti-metabolite agent which can inhibit thymidine phosphorylase. It has been used to treat patients with metastatic colorectal gastric or gastroesophageal cancer (Table 1.1, entry 45) [258–260].

Trametinib is an anti-neoplastic agent which is reported to inhibit mitogen protein kinase. This drug has been used to metastatic melanoma diagnosis (Table 1.1, entry 46) [261, 262]. Triptorelin acts as an antagonist of naturally occurring gonadotropin releasing hormone (GnRH) thereby stimulates the hormonal therapy of advanced and metastatic prostate cancer (Table 1.1, entry 47) [263–265]. Uracil mustard has been used for the diagnosis of the advance leukaemias, reticuloses and thrombocythemia (Table 1.1, entry 48) [266–269]. Vandetanib inhibits multi-targeted tyrosine kinase. This drug has potency to treat patients with advanced or metastatic medullary thyroid cancer (Table 1.1, entry 49) [270–272]. Vemurafenib is a protein kinase inhibitor. It was found effective in treatment of metastatic melanoma (Table 1.1, entry 50) [273–276].

1.5 Conclusions

Heterocyclic skeletons are very common in naturally occurring bioactive compounds. Many commercially available drug molecules consist of heterocyles. Among various other diseases cancer is still considered as one of the deadly disease. It can be cured if it diagonised in the early stage. Still there is no such medicine available in the market with 100% efficiency and selective against cancer but a large number of drugs have been used for the treatment of various cancers. In some instances, along with cancerous cells, these available drugs react with normal healthy cells too which causes side effects. Now-a-days, various drugs are being prescribed as potent chemotherapeutic agents. Among these available drugs many of them were synthesized in the laboratory whereas some were either isolated from natural sources or derived from important naturally occurring compounds. Interestingly, majority of these drugs are consisting of heterocyclic moiety as the main structural unit or as important subunits. In this chapter we have described the importance, activity and sources of a huge number of commercially available heterocyclic naturally occuring, natural product derived or synthetic anti-cancer drugs.

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Shashi Kiran Misra and Kamla Pathak*

2 Naturally occurring heterocyclic anticancer compounds

Abstract: Naturally occurring heterocyclic scaffolds are key ingredients for the development of various therapeutics employed for biomedical applications. Heterocyclic pharmacophores are widely disseminated and have been befallen in almost all categories of drugs for the alleviation of myriad ailments including diabetes, neurodegenerative, psychiatric, microbial infections, disastrous cancers etc. Countless fused heterocyclic anticancerous templates are reported to display antimetabolite, antioxidant, antiproliferative, cytostatic etc. pharmacological actions via targeting different signaling pathways (cell cycle, PI-3kinase/Akt, p53, caspase extrinsic pathway etc.), overexpressive receptors (EGRF, HER2, EGF, VEGF etc.) and physiological enzymes (topoisomerase I and II, cyclin dependent kinase etc.). A compiled description on various natural sources (plants, microbes, marine) containing anticancer agents comprising heterocyclic ring specified with presence of nitrogen (vincristine, vinblastine, indole-3-carbinol, meridianins, piperine, lamellarins etc.), oxygen (paclitaxel, halichondrin B, quercetin, myricetin, kaempferol etc.) and sulphur atoms (brugine, fucoidan, carrageenan etc.) are displayed here along with their molecular level cytotoxic action and therapeutic applications.

Keywords: antimetabolite; cytotoxic; heterocyclic scaffolds; overexpressive receptors; signal pathways.

2.1 Introduction

Heterocyclic compounds have two or more fused cyclic ring containing different kinds of atoms where carbon is essential and exhibit several biochemical actions required for survival. For instances, naturally occurring nucleic acid, vitamins, hormones, enzymes, proteins and peptides etc. all do have complexed heterocyclic ring in their basic structure. Heterocyclic pharmacophores are one of the significant key components present in most of the FDA approved anticancer drugs available in the world market [1]. Numerous natural occurring heterocyclic templates i.e.

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alkaloids, flavonoids, flavones, polyphenols etc. have been vigorously exploited to design novel therapeutics to combat myriad newly introduced ailments. The well-organized geometrical configuration of heterocyclic templates offers desirable substitution to modify their pharmacological activity [2]. Cancer, a disease of uncontrolled multiplication of cells has become a common threat in health science. Here a normal cell changes in to cancer cell due to one or more transformation in its DNA [3]. Countless heterocyclic compounds have been reported encompassing nitrogen, oxygen and sulphur atoms that possess significant anticancer actions. These heterocyclic scaffolds have potential to induce apoptosis, suppress proliferation and control cancerogenesis via affecting number of molecular mechanism and signal pathways [4]. Vinblastine, vincristine, taxol, phenethyl isothiocyanate, carrageenan, piperine, meridianins, quercetin, chalcones and many more heterocyclic compounds are extensively investigated for their chemopreventive actions. A comprehensive study on natural occurring anticancer heterocyclic compounds, their biological origin and mechanism of anticancer actions are discussed here.

2.2 N-based natural occurring heterocyclic molecules

Structural diversity and ethanobotanical significance of naturally occurring Nitrogen based heterocyclic compounds have portrayed their extensive applications in the management of versatile cancers. Several reports lay down significant importance of chemotherapeutics containing N-heterocycles in their basic structures. Active biological compounds belonging to indole, pyrrol, piperidine, pyrimidine, quinolone etc. are investigated for their chemopreventive actions [5]. Indole alkaloids i.e. vincristine, vinblastine, bufotenin, tryptamine, lysergic acid etc. are reported for their remarked biological actions including anticancer activity against breast, colorectal, prostate cancer and lymphoma by the virtues of their potential to change cell cycle progression [6]. Cruciferous vegetables (cabbage, broccoli, turnip, cauliflower, mustard green), marine sources (sponges, mollusca and acidians) and microorganisms (*Streptomyces* sp.) are been acknowledged for their dietary values and anticancer actions.

2.2.1 Vinka alkaloids (vincristine and vinblastine)

These vinca alkaloids are isolated from periwinkle plant *Catharanthus roseus*. Both vincristine and vinblastine are naturally occurring bioactive compounds and enriched with several biomedical efficiencies including anti-angiogenesis, antiproliferative and anticancer actions for the alleviation of leukemia and lymphoma. Nitrogen centered indole based these alkaloids cause inhibition of metaphase (mitosis), bind with tubulins and alter their dynamics (cell division, shape

and cell motility) associated with malignancies [7]. Naturally, these antineoplastic are produced in plants through the interaction between inherent vindoline and catharanthin as displayed in Figure 2.1.

2.2.2 Cruciferous vegetables

Cruciferous vegetables (cabbage, broccoli, turnip, cauliflower, mustard green etc.) contain cyclin-dependent kinase enzymes inhibitors accountable for diverse anti-neoplastic actions. These natural biomasses are enriched with indole-3-carbinol (I3C), a potent heterocycle has been explored for its anticancerous role in the management of colon, breast, cervical and prostate cancers. I3C targets signaling pathways including hormonal homeostatis and proliferation of cells (Figure 2.2). I3C reduces Rb phosphorylation, inhibits cycline-dependent p21 and p27 kinase enzymes that causes cell arrest thus regulates breast and prostate cancers [8].

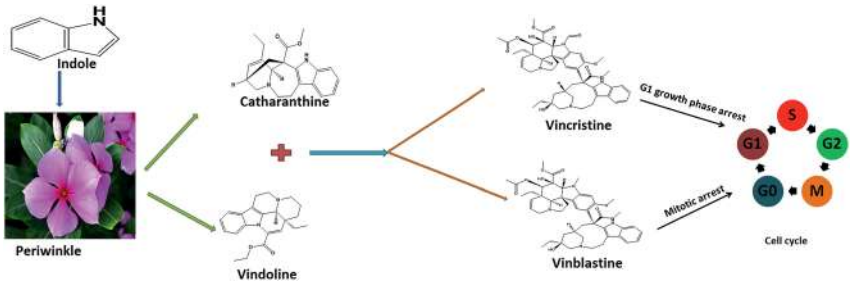

Figure 2.1: Anticancer mechanism of naturally synthesized vinca alkaloids.

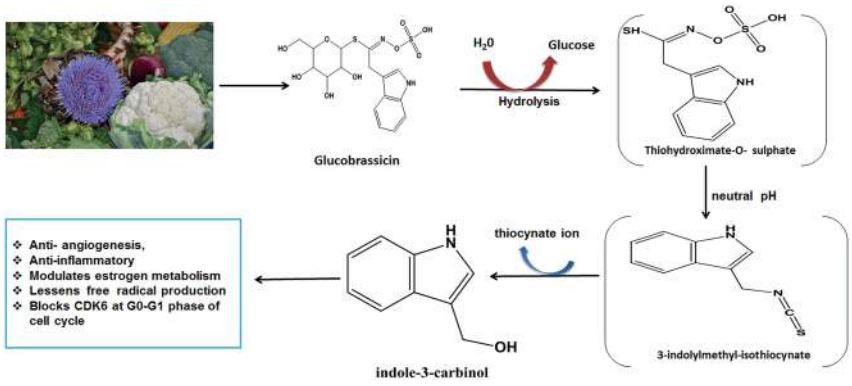

Figure 2.2: Biosynthesis of cruciferous indole-3-carbinolc and anticancer mechanism.

2.2.3 Marine sources (meridianins, dragmacidins and makaluvamines)

Indole based chemopreventive secondary metabolites; Meridianins are isolated from marine invertebrate named *Aplidium meridianum*. These compounds are reported to comprise remarked anticancerous action as they particularly target on CDK1 and CDK5 enzymes involved in cancer cells proliferation [9]. Spongosorites, deep marine sponges have been listed out for containing numerous bioactive bis-indole alkaloids such as dragmacidins, toposentins, hamacanthins etc. Dragmacidins cause inhibition of threonine/serine phosphoprotein phosphatase and arrest mitotic process at metaphase [10]. Zyzzya, another marine sponge is been identified to contain chemopreventive compounds known as 'makaluvamines'. Figure 2.3 illustrates compiles heterocyclic structures of meridianins, dragmacidins and makaluvamines.These heterocyclic compounds belong to iminoquinone class and possess *in vitro*/*in vivo* cell toxicity against diverse carcinomas due to topoisomerase II inhibition [11]. Reports find vital role of makaluvamine A, N and C for the management of ovarian, lung, breast and colon cancers and exhibit dose dependent DNA cleavage action [12].

2.2.4 Lamellarins and trabectedin

The lamellarins (LAMs) are isolated from diverse ranges of marine biota such as sponges, molluscs and ascidians. These molecules are group of numerous

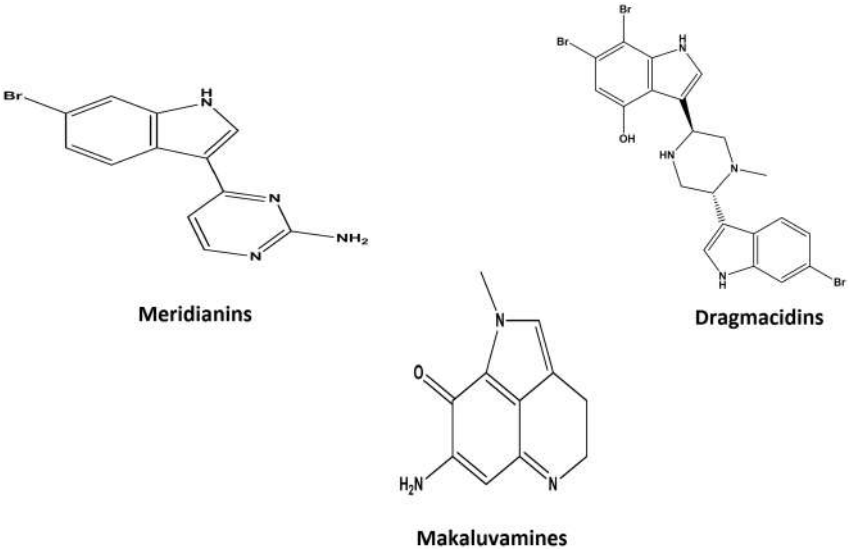

Figure 2.3: Marine indole based heterocyclic anticancer agents.

poly-aromatic pyrrole heterocyclic compounds. Lamellarins A-D was first isolated in the year 1985 from the marine mollusk gastropod, containing heterocyclic benzopyrano-pyrrolo-isoquinolinone moiety [13]. Further, other pyrrole tri-substituted Lamellarins (O-R) were identified from marine sponges *Dendrilla cactos* and *Dendrilla nigra* [14]. Although, all LAMs are potent cytotoxic but their antiproliferative efficiencies varies from one molecule to other. Report says that condensed penta/hexa heterocyclic compounds exhibit effective anticancer actions compared to tri-substituted pyrrole (Figure 2.4). For instance, LAM-D is highly potent anti-proliferative and LAM-O is poor anticancerous agent [15]. LAM-D is strong inhibitor of DNA topoisomerase I, and finds its place for the treatment of multidrug resistant tumor cells and prostate malignancy. A ternary complex between LAM-D and DNA topo-isomerase I form and get intercalated to the DNA that restricted functions of topo-isomerase I. A schematic illustration on mechanism of action of LAM-D is outlined in Figure. LAM-D potentially influences p-glycoproteins, induces proapotopic effect and causes massive depolarization of mitochondrial membrane potential of murine leukemic cells (P388CPT5 cells) [16].

Another marine tunicate 'trabectedin' is potent anticancerous agent and naturally obtained from *Ecteinascidia turbinate* (Caribbean Sea squirt) [17]. Trabectedin contains tetrahydroisoquinoline heterocyclic compound (illustrated in Figure 2.4) and was the first antineoplastic marine drug approved in European Union. It is potential action has been explored for the treatment of soft tissue sarcoma and ovarian cancers after failure of responses of the anthracyclines and pegylated liposomal doxobrubicin. In the year 2007, it gained market approval from European commission for the treatment of advanced soft tissue sarcoma. This marine heterocyclic molecule has direct action on transcription regulation and homologous recombination (DNA) repair systems. Moreover, it modulates the tumor microenvironment associated with breast cancer

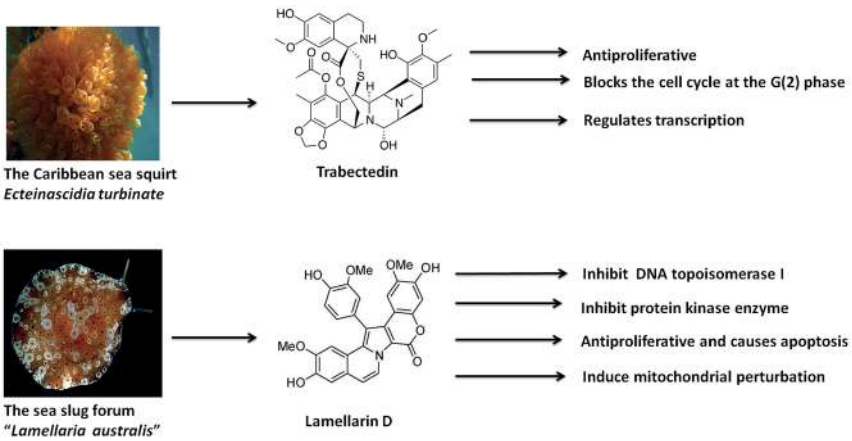

Figure 2.4: Biological source and anticancer mechanism of trabectedin and lamellarin D.

susceptibility genes (BRCA 1 and 2) [18]. Trabectedin has unique alkylating feature that affects cell processes related to proliferation, nucleotide excision repair, transcription etc. Doxil™ is recommended along with trabectedin in case of relapsed ovarian cancer. Literature envisages significant role of trabectedin in the disturbance of cell cycle. It blocks or arrests G1 and G2 sites of cell cycle. Additionally it inhibits overexpression of MDR1 (multidrug resistance) gene coding for p-gp (permeability glycoprotein) that causes cell resistance for most of the anticancerous drugs [19].

2.2.5 Piperine, sanguinorine, tetrandrine

Phytochemical piperine is an important dietary alkaloid, chiefly found in fruits and roots of black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) [20, 21]. Black pepper is considered as king of spices and has been utilized in Indian Medicine for the cure of respiratory and gastrointestinal disorders. Piperine's bitter taste and characteristic pungency positioned specifically in spices [22]. Piperine contained Nitrogen based piperidine heterocyclic components in its nucleus that is accountable for different pharmacological actions including anti-inflammatory and anti-neoplastic [23]. Piperine impairs the migration and T cell-activating function of dendritic cells [24]. leishmanicidal, antimetastatic and other immunosuppressive activities. The chemopreventive actions of piperine are shown by activation of apoptosis, cascade signaling, antiprolifiration, anti-angiogenesis, cell cycle arrest etc. [25]. Piperine, at molecular level affects numerous proteins engaged in the process of apoptosis, activates both extrinsic and intrinsic pathways thereafter suppresses tumor development and metastasis. Lai et al. (2012) administered piperine to xenografted mice bearing 4T1 breast cancer. Markedly reduced tumor growth via activating caspase-3-mediated intrinsic pathway and G2/M cell arrest was observed. Piperine reduces expression of metalloproteinases (MMP1, MMP3, MMP9, MMP13) in breast cancer [26]. Piperine is also effective against androgen dependent and independent prostate cancers cells PC3 and LNCaP, DU 145 respectively. It significantly reduced expression of nuclear factor (NF-kB), phosphorylated STAT-3 and impaired androgen receptors expressions (AR in LNCaP) [27]. Piperine markedly inhibits functions of p-gp (P-glycoprotein) and cytochrome CYP3A4 that are associated with drug (docetaxel) metabolism, resensitizes MDR (multidrug resistance) in cancer cells. Bioactive piperine stimulates apoptosis, effect on cell cycle and act as pro-oxidant via triggering production of reactive oxygen species (ROS) in cancerous conditions. Generated ROS causes depolarization of mitochondrial potential that liberates cytochrome C, activates caspases and induces both extrinsic and intrinsic apoptosis pathways. Thereafter, piperine exhibit antiproliferative action via blocking cell cycle through direct binding particularly at G1 phase. Piperine downregulates cycline D and upregulates p21 and p27 (cyclin dependent kinase inhibitors). Consequently, cell cycle arrest occurs owing to blockage of progression of S-G2-M phase [28].

Benzophenanthridine heterocyclic based sanguinorine alkaloid is obtained from papavarous plants *Chelidonium majus* and *Sanguinaria canadensis*. Sanguinorine is enriched with antibacterial, antineoplastic, antischistosomal, antifungal, anti-inflammatory and antiplatelets actions etc. [29] Potent chemopreventive action (less than 10 μmol) of sanguinorine has attracted attention of researchers for the management of different phases of apoptosis in myriad cancerous cells [30]. The heterocyclic alkaloid exhibit remarked antiangiogenic action in the treatment of breast cancer through sensitizing tumor necrosis factor (TNF) hence mediating ligand oriented apoptosis. It has been revealed that co-administration of cyclooxygenase-2 with sanguinarine enhanced anticancer effect in prostate cancer sufferer. At molecular level, sanguinarine gets interacted with glutathione, generates ROS and results cell toxicity [31]. Further, it is selective blocker of MKP-1(mitogen activated protein kinase phosphatase 1) that disrupts microtubule dynamics and causes damage in DNA [32]. Several pathways including NF-Kb suppresion, signal transducer inhibition, transcriptase 3 activation, CDKs downregulations, genes p21, p27 upregulation and Bcl-2 family modulation etc. are associated with the actions of sanguinarine alkaloids [33]. Figure 2.5 displays compiled heterocyclic structures of piperine, sanguinorine and tetrandrine.

Pharmacologically active tetrandrine, is a bisbenzylisoquinoline heterocyclic compound naturally occur in roots of *stephania tetrandra* [34]. This heterocyclic compound is enriched with various therapeutic actions including immunomodulating, anti-inflammatory, anti-hepatofibrogenic, neuroprotective, antiarrhythmic and anticancer

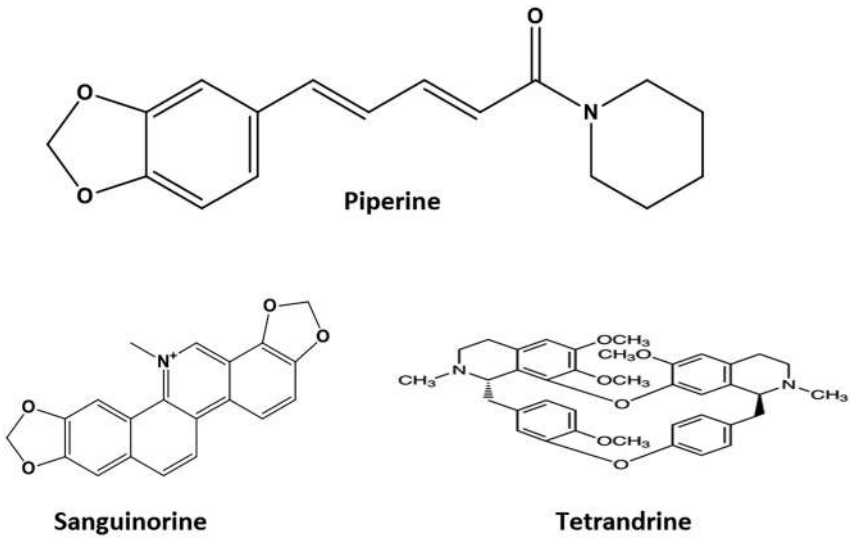

Figure 2.5: Chemical structure of anticancerous heterocyclic piperine (piperidine), sanguinorine (benzophenanthridine) and tetrandrine (bisbenzylisoquinoline).

activities in micromolar concentration [35]. This heterocyclic component induces cell cycle arrest and apoptosis in cancerous conditions including leukemia, colon, hepatoma, bladder and lung cancers. Further, it also inhibits VEGF expressions in glioma cells [36, 37].

2.2.6 Evodiamine, camptothecin, berberin

N-heterogeneous quinazolinocarboline alkaloid, evodiamine is isolated from Chinese herb named Wu-Chu-Yu (*Evodia rutaecarpa*). This naturally occurring herb is highly bioactive and possesses anti-anxiety, anti-inflammatory, anticancer and anti-allergic activities [38].

Essential physiological secretions including testosterone and catecholamine are related with the biological actions of evodiamine. Literatures signify strong cytotoxic actions of evodiamine against hepatoblastoma cell lines and colon cancers [39]. Moreover, this alkaloid has also potential actions including anti-proliferation, apoptosis induction and inhibition of metastasis associated with breast, prostate, leukemic T-lymphocytes, cervical and lung cancerous cells [40]. It alters balance of proapoptotic Bax proteins and anti-apoptotic Bcl-2 expressions and activate caspase effectors in myriad human melanoma cells (A375-S2), Leukemic/acute leukemia (U937/CCRF-CEM), breast cancer (NCI/ADR-RES), androgen dependent (LNCap) and independent prostate cancers (PC-3/DU145) etc. [41]. Cancerous cells treated with this alkaloid showed G2/M cell cycle arrest due to covalent interaction with DNA topoisomerase enzyme I. On consistent exposure to evodiamine results enhanced production of reactive oxygen species (ROS) and nitric oxide (NO) that trigger mitochondrial membrane depolarization and apoptosis [42]. Camptothecin is quinoline alkaloid containing Nitrogen atom in their basic nucleus (Figure 2.6). This alkaloid is very first isolated in 1958 from the Chinese plant *Camptotheca acuminate* and has been explored for the development of FDA approved anticancer drugs such as irinotecan (Camptostar™) and topotecan (Hycam™) for the treatment of colorectal and ovarian cancers respectively [43]. Camptothecin inhibits DNA topoisomerase I and interrupt cell division process. Apart from anticancerous activity, this heterocyclic molecule exhibits antiviral and antiprotozoal activities [44]. Campothecin and its derivatives are potent topoisomerase I blockers and known as topoisomerase I poisons owing to their irreversibly binding with topoisomerase I enzyme, required in higher eukaryotes for unwinding of DNA during cell replication that causes cell death (Figure 2.6).

Another, isoquinoline heterocyclic chemopreventive component 'berberine' is isolated from varieties of Chinese herbs including *Phellodendron chinense schneid*, *Coptidis japonica rhizome* and *Phellodendron amurense* [45]. Berberine has numerous pharmacological activities such as antidiabetics, antibacterial, anti-inflammatory, anti-atherosclerotic, neuroprotective and antilipidimic [46, 47]. Literatures find important role of berberine for the treatment of polycystic ovary syndrome and

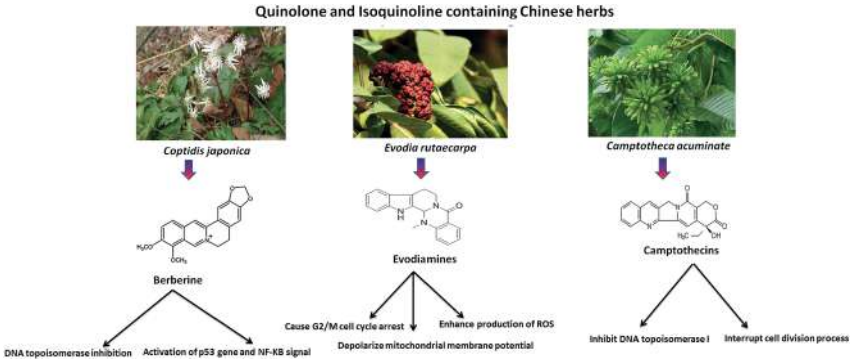

Figure 2.6: Biological sources and anticancer mechanism of berberine, evodiamines and camptothecins.

gastrointestinal disorders. Chemopreventive actions of berberine include apoptosis, cell cycle arrest, DNA topoisomerase inhibition, mitochondrial potential depolarization, production of ROS and activation of p53 gene and NF-KB signal [48, 49]. At molecular level, berberine specifically binds with oligonucleotides and inhibits topoisomerase on account for anti-proliferation action (Figure 2.6). Wang et al. (2010) explained that berberine can induce autophagy and apoptosis in hepatic carcinoma cells (MHCC97-L and HepG2). Induced cell death was observed due to activation of mitochondrial apoptosis (HepG2 cell lines) and increased Bax expression (MHCC97-L cells). Further, subsequent stimulation of caspases-3 & 9, induction of endoplasmic reticulum stress, release of cytochrome C into cytosol and development of permeable transition pores were indicated in affected cells [50].

2.2.7 Other natural heterocyclic anticancerous compounds

Matrine heterocyclic compound is isolated from Chinese *Sophora flavescens* plant and exhibit anticancer action. It is quinolizidine alkaloid and inhibits Toll-like receptor 4 (TLR4) signaling that is expressed in various cancerous activities. It upregulates glutamate transporters, reduces levels of adhesive components and chemokines through inhibiting NF-kβ [51]. The anticancer spectrum of this molecule is wide and has been investigated against mitigation of lung, liver, breast, pancreatic and gallbladder carcinomas owing to its antiproliferative and signal pathways inhibitory actions [52]. Another benzophenanthridine heterocyclic molecule 'fagaronine' is extracted from the plant *Fagara zanthoxyloides* (Rutaceae). The compound has been found to be efficient against leukemia via controlling cell differentiation, proliferation and inhibition of DNA polymerase and reverse transcriptase activity [53]. Pyrrolophenanthridine nucleus containing 'Lycorine' is active constituent present in the plant *Lycoris radiate* (Amaryllidaceae). It has drawn high attention in the field of medicinal chemistry owing

to possessing several pharmacological values such as anti-viral, antimicrobial, anti-carcinogenic, anti-inflammatory etc. At molecular level, it suppresses topoisomerase, acetylcholinesterase followed by control of circadian period length. The exclusive chemical property, multifunctional biological actions and minimum toxicity of lycorine present potential usage as chemopreventive tool [54]. Figure 2.7 exhibited different natural heterocyclic based nitrogen atom containing anticancer compounds.

2.3 Oxygen based natural occurring heterocyclic molecules

Oxygen containing heterocyclic compounds are important class in medicinal chemistry due to vast natural abundance and myriad pharmacological actions i.e. anticancer (taxol), cardiac (digoxin), immunosuppressant (cyclosporine A), hypolipidemic (lovastatin) etc. [55]. Few natural occurring oxygen containing heterocyclic compounds are discussed here.

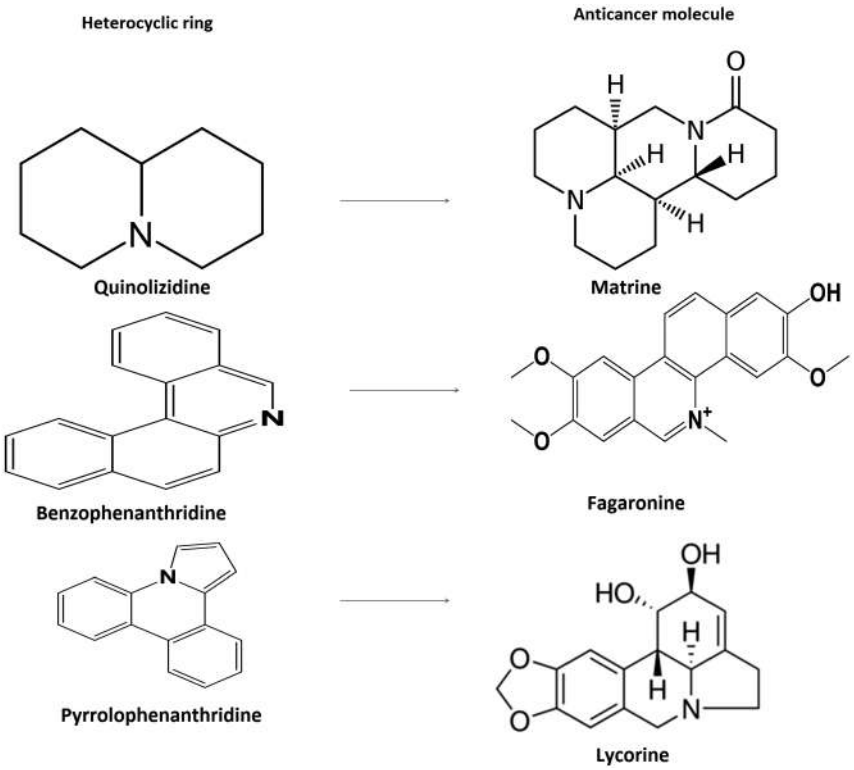

Figure 2.7: Heterocyclic derived anticancerous matrine, fagaronine and lycorine.

2.3.1 Paclitaxel

Widely explored oxygen containing tetracyclic diterpenoid heterocyclic paclitaxel has limitless applications in cancer management (Figure 2.7). Paclitaxel is FDA approved microtubule inhibitor (Taxol®) and intended for the cure of lung, ovarian, breast and Kaposi's sarcoma [56]. It has potential to arrest mitotic cell division and causes multipolar division. Multifunctioning anticancerous paclitaxel is naturally obtained diterpenes from bark of Pacific Yew (*Taxus brevifolia*) and English Yew (*Taxus baccata*) trees. At molecular level paclitaxel stabilizes microtubulins, disturbs their basic functions and induces inhibition of depolymerization of microtubules. Taxanes are potent antineoplastic compounds but their poor water solubility and high cell toxicity limit frequent applications and dictate designing of novel clinically active pharmaceutical products [57]. To overcome the above issues, nanotechnological based liposomes, nanoparticles, micelles and emulsions of paclitaxel have been formulated and evaluated against diverse malignancies [58]. For instance, Abraxane® a novel nanoformulation containing paclitaxel and albumin is now approved by USFDA for the management of metastatic breast cancer. Paclitaxel encourages microtubular assembly that consists of repetitive subunits of α/β-tubulin dimers. Initially, at prophase paclitaxel let the microtubules to form spindle after pulling the chromosomes near the poles. Later on, the spindle structure gets dissolved due to the depolymerization [59]. Report says that low temperature (cold) and calcium ions trigger the activity of paclitaxel against cancerous cells [60].

2.3.2 Halichondrin B

Marine macrolides have high potential to exhibit numerous biomedical usages owing to their cidal actions on fungi, viruses, nematodes, malarial parasites and cancerous cells. Basically, these are polyketide heterogenous molecules (Figure 2.8) isolated from sponges, marine invertebrates, algae and dinoflagellates. For instance, polyether macrolide 'halichondrin B' is obtained from *Halichondria okadai*, a Japanese sponge contains notable pharmacological actions including antineoplastic activity [61]. This heterocyclic polyether macrolide potentially inhibits proliferation of cancerous cells by the virtue of cell cycle arrest. It progressively checks G2/M through inhibiting tubulin polymerization [62]. Its structural analogue Eribulin mesylate found highly chemopreventive against metastatic breast cancerous cells while co-administered with cisplatin [63]. Tubulin and microtubules are the primary targets of halichondrins B and eribulin as both cause microtubule polymerization thus disturb tubulin dynamics. Unstable tubulin dynamics plays crucial role in the functions and assembly of mitotic spindles and affects cell proliferation [64].

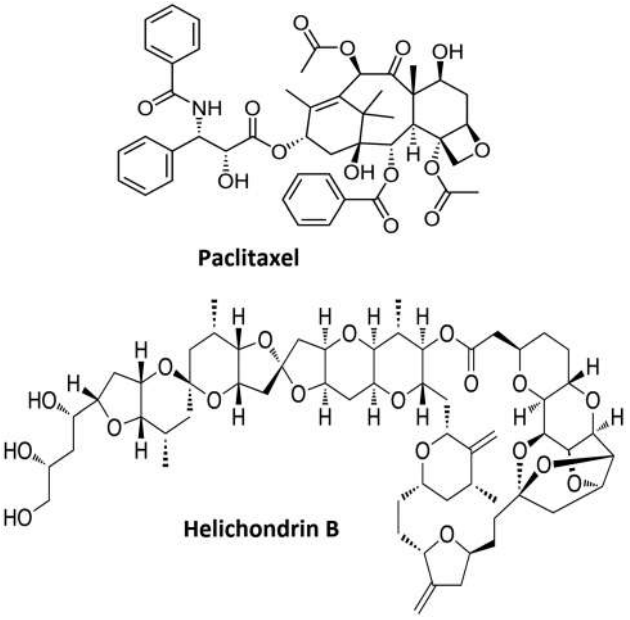

Figure 2.8: Chemical structure of paclitaxel and helichondrin B.

2.3.3 Quercetin, myricetin and kaempferol

Phytochemicals such as heterocyclic flavones, flavonoids and polyphenols all are highly explored for their inherent medicinal values including antineoplastic actions. Oxygen atom containing quercetin heterocyclic compound (3,3′,4′,5,7-pentahydroxy-flavone) is widely occur in daily food products such as nuts, vegetables and teas [65]. Remarked pharmacological values of quercetin related to cancer are identified such antioxidant, antiproliferation and anti-inflammations are reported [66]. Chemically, it is 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one and containing heterocyclic pyrone ring [67]. Bioactive flavonoid 'Quercetin' is highly effective pharmacophore owing to presence of two antioxidant molecules that can remove generated free radicals in the microenvironment of cancerous cells and enable to join transitional metal ions (Figure 2.9). Hydroxyl functional group adorned catechol molecule at position 3 in the structure of quercetin scavenges free radical that is highly required for the protection of osteoporosis and various malignancy treatments. At molecular level, quercetin modulates mitochondrial apoptosis through inducing p53 genes that results upregulation of Bax, downregulation of Bcl-2 followed by caspase-3 activation. Consequently, apoptosis occurs in the cancerous cells leading to anticancer action [68]. Literature envisages potential anticancer action of dietary polyphenol quercetin on leukemic cell lines isolated from chronic lymphocytic leukemia (CLL) sufferer. It

activates the effect of first line anticancer drug 'fludarabine' in the cure regimen of CLL. It downregulates protein levels associated with Mcl-1 mRNA and causes protein degradation with minimum damage of normal peripheral blood components and cells [69]. Additionally, quercetin is found to cause cell death through G0/G1 cell arrest in case of leukemia, inhibition of G2/M phase in esophageal adenocarcinoma and blocking of S phase in colorectal cancer [70–72].

Another flavonoid containing chemopreventive heterocyclic compound 'myricetin' falls under polyphenolic category. Myristine is very common in vegetables, berries, tea, wine etc. belonging to plant family Anacardiaceae, Primulaceae, Pinaceae, Myricaceae [73]. Polyphenolic myricetin was first isolated from the bark of *Myrica nagi* in the late eighteenth century. It is light yellow in color with poor solubility in water (16.6 μg/ml) and have high (357 °C) melting point [74]. Myricetin has been highly explored for its antioxidant and nutraceuticals values. Variety of biomedical applications including analgesic, antitumor, antidiabetics, hepatoprotective, anticancerous actions etc. have also been reported (Figure 2.9). Phytochemical myricetin is chemically related to other heterocyclic polyphenolic herbal molecules such as morin, quercetin, fisetin and kaempferol. All of them were prominently explored for their antioxidant and nutraceuticals properties. An anticancer action of myricetin has been declared for myriad cell lines including skin,

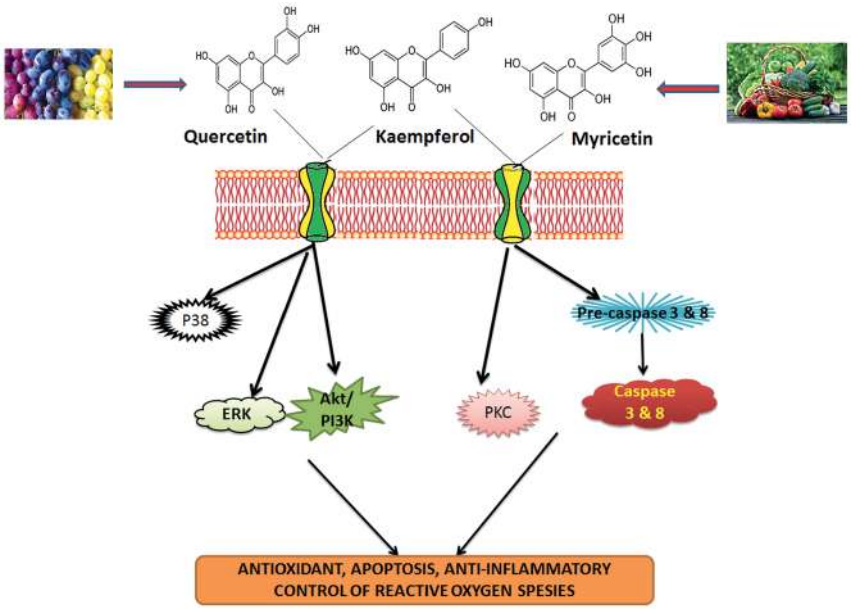

Figure 2.9: Anticancer mechanism of oxygen based polyphenolic heterocyclic quercetin, kaempferol and myricetin.

hepatic, colon and pancreatic cancers [75]. At molecular level, myricetin inhibits topoisomerase I and II (K562 leukemia cell line), tumor promoter induced cells via restricting mitogen activated protein kinase (MEK), Akt, JAK1 and MKK4 kinase actions (skin cancer) at minimum concentration (10 Mm). Its combination with resveratrol exhibited synergistic efficacy for epidermal growth hormone induced transformation [76]. Moreover, myricetin suppresses 12-O-tetradecanoylphorbol-13-acetate (TPA) and epidermal growth hormone encouraged phosphorylation of ERK (extracellular signal regulated kinase) and p90 (ribosomal S6) kinase associated with skin cancer [77]. Further, with combination of food mutagen also displays anti-genotoxic actions in lymphocytes [78]. Labbe et al. (2009) studied anticancerous and cytotoxic effects of myricetin and related flavonoids against Medulloblastoma, a common brain tumor. This abnormality predominantly occurs in children and is highly metastatic. HGF (hepatocyte growth factor) and tyrosine kinase are emerged as primary components in the proliferation of medulloblastoma that is inhibited by flavonoid containing dietary substances [79].

Common fruits and vegetables such as grapes, strawberries, apples, citrus ones, beans, cabbage, broccoli etc. are supplemented with kaempferol heterocyclic compound (Figure 2.9) that has countless pharmacological actions [80]. Diverse medicinal plants such as *Sophora japonica*, *Euphorbia pekinensis*, *Equisetum species* and *Ginkgo biloba* etc. are figured out for containing kaempferol heterocyclic component [81]. Kaempferol has been explored for the treatment of inflammatory induced disorders such as intervertebral disc damages; post menopause bone loss, colitis and lung injury [82]. Several researches are reported that suggested potential biomedical usage of bioflavonoid kaempferol i.e. antidepressant, Parkinson, Alzheimer, ischemic stroke and alleviation of dermal fibroproliferative disorders [83]. Anticancer actions of kaempferol have been reported against esophageal, breast, cervical, liver, ovarian, bladder carcinomas and leukemia. At molecular level, apoptosis was observed by the virtue of promoter of caspases (pre-caspases 3, 8, 9 factors) and antioxidant actions by inhibiting the process of ROS. Kaempferol also activates immunity of host, restricts growth of cancerous blood vessels and potentiates sensitivity of chemotherapeutic drugs [84]. Heterocyclic kaempferol causes G0/G1 phase arrest and downregulates Bcl-2 receptor, thus inhibit expression of caspase-9 and triggers apoptosis for the alleviation of esophageal cancer [85].

Further, it suppresses breast cancer progression induced by triclosan and inhibits insulin growth factor (IGF1) and estrogen E2 receptor associated with caspase-9 pathways [86]. Both acute myeloid leukemia and chronic lymphocytic leukemia are reported to be managing with kaempferol bioflavonoid. It has been mentioned that growth of human leukemia (HL-60) are suppressed by inhibiting DNA repair proteins expression including O-6-methylguanin-DNA-methyltransfersa and DNA-dependent protein kinase [87].

2.3.4 Chalcones

Chalcones are metabolic precursors of few flavonoids and isoflavanoids and found as important plant constituent in fruits, vegetables and spices majority of plant family leguminosae, Moraceae, Asteraceae [88]. These heterocyclic compounds have common pharmacophore i.e. 1,3-diaryl-2-propen-1-one, that is an essential basic moiety for the execution of variable pharmacological actions including anti-inflammatory, anticancer, antioxidant, antimicrobial, antidiabetics and anti-malarial actions [89]. Chemically, these natural occurring heterocyclic compounds contain open chain flavonoid adorned with two aromatic rings. Butein, isoliquiritigenin and isobavachalcone (Figure 2.10) are prominently explored natural anticancerous chalcones obtained from *Rhus* sp. bark, licorice roots and *Psoralea* extract respectively. Table 2.1 summarizes anticancer action of natural occurring chalcones.

2.3.5 Heterocyclic antibiotics (doxorubicin, bleomycin and dactinomycin)

Anthracycline derived cytotoxic doxorubicin antibiotic is collected from culture of soil fungi '*Streptomyces peucetius*'. It is widely used in the diverse neoplastic conditions i.e. leukemia (both in acute lymphoblastic and myeloblastic), neuroblastoma, Wilms' tumor, carcinoma (breast, ovarian, bladder, thyroid and gastric) etc. At molecular level, doxorubicin binds with nucleic acids, forms complexes with DNA and inhibits activity of topoisomerase II thus hampers their functions [98]. It activates molecular signal i.e. AMP-activated protein kinase inducing apoptosis and affects caspase channels by altering Bcl-2/Bax ratio and breaks DNA genomics [99].

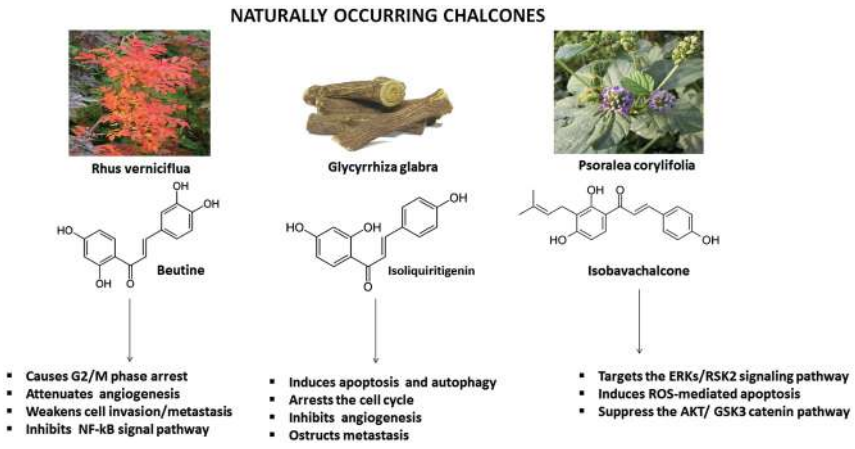

Figure 2.10: Anticancer mechanism of naturally occurring chalcones.

Table 2.1: Various chalcones utilized for anticancerous usage.

Chalcones	Objective	Research outcome	Anticancer usage	Ref.
Butein	Evaluation of constitutive and inducible effects of butein against tyrosine kinase and phosphatase followed by regulated growth of tumor cells	Heterocyclic butein caused downregulation of STAT-3 regulated genes expression including Bcl-2, Mcl-1, cyclin D1 that mediated suppression of tumor growth and induced apoptosis.	Multiple myeloma	[90]
	The anticancer potential activity of butein was evaluated on viability, apoptosis and migration of squamous cell carcinoma (OSCC).	A profound action on carcinoma cell proliferation, antioxidant and anti-migratory effects were observed that suggested suppression of OSCC through butein.	Oral squamous cell carcinoma	[91]
	Analysis of anticancer activity of butein through checking production of reactive oxygen species in breast cancer.	A significant reduction in the level of ROS observed that led to butein reduction of ROS level owing to release of N-acetyl-L-cysteine (ROS scavenger).	Treatment of breast cancer	[92]
Isoliquiritigenin	Mechanism behind induction of cell cycle arrest (G2/M) and cytotoxicity against HeLa human cervical cells were analyzed for isoliquiritigenin.	Research outcomes suggested inhibition of topoisomerase II, DNA damage and metaphase arrest by the action of isoliquiritigenin.	Used as topoisomerase II poison in cancer treatment	[93]
	Antiproliferation and cell apoptosis actions of isoliquiritigenin against prostate cancer were studied out.	Outcomes obtained from molecular studies revealed G2/M cell cycle arrest that was reliant on reduction in cyclin dependent kinase enzyme (CDK1).	Active against prostate cancer	[94]
	Investigation of antitumor activity of isoliquiritigenin and its active metabolite tetrahydrochalcon against lung cancer xenografted model.	Both antitumor action and inhibition of cancer cell migration were observed that occurred by the interference of isoliquiritigenin with Src kinase activity in lung cancerous cells.	Antitumorigenic action on lung cancer	[95]
Isobavachalcon	Investigation of anticancerous action of isobavachalcon against tongue squamous cell carcinoma (Tca8113 cells).	Western blot studies revealed inhibitory action of Isobavachalcon in cell migration and invasion. It caused downregulation of expression of matrix metalloproteinase (MMP 2 & 9).	Treatment of tongue squamous cell carcinoma.	[96]
	Investigation of anticancer action of isobavachalcon against prostate cancer by targeting antioxidant thioredoxin reductase 1 enzyme (TrxR1).	It caused ROS mediated apoptosis through interaction with Sec (selenocysteine) contained TrxR1 enzyme. Knocking down of this enzyme has sensitized prostate cancerous cells for isobavachalcon action.	Prostate cancer	[97]

Glycopeptide antibiotic bleomycin is isolated from fungus culture '*Streptomyces verticullum*' (Figure 2.11). It is also a broad spectrum heterocyclic antibiotic and vastly employed to treat neoplasms in humans. Interestingly, it contains nitrogen, oxygen and sulphur atoms in its structure and the bithiazole portion interact profoundly with DNA (non-covalently) [100]. Bleomycin is recommended in Hodgkin's lymphoma and various cancers including ovarian, testicular, and cervical. It is advised to administer inside the chest to prevent recurrence of fluid around the lungs (malignant pleural effusion), a fatal condition in cancer [101]. Bleomycin is a nonhemeprotein and causes cleavage/breaking of DNA strands. It also prevents association of thymidine into DNA, assists production of free radicals (superoxides and hydroxides) that cleave DNA [102]. Another fungal origin dactinomycin (Actinomycin D) is composed of heterocyclic phenoxazine ring. It is a natural chromopeptide made up of chromophore and pentapeptide lactone rings. Naturally it is obtained from genus *Streptomyces parvulus* via fermentation. Dactinomycin is one of the oldest chemopreventive antibiotics utilized for the management of Wilm's tumor, Ewing's sarcoma, rhabdomyosarcoma and gestational trophoblastic disorder [103]. Alike, both antibiotics (doxorubicin and bleomycin), it also targets DNA, hinders RNA synthesis via transcription process thus affects protein synthesis [104].

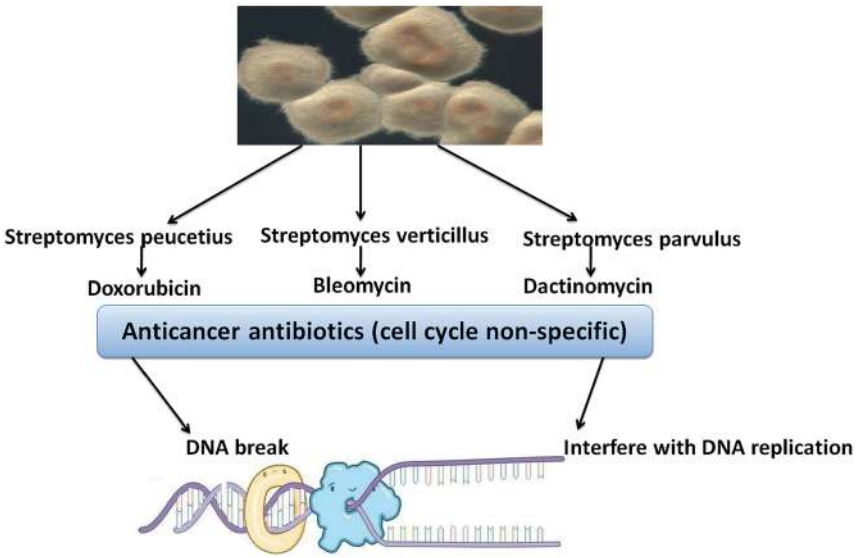

Figure 2.11: Anticancer mechanism of heterocyclic antibiotics produced by microorganism Streptomyces sps.

2.4 Sulphur based natural occurring heterocyclic compounds

Mangroves, microalgae, actinobacteria, halophytes etc. are important oceanic assets that contribute important ethanobotanical and pharmacological virtues owing to constitution of polyphenolic and sulphated polysaccharides. This category of biomass is diverse, highly productive and explored for the development of newly anticancerous therapeutics. Further, these natural sources are filled with antioxidant and immunostimulation actions [105].

Mangroves are generally grown in excessive temperate region specified by estuarine swamps enriched with high salt and low nutrients. This plant community experience high radiation and capable to flourish under environmental stress conditions that results in generation of ROS (reactive oxygen species) along with metabolites. To combat generated ROS, these plants produce several defense substances including enzymes and polyphenolic compounds. The explored polyphenols and sulphated polysaccharides from the mangroves and other marine resources have potential to activate macrophage that stimulate apoptosis and check DNA damage [106, 107].

2.4.1 Brugine

Sulphur containing brugine alkaloid consisting 1,2-dithiolane ring (Figure 2.12) is isolated from bark of mangrove species of *Bruguiera sexangula* and *Bruguiera gymnorrhiza* that displayed anticancerous efficacy against lexis lung carcinoma and sarcoma 180. Above mangrove species are also explored for cancer effective tannins [108]. *(Bruguiera gymnorhiza)* Rhizophoraceae is widely distributed in the wild forest of Sunderbans India, Indonesia, Malaysia, Mauritius and China. The bark and root decoction of this mangrove are employed for the treatment of fever, diabetes, diarrhea and liver disorders [109].

2.4.2 Fucoidan

Marine algae are always inevitable traditional remedies in the region of eastern hemisphere. This natural biota is abundant and overloaded with diverse biological components including terpenes, phytocyanin, polysaccharide fucosterol etc. that exhibited myriad health benefits including pharmacological actions such as antioxidant, neuroprotective, anti-inflammatory, antineoplastic and antiobesity [110, 111]. Brown algae (*Undaria pinnatifida*, *Porphyra hateanensis*, *Sargassum fusiforme and Laminaria japonica*) contain numerous biological components such as gepsin, alginic acid, porphyran, oligosaccharides that protect seaweed from bacterial invasion [112]. Sulphated carbohydrate porphyran is approximately related to agarose with a linear

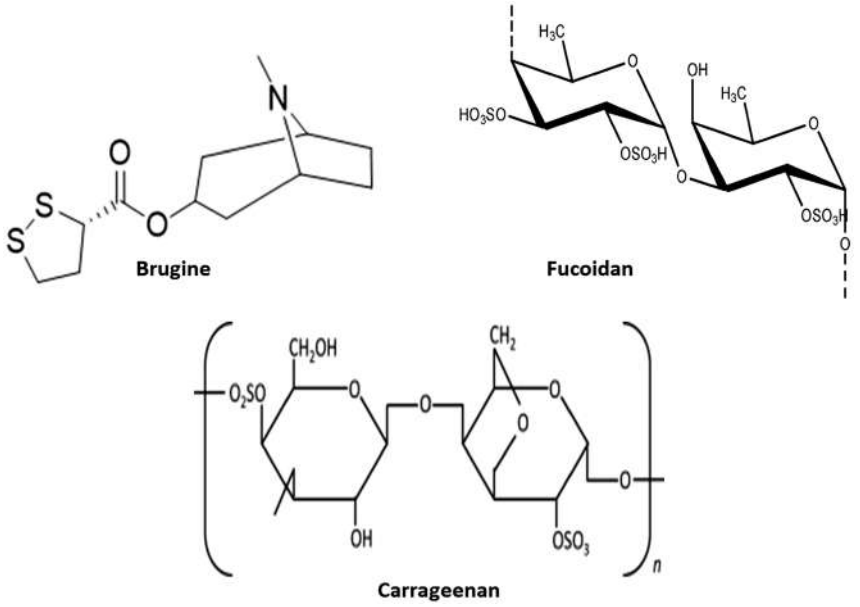

Figure 2.12: Sulphur atom containing chemopreventive natural heterocyclic compounds.

backbone and specified with ester sulphate [113]. *P. hateanensis* consists of sulphated galactam fraction F1 that on intraperitoneal administration remarkably reduced lipid peroxidation owing to its superior antioxidant activity. Further it was found to augment actions of superoxide dismutase and glutathione peroxidase, thus total antioxidant capacity was improved in aging mice [114]. Fucoidan and fucoxanthin are two major metabolite of brown algae or phaeophyceae that also exhibited anticancer actions [115]. Fucoidan is sulphur containing complex heterocyclic molecule extracted from seaweed brown algae (Figure 2.12). Extracellularly it is enriched with fucose and well proved for anticancer action. The structure of sulphated heteropolysaccharide fucoidan highly depends on isolated species source [116]. Table 2.2 represents species based anticancer action of fucoidan.

Fucoidan activates the intrinsic and extrinsic processes of apoptosis followed by augmenting immune responses. Thereafter, suppression of angiogenesis occurs due to blockage of adhesion of cancerous cells to the human platelets. Literature envisages superior anticancer action of oversulphated fucoidan to the neutral fucoidan against carcinoma.

2.4.3 Carrageenan

Irish moss, a seaweed is comprised of carrageenan, a sulphated polysaccharide heterocyclic compound. The red algae species *Chondrus crispus*, Gigartinaceae has been

Table 2.2: Diverse variety of brown algae exhibiting anticancer activity.

Species of red/brown algae	Type of cancer	Mechanism of action	Ref.
Sargassum thunbergii	Ehrlich carcinoma	Fucoidan increased chem-iluminescence and phagocytosis action of macrophages.	[117]
Fucus vesiculosus	Management of Sarcoma 180 cells and Lewis lung carcinoma	It suppressed chemotactic and mitogenic action on VEGF (vascular endothelial growth factor 165) through binding with cell surface.	[118]
Cladosiphon okamuranus	Treatment of MKN45 stomach cancerous cells	Growth inhibition of stomach cancerous cells was observed without affecting healthy cells.	[119]
Undaria pinnatifida, Miyoek	Hepatocellular, alveolar, prostate and cervical carcinoma	O-acetylated sulphated galactofucan fucoidan showed antitumor action against different carcinoma cells by the virtues of its exclusive chemical composition.	[120]
Fucus evanescens	Lewis lung adenocarcinoma	Dose dependent antimetastatic and anticancerous actions.	[121]

explored for the extraction of polysaccharide galactans or carrageenan (Figure 2.12). The classification of carrageenan (i.e. kappa, iota, and lambda) is based on the position of attached ester sulphate groups and 3, 6-anhydro-D-galactose moiety [122]. In recent years multifunctional qualities (biocompatibility, biodegradability and non-toxicity) and biomedical usage (anticoagulant, antimicrobial, antiviral and antioxidant) of this sulphated polysaccharide have been extensively investigated [123]. Reports have evident immunomodulatory and antineoplastic efficacy of carrageenan owing to its virtue to destabilize linking of glycosaminoglycans with extracellular matrix proteins. Consequently, eliminate of chance of cancer cell adhesion to the matrices and limit the process of metastasis [124]. Although, anticancer efficacy is highly depends on molecular weight, chemical structure, position of sulfation and its quantity. Lower molecular weight comprising lambda carrageenan exhibits superior anticancer action compare to other two i.e. kappa and iota [125]. Tumor inhibition effect of low molecular weight carrageenan (lambda, 9.3 kDa) admixed with 5-fluorouracil was investigated on S180 tumor transplanted mice. Result revealed enhanced antitumor action of 5-fluorouracil due to conjugated lambda carrageenan and suggested as an adjuvant in chemotherapy [126]. The study was extended to investigate immunomodulation and antitumor efficacy of different molecular weight (15, 140, 240, 650 and 9.3 kDa) lambda carageenan against hepatocellular H-22 tumor transplanted mice that depicted cell cycle arrest at G1 and G2/M phase. Carrageenan containing adjuvant significantly augmented production of anti-ovalbumin antibodies in mice [127].

2.4.4 Phytoalexins and phenethyl isothiocyanate

Phytoalexins are secondary metabolites of cruciferous plants and *de novo* synthesized in a response of fungal infection and environmental stress in the plant. Phytoalexins impart biological actions including antimicrobial and cytotoxicity [128]. Camalexin is heterocyclic indole derived phytoalexin (Figure 2.13), which accumulates in cruciferous plants (i.e. *Arabidopsis thaliana*, Brassicaceae) and exhibits defense mechanism against plant (fungi) and human pathogen (trypanosoma) [129]. Moreover, anticancer activity of camalexin is also reported against leukemia and prostate and breast cancers. Camalexin mediates formation of ROS, aggressively reduces cell proliferation followed by augmented apoptosis in metastatic prostate cancer sufferer irrespective to disturb normal cells. The potency of camalexin can be increased by addition of hydrogen peroxide that potentiates formation of reactive oxygen species and results prominent cytotoxic action [130]. Time and concentration dependent anti-leukemic action of camalexin was reported by Mezencev & coworker. Cytotoxicity studies revealed formation of ROS, activation of caspase 8, 9 and 3/7 processes, dissipation of mitochondrial membrane potential and apoptosis in Jurkat leukemic cells in a micromolar range [131, 132]. Cruciferous vegetables also contain high content of sulphurated phytochemicals i.e. glucosinolates, that are accountable for anticancer actions. Glucosinolates after metabolic degradation results chemopreventive isothiocyanates heterocyclic compounds. Numerous anticancerous mechanisms such as mediation of carcinogen metabolism, initiation of apoptosis, inhibition of migration of cancer cells, cell cycle arrest, blocking of malignancy associated signal pathways etc. have been recommended by isothiocynates [133]. Sulphur containing cruciferous phytochemical is phenethyl isothiocyanate, a gluconasturtiin prevents tumorogenesis and initial step of carcinogenesis (Figure 2.13). It targets multiple proteins that are required for cancer promoting mechanisms including proliferation, progression and lastly metastasis. Phenethyl isothiocyanate (PEITC) accompanied with other conventional anticancerous therapeutics effectively treats cancer [134].

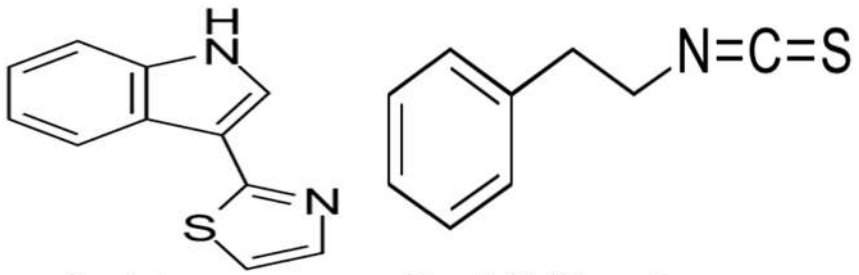

Figure 2.13: Cruciferous sulphur atom encompassing anticancerous camalexin and phenyl isothiocyanate.

2.5 Conclusions

Heterocyclic natural compounds have played crucial roles for the management of several cancers. These compounds are good candidates for designing of biologically active substances that effectively interact with targets (cancerous site) and disturb signal processes accountable for cancerogenesis. Their natural abundance, relative ease of structural modification and precise anticancer action further qualify their suitability for starting up development of anticancer drug development. This study has focused on significance of natural occurring nitrogen, oxygen and sulphur containing heterocyclic compounds utilized for anticancer action.

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3 Lawsone (2-hydroxy-1,4-naphthaquinone) derived anticancer agents

Abstract: 2-Hydroxy-1,4-naphthaquinone, commonly known as lawsone, represents an extremely important biologically active naturally occurring compound. It can easily be isolated from *Lawsonia inermis* (henna) tree leaf extract. Last decade has seen tremendous applications of lawsone as a starting component for the preparation of various organic scaffolds. Many of these synthesized scaffolds showed a wide range of biological activities including potential activities towards several cancer cell lines. This review deals with diverse synthetic methods of lawsone derived scaffolds and their screening against different anti-cancer cell lines along with promising results.

Keywords: 2-hydroxy-1,4-naphthaquinone; anti-cancer; anti-tumor; β-lapachone; lapachol; lawsone.

3.1 Introduction

Lawsone or 2-hydroxy-1,4-naphthaquinone (**1**) is a naturally occurring naphthoquinone derivative. Lawsone is the major constituent of *Lawsonia inermis* (henna) tree and thus it can be isolated easily from the henna leaf extract [1]. Henna leaf extract has been reported to possess anti-cancer activities [2]. Fieser and Martin [3] synthesized lawsone in their laboratory from the reactions of ammonium 1,2-naphthoquinone-4-sulfonate with conc. sulfuric acid followed by hydrolysis with NaOH. Recently, in 2019, Sivakumar *et al.* [4] synthesized lawsone from α-naphthol. The hydroxyl (–OH) group at the C-2 position of lawsone tends to reduce its electrophilic potential. Lawsone has been used as an important precursor for the synthesis of various pharmaceutical compounds [5]. Lawsone itself and many of its derivatives show prominent biological activities. Figure 3.1 represents a glimpse of 2-hydroxy-1,4-naphthaquinone derived scaffolds having a wide range of pharmacological activities, which include anti-malarial [6, 7], anti-leishmanial [8], anti-protozoal [9], anti-plasmodial [10], antiparasitic [11], anticancer [12],

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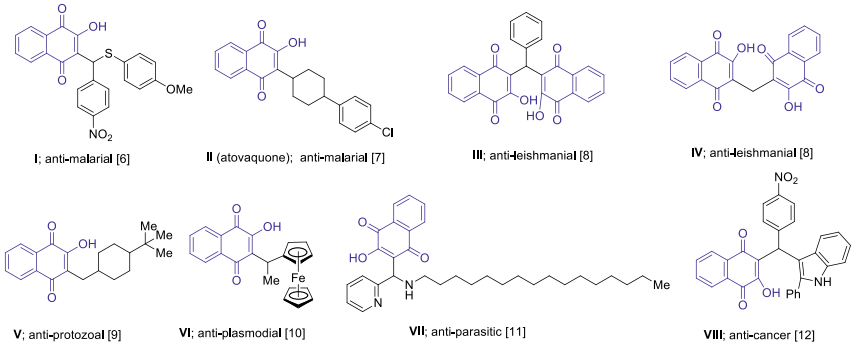


Figure 3.1: Few examples of 2-hydroxy-1,4-naphthaquinone derived synthetic compounds bearing significant and diverse biological activities.

etc. It is proposed that the biological activities of lawsone are due to the oxidative stress generation [1]. It can also be used to elevate the plasma levels of blood urea and creatinine [13]. Lawsone can cause depletion of intracellular glutathione [14]. Many naturally occurring bioactive compounds such as lapahol, nor-lapahol, α-lapachone, β-lapachone, nor-α-lapachone, nor-β-lapachone, avicequinone B, kigelinone, streptocarpone, dunniol, duninone, α-duninone, lapinone, lomatiol, maturone etc. can also be synthesized in the laboratory starting from 2-hydroxy-1,4-naphthaquinone or its derivatives (Figure 3.2) [15, 16]. Some metal complexes of lawsone showed prominent anti-cancer

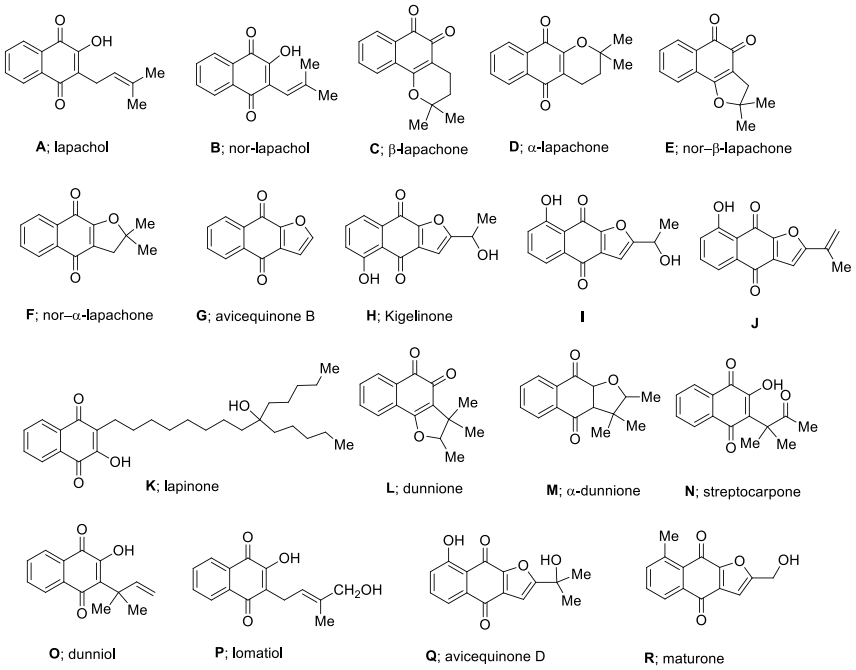


Figure 3.2: Representative examples of naturally occurring bioactive compounds synthesized from 2-hydroxy-1,4-naphthaquinone and its derivatives.

activities [17]. Few dunnione [18] as well as lapinone [19] derivatives are found to possess significant anti-malarial efficacies. Starting from 2-hydroxy-1,4-naphthaquinone, various other lapachol and lapachone derivatives have also been synthesized that exhibit excellent anticancer activity against different cancer cell lines [20]. Literature studies revealed that many organic scaffolds bearing the 1,4-naphthoquinone moiety possess promising anticancer activities against various human cancer cell lines [21, 22]. Some derivatives of 2-hydroxy-1,4-naphthaquinone show prominent efficacies against Chagas disease [23–27]. In continuation of our strong interest on bioactive scaffolds [28–40], in this manuscript we have highlighted the anti-cancer efficacies of various synthetic compounds which were prepared by using 2-hydroxy-1,4-naphthaquinone as the important basic component. To the best of our knowledge, there is no such literature is available on the same topic. Therefore, the present manuscript is expected to attract the interests of many researchers working with lawsone or its derivatives and subsequent biological applications.

3.2 Synthesis of lawsone derived synthetic organic scaffolds having anticancer activities

3.2.1 Synthesis of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*] quinazoline-7,8-diones

In 2013, Wu *et al.* [41] synthesized a series of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-dione derivatives (**4**) *via* one-pot three-component reactions of 2-hydroxy-1,4-naphthoquinone (**1**), various aromatic aldehyde (**2**) and 3-amino-1,2,4-triazole (**3**) in the presence of a catalytic amount of sulfamic acid under neat conditions at 110 °C (Figure 3.3). Reactions were completed within 90 min. All the synthesized compounds were isolated in excellent yields and their anti-proliferative activity was screened against the human gastric carcinoma cell line SCG7901, hepatoma cell line HepG2 and compared the IC_{50} (the concentration required for 50% inhibition of cell viability) values with the potent antitumor drug Doxorubicin. It was found that among all the 10 synthesized scaffolds, compounds **4b**, **4g**, **4h**, **4j** exhibit promising antitumor activity against the cancer cell lines mentioned above. It was observed that the electron donating substituent on aromatic ring at the C-6 position showed better efficacies as compared to electron withdrawing substituents. The research group also synthesized 15-(4-chlorophenyl)-[1,2,4]triazolo[1′,5′:1,2]pyrimido[5,4-*a*]benzo[*c*]phenazine (**6**) from the reactions of compound **4c** and *o*-phenylenediamine (**5**) under neat condition at 100 °C (Figure 3.4). However, this compound (**6**) showed less efficacy than all the 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-dione derivatives (**4**).

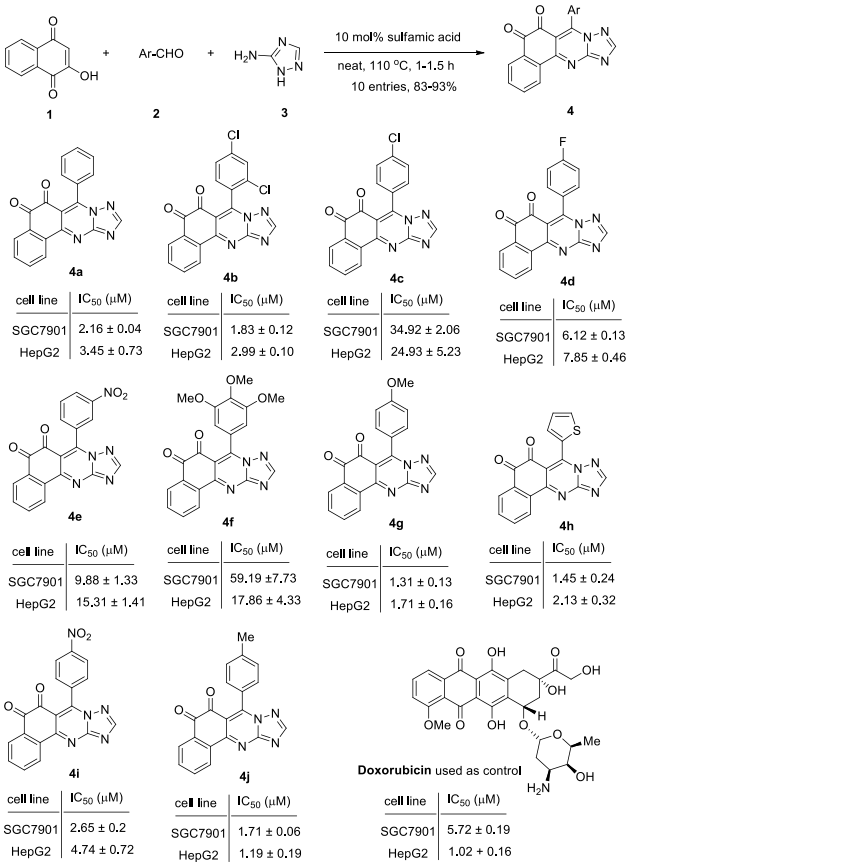


Figure 3.3: Solvent-free synthesis of 6-aryl-benzo[*h*][1,2,4]-triazolo[5,1-*b*]quinazoline-7,8-diones as antitumor agents.

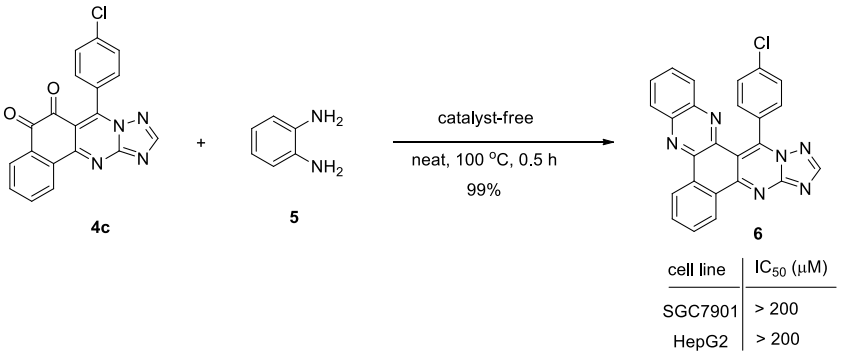


Figure 3.4: Solvent-free synthesis of 15-(4-chlorophenyl)-[1,2,4]triazolo[1′,5′:1,2] pyrimido[5,4-*a*] benzo[*c*]phenazine under neat condition.

3.2.2 Synthesis of 13-aryl-13*H*-benzo[*g*]benzothiazolo[2,3-*b*] quinazoline-5,14-diones

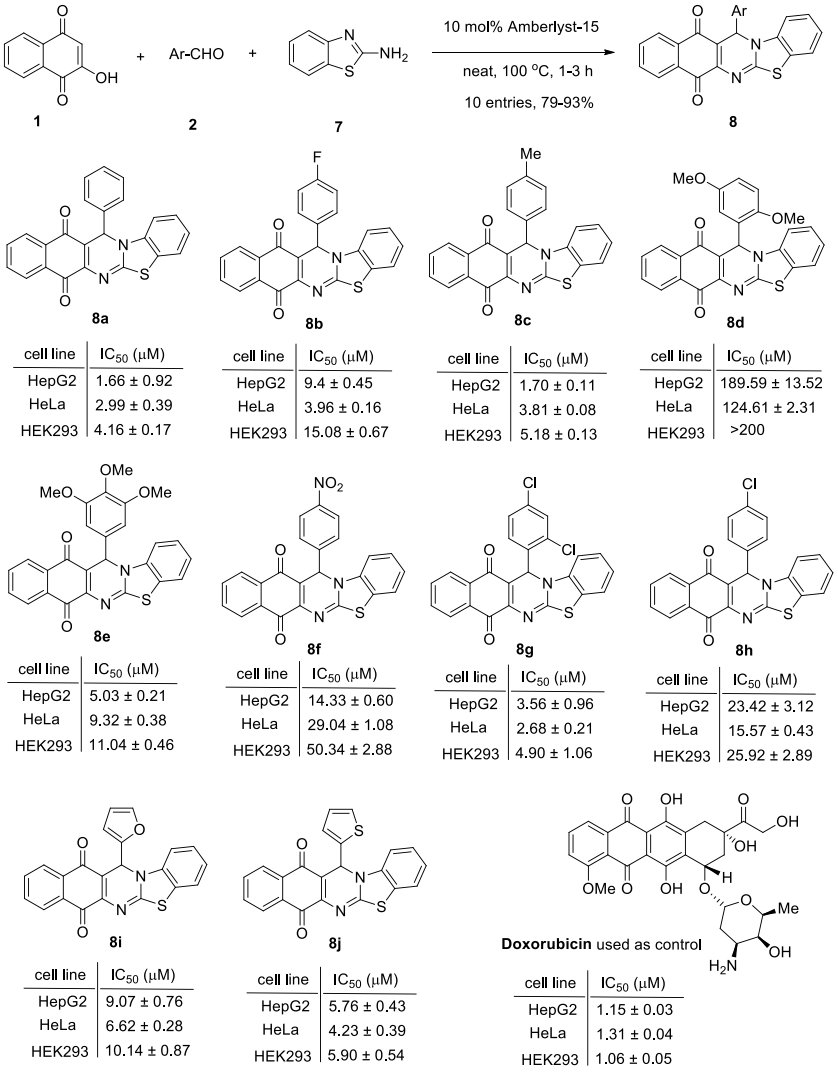
The very next year, Wu *et al.* [42] further synthesized another series of biologically promising compounds *i.e.,* 13-aryl-13*H*-benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-5,14-diones (**8**) in excellent yields from the reactions of 2-hydroxy-1,4-naphthoquinone (**1**), various aromatic aldehydes (**1**) and 2-aminobenzothiazole (**7**) using a catalytic amount of amberlyst-15 as an efficient heterogeneous catalyst under solvent-free conditions at 100 °C (Figure 3.5). *In vitro* anti-proliferative activity of all the synthesized compounds was screened against two human cancer cell lines HepG2 (liver cancer) and HeLa (cervical cancer) and their IC_{50} values were compared with the potent anticancer drug doxorubicin. From this study, it is evident that compounds **8a**, **8b**, **8c**, **8e**, **8g**, **8i** and **8j** possess good to moderate anti-proliferative activity against these two tumor cell lines. It is noteworthy to mention that all the synthesized compounds showed lesser cyto-toxicity against non-cancerous HEK293 cells.

3.2.3 Synthesis of benzo[*g*]chromenes

Perumal *et al.* [43] developed a simple and ultrasound-assisted method for the synthesis of a series of 5,10-dihydro-4*H*-benzo[*g*]chromenes-3-carbonitrile derivatives (**10**) *via* one-pot three-component reactions of 2-hydroxy-1,4-naphthoquinone (**1**), various substituted benzaldehydes (**2**) and malononitrile (**9**) in polyethylene glycol (PEG) using a catalytic amount of copper triflate (Figure 3.6). All the synthesized products afforded excellent yields within just 10 min. Anti-cancer activity of the synthesized compounds was evaluated against cervical cancer cell line (Hela cell). Among all the tested compounds, the compounds **10c**, **10g**, **10h**, **10i** and **10j** showed higher cytotoxicity than that of potent anticancer drug doxorubicin. Structure activity relationship studies (SARS) revealed that the presence of electron-withdrawing substituent on the aromatic ring at the 4th position of these scaffolds reduces the anticancer activity, whereas electron-donating substituent increases the same.

3.2.4 Synthesis of 7,7-*a*-dihydro-5*H*-benzo[*g*]benzofuro[3,2-*c*] chromene-5,13(12*aH*)-dione derivatives

In 2002, Costa and his collaborators [44] evaluated anticancer activity of 7,7-*a*-dihydro-5*H*-benzo[*g*]benzofuro[3,2-*c*]chromene-5,13(12*aH*)-dione derivatives (**14a**–**d**) against MCF-7 breast cancer cells. Initially, they carried out the reaction between 2-hydroxy-1,4-naphthaquinone (**1**) and acrolein (**11**) in the presence of phenylboronic acid and glacial acetic acid which afforded the corresponding 2*H*-benzo[*g*]chromene-5,10-dione (**12**) in toluene under reflux conditions. The desired compounds (**14a**–**d**)



cell line	IC_{50} (μM)
HepG2	1.66 ± 0.92
HeLa	2.99 ± 0.39
HEK293	4.16 ± 0.17

cell line	IC_{50} (μM)
HepG2	9.4 ± 0.45
HeLa	3.96 ± 0.16
HEK293	15.08 ± 0.67

cell line	IC_{50} (μM)
HepG2	1.70 ± 0.11
HeLa	3.81 ± 0.08
HEK293	5.18 ± 0.13

cell line	IC_{50} (μM)
HepG2	189.59 ± 13.52
HeLa	124.61 ± 2.31
HEK293	>200

cell line	IC_{50} (μM)
HepG2	5.03 ± 0.21
HeLa	9.32 ± 0.38
HEK293	11.04 ± 0.46

cell line	IC_{50} (μM)
HepG2	14.33 ± 0.60
HeLa	29.04 ± 1.08
HEK293	50.34 ± 2.88

cell line	IC_{50} (μM)
HepG2	3.56 ± 0.96
HeLa	2.68 ± 0.21
HEK293	4.90 ± 1.06

cell line	IC_{50} (μM)
HepG2	23.42 ± 3.12
HeLa	15.57 ± 0.43
HEK293	25.92 ± 2.89

cell line	IC_{50} (μM)
HepG2	9.07 ± 0.76
HeLa	6.62 ± 0.28
HEK293	10.14 ± 0.87

cell line	IC_{50} (μM)
HepG2	5.76 ± 0.43
HeLa	4.23 ± 0.39
HEK293	5.90 ± 0.54

cell line	IC_{50} (μM)
HepG2	1.15 ± 0.03
HeLa	1.31 ± 0.04
HEK293	1.06 ± 0.05

Figure 3.5: Solvent-free synthesis of 13-aryl-13*H*-benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-5,14-diones as anti-proliferative agents.

were synthesized from the reaction of 2-chloromercurio-4,5-methylenedioxyphenol (**13**) and freshly prepared compound **12** in the presence of a catalytic amount of $PdCl_2$ and LiCl in acetone (Figure 3.7). Compound **14a** and **14d** showed prominent anti-cancer activity. In another study, the same group showed that these type of pentacyclic 1,4-naphthoquinone derivatives are more potent anti-cancer agents than the lapachol (**A**) and even α-lapachone (**D**) [45].

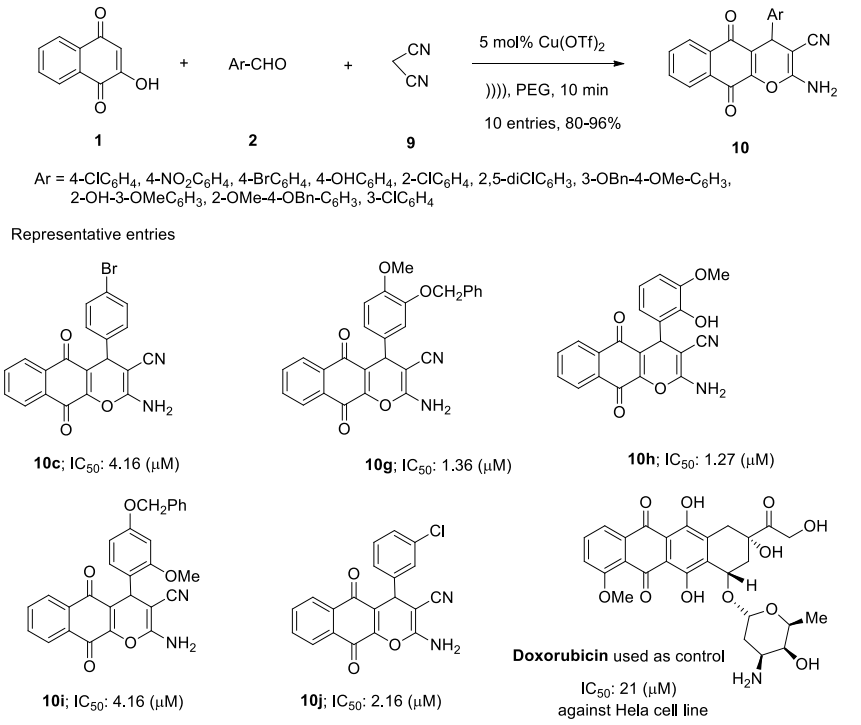


Figure 3.6: Ultrasound-assisted synthesis of benzo[*g*]chromenes as antitumor agents.

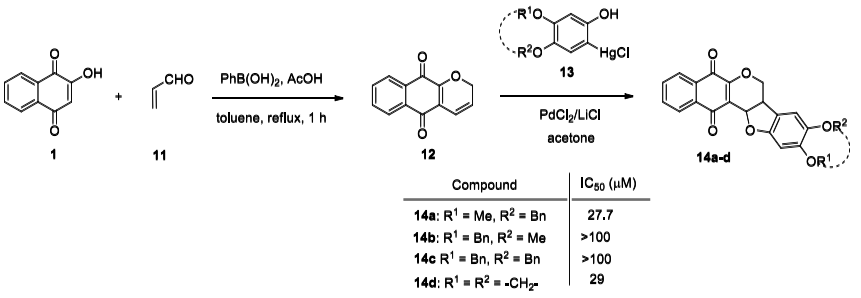
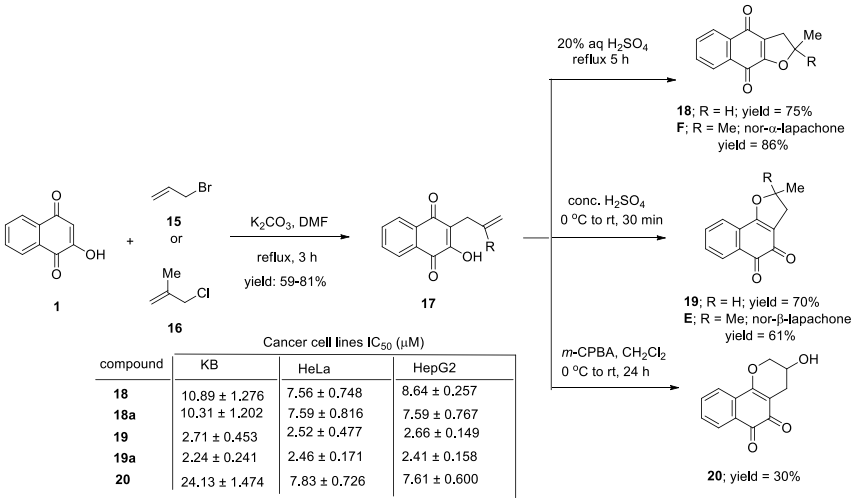


Figure 3.7: Synthesis of 7,7*a*-dihydro-5*H*-benzo[*g*]benzofuro[3,2-*c*]chromene-5,13(12*aH*)-dione derivatives starting from 2-hydroxy-1,4-naphthaquinone.

3.2.5 Synthesis of pyrano- and furanonaphthoquinones

In 2003, Kongkathip *et al.* [46] synthesized furanonaphthoquinones (**18**, **F**, **19**, **E**) as well as hydroxypyrano-naphthoquinone (**20**) from *C*-alkylated products (**17**) prepared by the reactions of 2-hydroxy-1,4-naphthaquinone (**1**) and allyl bromide (**15**) or



Cancer cell lines IC_{50} (μM)

compound	KB	HeLa	HepG2
18	10.89 ± 1.276	7.56 ± 0.748	8.64 ± 0.257
18a	10.31 ± 1.202	7.59 ± 0.816	7.59 ± 0.767
19	2.71 ± 0.453	2.52 ± 0.477	2.66 ± 0.149
19a	2.24 ± 0.241	2.46 ± 0.171	2.41 ± 0.158
20	24.13 ± 1.474	7.83 ± 0.726	7.61 ± 0.600

Figure 3.8: Synthesis of furanonaphthoquinones as well as hydroxypyrano-naphthoquinone starting from 2-hydroxy-1,4-naphthaquinone.

2-methyl-allyl chloride (**15a**) in dimethyl formamide (DMF) under reflux conditions (Figure 3.8). Compound **18** and nor-α-lapachone (**F**) were synthesized *via* the cyclization of *C*-alkylated products (**17**) using 20% aqueous sulfuric acid under reflux conditions. By using concentrated sulfuric acid, *C*-alkylated products (**17**) were cyclized to furano-1,2-naphthoquinones (**19** and nor-β-lapachone **E**) at 0 °C to room temperature. Compound **20** was prepared *via* the epoxidation of compounds **17** by using *m*-chloroperbenzoic acid in dichloromethane at 0 °C to room temperature. This reaction took 24 h for completion. Anti-cancer activity of the synthesized compounds was assayed against three different cancer cell lines (KB, HeLa and HepG2). It was found that compound **18**, nor-α-lapachone (**F**) and **20** possess moderate anti-cancer efficacy whereas compound **19** and nor-β-lapachone (**E**) are promising anticancer agents as they showed significant anticancer activity against all three examined cell lines. But unfortunately, these synthesized compounds showed almost equal cytotoxicity against normal cell lines as well. They also carried out the reactions of 2-hydroxy-1,4-naphthaquinone (**1**) and α-bromoacetate ethyl ester (**21**) in DMF which yielded the corresponding *C*-alkylated product (**22**). Reduction of the ester function in compound **22** by using ethanolic solution of sodium borohydride generated the intermediate **23** which on cyclization by using 20% aqueous sulfuric acid afforded the corresponding unsubstituted furanonaphthoquinone (**24**) (Figure 3.9). Interestingly, the compound **24** showed very less toxicity against normal cells (IC_{50} = 84.15 μM) but exhibited strong cytotoxicity against human cervical carcinoma cells (IC_{50} = 9.25 μM). Prasad *et al.* [47] were able to functionalize furanonaphthoquinone successfully and thus prepared a series of furanonaphthoquinone-1,2,3-triazole hybrids. They investigated the anticancer activity of these compounds against various cancerous cell lines and found that

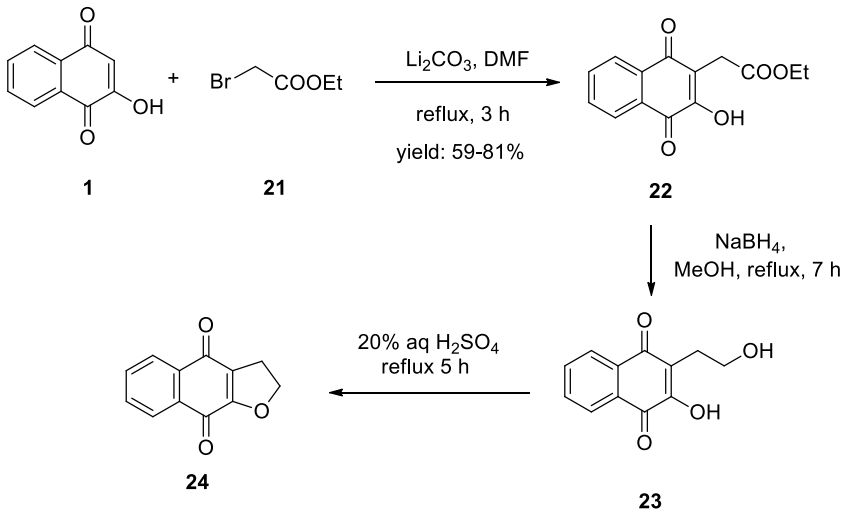
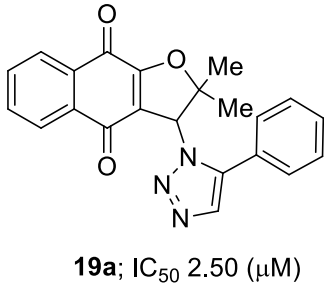


Figure 3.9: Synthesis of furanonaphthoquinones as well as hydroxypyrano-naphthoquinone starting from 2-hydroxy-1,4-naphthaquinone.



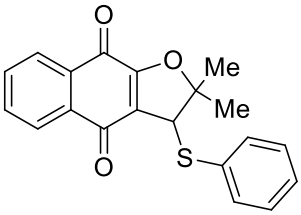
19a; IC_{50} 2.50 (μM)

Figure 3.10: Furanonaphthoquinone-1,2,3-triazole hybrid as potent anticancer agent.

compound **19a** possesses significant anticancer activity against MCF-7 cell line (Figure 3.10). Da Silva Júnior and his group [48] synthesized thiophenyl derivative of furano-naphthoquinone (**19b**) which showed excellent anticancer activity against a variety of cancerous cell lines with very low IC_{50} values (Figure 3.11).

3.2.6 Synthesis of 14-aryl-12*H*-dibenzo[*a*,*h*]xanthene-12,13(14*H*)-diones

Hueso-Falcón *et al.* [49] synthesized 14-aryl-12*H*-dibenzo[*a*,*h*]xanthene-12,13(14*H*)-dione derivatives (**26**) from one-pot three-component reactions of 2-hydroxy-1,4-naphthaquinone



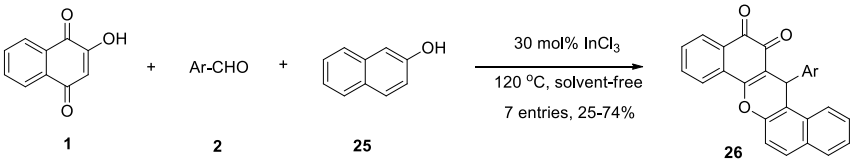
19b

Figure 3.11: Thiophenyl derivative of furano-naphthoquinone as promising anticancer agent.

(**1**), aromatic aldehydes (**2**), and β-naphthol (**25**) using a catalytic amount of indium chloride ($InCl_3$) under solvent-free conditions (Figure 3.12). Anticancer activity of the synthesized compounds was evaluated against two tumoral cell lines *i.e.*, HEL (human erythroleukemia) and MCF7 (human breast cancer MCF-7). Among these tested scaffolds, compound **26a** and **26d** showed significant anticancer activity.

3.2.7 Synthesis of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione derivatives

Khurana and his research group [50] reported a simple and ionic liquid (bmim[HSO_4]) catalyzed protocol for the efficient synthesis of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione derivatives



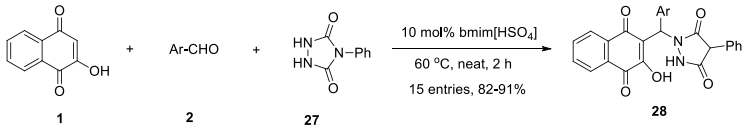
compound	Ar	Cancer cell lines IC_{50} (μM)	
		HEL	MCF7
26a	4-BrC_6H_4	1.2 ± 0.7	8.5 ± 1.9
26b	C_6H_5	> 10	> 10
26c	4-$NO_2C_6H_4$	4.0 ± 2.0	> 10
26d	4-ClC_6H_4	1.6 ± 0.7	1.9 ± 1.0
26e	4-FC_6H_4	7.25 ± 2.5	> 10
26f	2-FC_6H_4	3.8 ± 1.9	> 10
26g	2-thienyl	> 10	> 10

Figure 3.12: Synthesis of 14-aryl-12*H*-dibenzo[*a*,*h*]xanthene-12,13(14*H*)-diones under solvent-free conditions.

(**28**) *via* one-pot three-component reactions of 2-hydroxy-1,4-naphthaquinone (**1**), aromatic aldehydes (**2**) and 4-phenylurazole (**27**) under solvent-free conditions at 60 °C (Figure 3.13). All the synthesized compounds showed good antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl radical scavenging study. Anti-cancer activity of the synthesized compounds were also evaluated against five human cancer cell lines *viz*., T47D (breast cancer), HCT-15 (colon cancer), NCI-H522 (lung cancer), HepG-2 (liver cancer) and PA-1 (ovary cancer). Among all, compounds **28a**, **28b**, **28c**, and **28d** do possess significant anticancer activity.

3.2.8 Synthesis of 2-benzyllawsone

Kumar *et al.* [51] synthesized 2-benzyllawsone (**30**) by the benzylation of 2-hydroxy-1,4-naphthaquinone (**1**) using benzyl bromide (**29**) in dry acetone (Figure 3.14). Anti-cancer activity studies revealed that 2-benzyllawsone possesses significant anticancer activity by inducing apoptosis through topoisomerase-II inhibition. It was also found that 2-benzyllawsone has very less cytotoxicity against normal cells.



Ar = 4-ClC$_6$H$_4$, 4-FC$_6$H$_4$, 4-MeC$_6$H$_4$, 4-BrC$_6$H$_4$, 4-NO$_2$C$_6$H$_4$, 3-BrC$_6$H$_4$, 2-MeC$_6$H$_4$, 4-OMeC$_6$H$_4$, 3-ClC$_6$H$_4$, 2-ClC$_6$H$_4$, 2-naphthyl, 3-MeC$_6$H$_4$, 4-CF$_3$C$_6$H$_4$, 4-CNC$_6$H$_4$, 2,4-diClC$_6$H$_3$

compound	Ar	Cancer cell lines IC_{50} (μM)					
		T47D	HCT-15	Ovary PA-1	HepG2	NCI H-522	Normal cell
28a	3-BrC$_6$H$_4$	8 ± 3.9	40 ± 1.08	10.43 ± 2.11	9.67 ± 2.57	16 ± 2.5	75.49 ± 9.12
28b	2-MeC$_6$H$_4$	5.34 ± 1.16	43 ± 2.1	10.2 ± 2.10	45 ± 7.23	54 ± 9.2	10.2 ± 1.3
28c	2-naphthyl	15 ± 2	20 ± 3.4	9.12 ±1.11	95.23 ± 2.12	14 ± 3.7	180 ± 4.78
28d	3-MeC$_6$H$_4$	8.57 ± 1	44 ± 4.9	5.6 ±2.8	7.89 ± 3.1	10.2 ± 1.3	82.59 ± 3.19

Figure 3.13: Synthesis of 14-aryl-12*H*-dibenzo[*a*,*h*]xanthene-12,13(14*H*)-diones under solvent-free conditions.

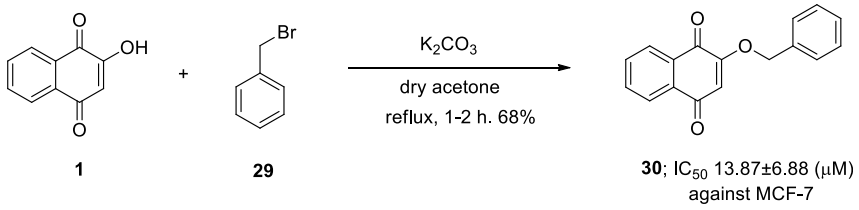


Figure 3.14: Synthesis of 2-benzyllawsone as potent anticancer agent.

3.2.9 Synthesis of bislawsone derivatives

In 2020, Novais *et al.* [52] reported antibacterial activity of bislawsone derivatives. In 2015, Prof. Brahmachari [53] synthesized a series of 3,3′-(aryl/alkyl-methylene) *bis*(2-hydroxynaphthalene-1,4-dione) derivatives from the pseudo three-component reactions between two equivalents of 2-hydroxy-1,4-naphthaquinone (**1**) and one equivalent of various aryl or aliphatic aldehydes (**2**) using sulfamic acid as catalyst in ethanol–water mixture as solvent at room temperature (Figure 3.15). All the reactions proceeded smoothly and afforded the corresponding products with excellent yields. Later on, the anti-cancer activity of the synthesized compounds was evaluated [54]. Among all the synthesized compounds, compound **31a** showed prominent anti-cancer activity against human glioblastoma cells. Very recently, in 2020, Ribeiro *et al.* [55] synthesized another series of nonsymmetrical 3,3′-(aryl/alkyl-methylene)bis-2-hydroxy-1,4-naphthoquinones that showed promising cytotoxic efficacies against prostate cancer cells.

3.2.10 Synthesis of 3-(aminomethyl)naphthoquinones

Some 3-(aminomethyl)naphthoquinone derivatives were found to possess significant anti-tuberculosis activities [56]. In 2016, Da Silva *et al.* [57] synthesized a series of structurally diverse 3-(aminomethyl)naphthoquinone derivates (**33**) from one-pot three-component reactions of 2-hydroxy-1,4-naphthaquinone (**1**), aldehydes (**2**) and various amines (**32**) in ethanol at room temperature (Figure 3.16). All the synthesized

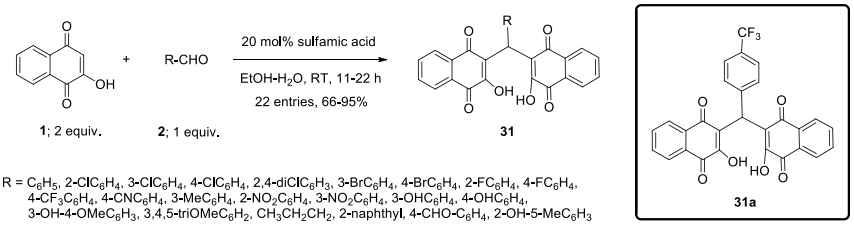


Figure 3.15: Synthesis of 3,3′-(aryl/alkyl-methylene)*bis*(2-hydroxynaphthalene-1,4-dione) derivatives as potent anticancer agent.

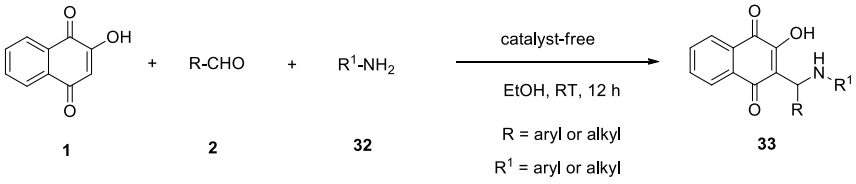


Figure 3.16: Synthesis of 3-(aminomethyl)naphthoquinones as anti-cancer agents.

compounds were evaluated for their anti-cancer activity and showed moderate to good cytotoxicity against number of cancerous cells tested. Mahal *et al.* [12] showed that fatty alkyl substituted compounds **VII** and **33a**, **33b** (Figures 3.1 and 3.17) possess significant anticancer activity as they can increase the formation of reactive oxygen species (ROS) in cancer cells. Novel platinum(II) complexes of 3-(aminomethyl)naphthoquinone also showed prominent anti-cancer efficacies [58]. Ahmad *et al.* [59] synthesized ferrocene modified 3-(aminomethyl)naphthoquinone (**34**) which showed better anti-cancer efficacy than that of **33a** and **33b** (Figure 3.18). Furthermore, Pereira *et al.* [60] found that 2-hydroy-3-anilino-1,4-naphthoquinones (**33**) are less toxic against normal cells paving the way for the development of new drug molecules.

3.2.11 Synthesis of (2*E*,4*E*)-1,4-dioxo-1,4-dihydronaphthalen-2-yl-5(benzo[*d*][1,3]dioxol-5-yl)penta-2,4-dienoate

Paengsri and Baramee [56] carried out the reaction between 2-hydroxy-1,4-naphthoquinone (**1**) and (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)penta-2,4-dienoyl chloride (**35**) under refluxing conditions which afforded the corresponding ester (**36**) having significant anti-cancer activity (Figure 3.19).

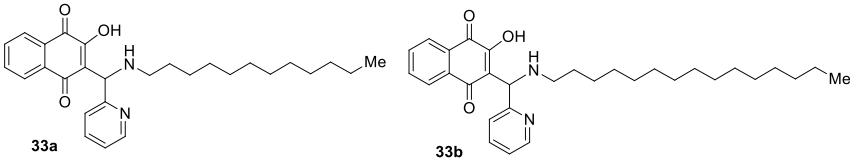


Figure 3.17: Fatty alkyl substituted 3-(aminomethyl)naphthoquinones with improved anti-cancer efficacy.

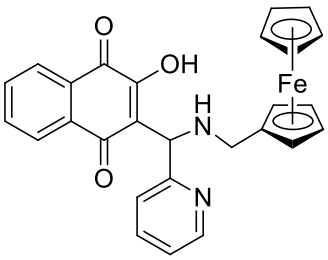


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Figure 3.18: Ferrocene substituted 3-(aminomethyl)naphthoquinones with enhanced anti-cancer efficacy.

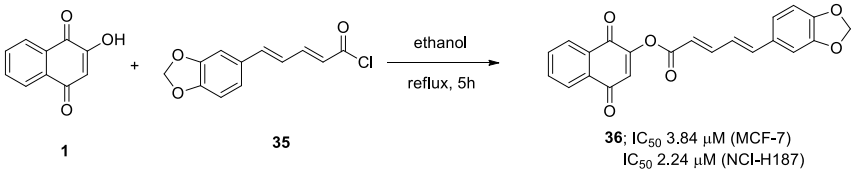


Figure 3.19: Synthesis of (2*E*,4*E*)-1,4-dioxo-1,4-dihydronaphthalen-2-yl-5(benzo[*d*][1,3]dioxol-5-yl) penta-2,4-dienoate as anti-cancer agent.

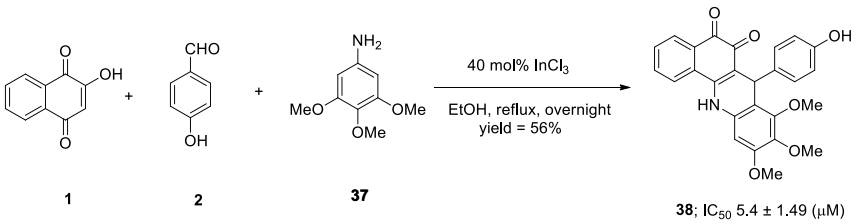


Figure 3.20: Synthesis of 7-(4-hydroxyphenyl)-8,9,10-trimethoxybenzo[*c*]acridine-5,6(7*H*,12*H*)-dione as potential anticancer agent.

3.2.12 Synthesis of benzo[c]acridine-5,6(7*H*,12*H*)-dione derivative

Golmakaniyoon *et al.* [61] synthesized 7-(4-hydroxyphenyl)-8,9,10-trimethoxybenzo[c] acridine-5,6(7*H*,12*H*)-dione (**38**) from the one-pot three-component reactions of 2-hydroxy-1,4-naphthoquinone (**1**), 4-hydroxybenzaldehyde (**2**) and 3,4,5-trimethoxyaniline (**37**) using indium chloride as the catalyst in ethanol under refluxing conditions (Figure 3.20). The synthesized compound showed excellent anticancer activity against MCF-7 breast cancer cells.

3.2.13 Synthesis of 2-ethyl-7-(4-methylpentyl)naphtho[1,2-*b*] furan-4,5-dione

Corral *et al.* [62] carried out the reaction of 7-substituted 2-hydroxy-1,4-naphthaquinone (**1**) and butyraldehyde (**2**) which afforded the corresponding 2-hydroxy-3-alkenyl-terpenylnaphthoquinone (**39**). Reaction with mercuric acetate in glacial acetic acid, compound **39** yielded a cyclized product *viz.*, 2-ethyl-7-(4-methylpentyl)naphtho[1,2-*b*] furan-4,5-dione (**41**) *via* the formation of intermediate **40** (Figure 3.21). Growth inhibition study against human lung (A-549) and colon (HT-29) carcinoma cells revealed that the compound **41** possess excellent anticancer activity.

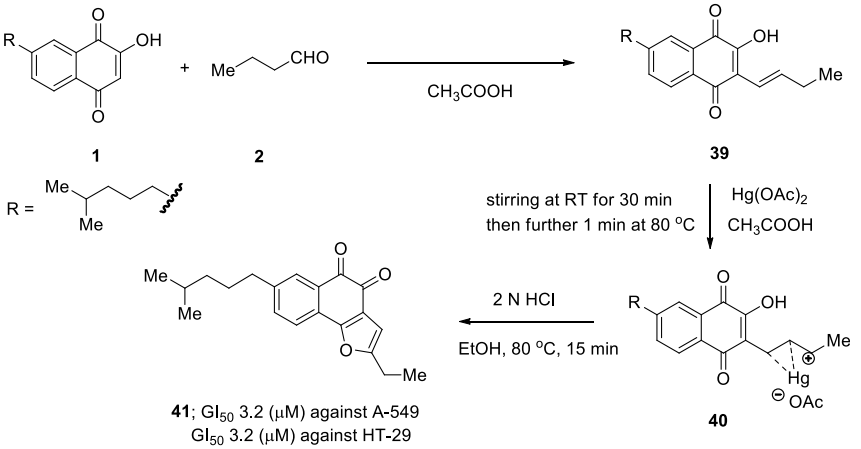


Figure 3.21: Synthesis of 2-ethyl-7-(4-methylpentyl)naphtho[1,2-*b*]furan-4,5-dione as promising anticancer agent.

3.3 Synthesis of lawsone derived natural products having anticancer activities

3.3.1 Synthesis of lapachol and related derivatives

Lapachol (**A**) was first isolated from *Tabebuia avellanedae* (Bignoniaceae) in 1882 [63]. Along with other biological activities, various lapachol derivatives were found to possess significant anti-cancer efficacies [63]. Compounds **42**–**46** are some examples of lapahol derivatives exhibiting potential anti-cancer activities (Figure 3.22) [43]. Sun *et al.* [64] synthesized lapachol (**A**) starting from 2-hydroxy-1,4-naphthaquinone (**1**). First, lithium salt of 2-hydroxy-1,4-naphthoquinone (**47**) was prepared *in situ* by adding lithium hydride to the frozen solution of lawsone (**1**) in dimethyl sulfoxide. The salt was then alkylated with 1-bromo-3-methyl-2-butene (**48**) which on washing with acid afforded the desired lapachol (**A**) with 40% yields (Figure 3.23). Eyong *et al.* [65] synthesized lomatiol (**P**) by the oxidation of lapachol (**A**) using SeO_2 as the oxidizing agent (Figure 3.24). Both lapachol and lomatiol showed prominent anti-cancer activity against human dendroglioma cancer cell (Hs683) with IC_{50} values 9 and 6 μM respectively [66].

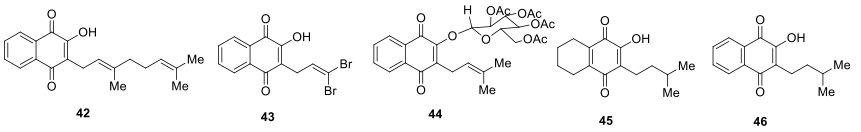


Figure 3.22: Lapachol derived compounds having promising anti-cancer activities.

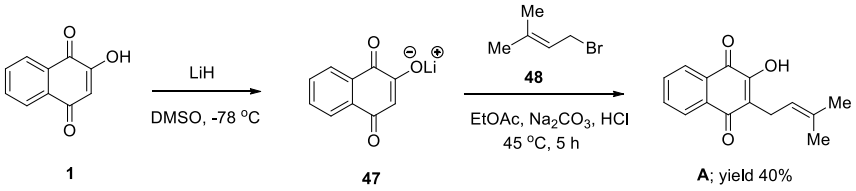


Figure 3.23: Synthesis of lapachol from 2-hydroxy-l,4-naphthoquinone.

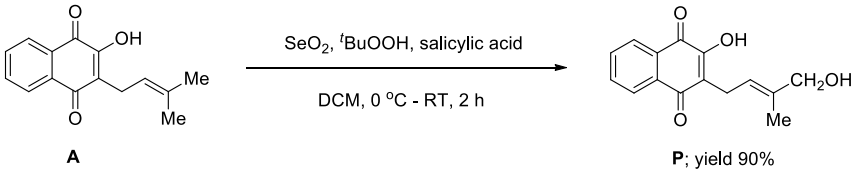


Figure 3.24: Synthesis of lomatiol from lapachol.

3.3.2 Synthesis of lapachones and related derivatives

β-Lapachone (**C**) was isolated from the bark of *T. avellanedae* [67]. This compound was found to possess a wide range of biological efficacies including antibacterial [68], antifungal properties [69], anti-inflammatory [70], trypanocidal [71, 72], etc. activities. Starting from 2-hydroxy-1,4-naphthoquinone (**1**), synthesis of β-lapachone (**C**) was accomplished by using Heck reaction through the formation of lapachol (**A**) (Figure 3.25). Firstly, 2-hydroxy-1,4-naphthoquinone (**1**) coupled with 1-bromo-3-methyl-2-butene (**48**) in the presence of a catalytic amount of $Pd(PPh_3)_4$ with triethylamine in dioxane which afforded lapachol (**A**) with 61% yield after 4 h at room temperature [73]. In the next step, β-lapachone (**C**) was synthesized *via* the sulfuric acid mediated cyclization of lapachol (**A**) in dichloromethane at 0 °C. This *ortho*-quinoid compound has also been regarded as a very promising anticancer agent [74–77].

Free radical initiated bromination of β-lapachone (**C**) with *N*-bromosuccinimide (NBS) generated 3,4-dibromo-β-lapachone (**49**) which on reaction with sodium azide afforded the corresponding azido derivative **50**. Da-Silva Jr. *et al.* [78] synthesized β-lapachone-based 1,2,3-triazole derivatives (**52a–52d**) from the reactions of

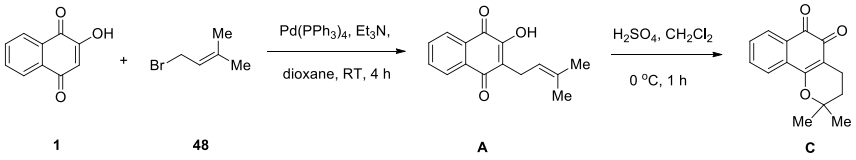
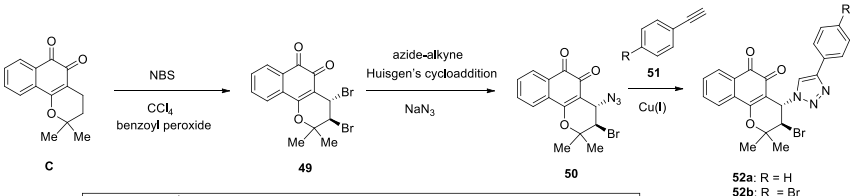


Figure 3.25: Synthesis of β-lapachone starting from 2-hydroxy-1,4-naphthoquinone.

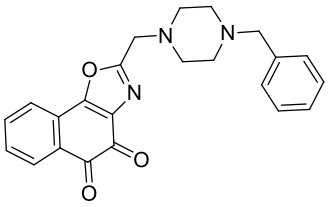
compound **50** and terminal alkynes (**51**) *via* Cu(I) catalyzed azide-alkyne Huisgen's cycloaddition reaction [79, 80] (Figure 3.26). Anti-cancer activity of the synthesized compounds was evaluated against five cancer cells from different parts of the body *viz*., PBMC (peripheral blood mononuclear cells), HL-60 (leukemia) MDA-MB435 (breast cancer), HCT-8 (colon cancer) and SF295 (central nervous system). Compound **50**, **52a**, **52b**, **52c**, & **52d** showed very low IC_{50} values indicating their high potential of anti-cancer activities.

In a recent review, Costa *et al.* [81] summarize various anticancer activities of β-lapachone and their related derivatives. Li *et al.* [82] synthesized 2-((4-benzylpiperazin-1-yl)methyl)naphtho[2,1-*d*]oxazole-4,5-dione (**53**) which shows potential anticancer activity against lung cancer cell (Figure 3.27). Dias *et al.* [83] observed anticancer activity of some iodine derivatives of β-lapachone (**54**) as well as α-lapachone (**55**) against colon cancer HCT-116 cells *via* ROS-induced apoptosis (Figure 3.28). In 2017, Hussain and Green [20] compiled literatures related to the patents filed on lapachol and lapachone analogs having a range of biological activities. Ashwell *et al.* [84] prepared a huge library comprising more than hundred β-lapachone analogs and examined their anticancer



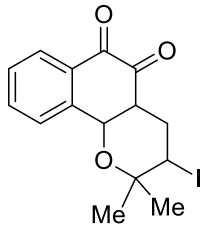
compound	Cancer cell lines IC_{50} (μM)				
	PBMC	HL-60	MDA-MB435	HCT-8	SF295
50	3.58	1.43	1.54	2.42	1.93
52a	2.08	1.01	1.22	1.37	1.55
52b	4.01	1.67	1.99	2.24	2.33
52c	3.37	1.20	1.20	2.26	1.76
52d	2.52	1.88	1.92	3.21	3.01

Figure 3.26: Synthesis of β-lapachone-based 1,2,3-triazole derivatives as a promising anticancer agents.

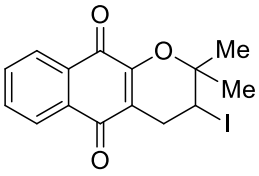


53; IC_{50} 4.6 ± 1.0 (μM)

Figure 3.27: 2-((4-Benzylpiperazin-1-yl)methyl)naphtho[2,1-*d*]oxazole-4,5-dione possessing promising anti-cancer activity.



54; IC_{50} 0.23-0.47 (μM)



55; IC_{50} 2. 52-3.67 (μM)

Figure 3.28: Iodine derivatives of β-lapachone and α-lapachone as anticancer agent.

activities towards various cancer cells *viz*., lung cancer cells (A549), colon cancer cells (DLD-1 and HT-29), leukemia cells (K-562), prostate cancer cells (DU-145), and pancreatic cancer cells (PACA-2). Many of them showed prominent anti-cancer efficacies. Flick *et al.* [85] reported the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitory activity of β-lapachone (**C**). Kim *et al.* [76] showed that β-lapachone has the potential inhibitory effects on the activities of the cytochrome P450 enzymes. Yang *et al.* [86] demonstrated antitumor activity of β-lapachone by inhibiting epithelial-to-mesenchymal transition in NAD(P)H quinone oxidoreductase-1 (NQO1) positive breast cancer cells. Pardee *et al.* [74] proposed that the anti-cancer activity β-lapachone may be due to its ability to produce ROS. Without causing the death of non-transformed cells, β-lapachone can selectively induce apoptosis in cancer cells [75]. Figure 3.29 represents some β-lapachone derivatives having significant anti-cancer efficacies [84, 87]. In 2003, Kongkathip *et al.* [46] synthesized and evaluated anti-cancer activities of both nor-β-lapachone (**E**) as well as

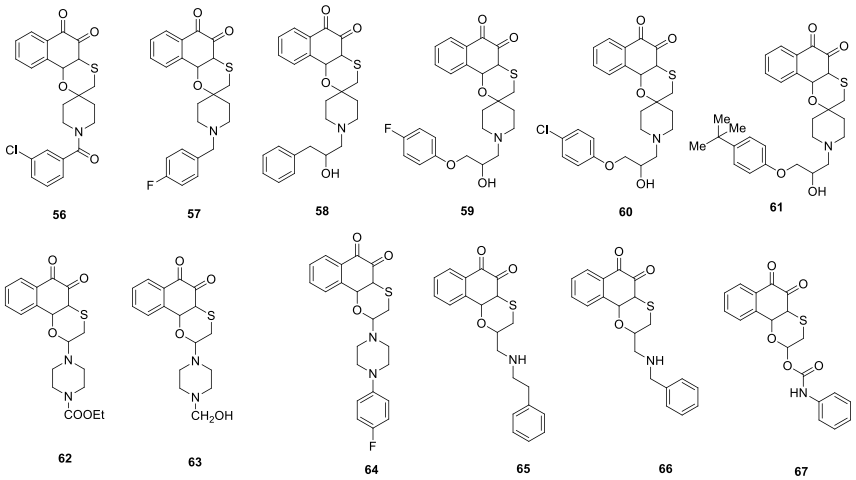


Figure 3.29: Some β-lapachone derivatives having significant anti-cancer efficacies.

nor-β-lapachone (**F**) (Figure 3.8). Nor-β-lapachone also possesses similar anticancer activity like β-lapachone [88]. Da Silva Júnior *et al.* [88] reported significant anticancer activity of some arylamino derivatives of nor-β-lapachone (**68**–**71**) (IC_{50} < 1 μM) (Figure 3.30).

3.3.3 Synthesis of avicequinone B and related derivatives

Avicequinone B or naphtho[2,3-*b*]furan-4,9-dione (**G**) possesses several biological activities. This compound was isolated from mangrove tree such as *Avicennia alba* and *Avicennia marina* [89]. Prateep *et al.* [90] synthesized avicequinone B (**G**) from 2-hydroxy-1,4-naphthoquinone (**1**) by using methyl vinyl sulfone (**72**) in dichloromethane under refluxing conditions (Figure 3.31). They also reported that the avicequinone B (**G**) has the potency to suppress the survival and induced anoikis (a subset of apoptosis) in human lung cancer cells. Kigelinone (**H**) was isolated from *Kigelia africana* showed prominent antitumor activity [91].

Jiménez-Alonso *et al.* [92] reported a facile protocol for the efficient synthesis of 2-amino-3-aryl/alkyl substituted avicequinone B derivatives (**74**) *via* one-pot three-component reactions of 2-hydroxy-1,4-naphthoquinone (**1**), various aromatic or aliphatic aldehyde (**2**) and isocyanides (**73**) using catalytic amounts of ethylenediamino diacetate in toluene under refluxing conditions (Figure 3.32). The reaction is quite fast and completed within 30 min to finally furnish the desired products in excellent yields. Growth inhibition ability of the synthesized compounds was evaluated against three human tumor cell lines (MCF7, MCF7/BUS, and SK-Br-3). GI_{50} (50%

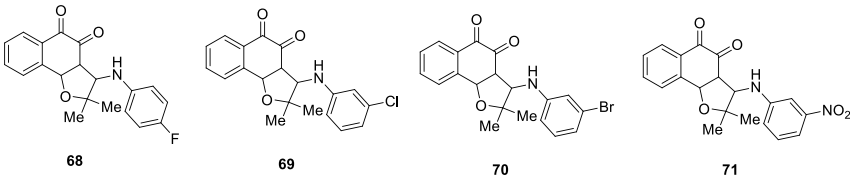


Figure 3.30: Arylamino derivatives of nor-β-lapachone having significant anticancer activities.

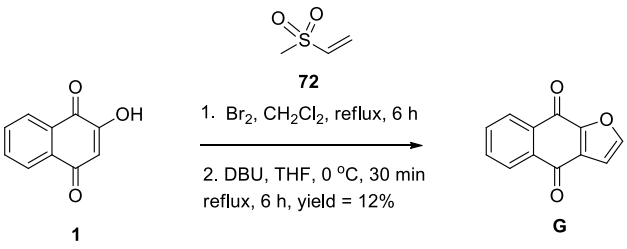
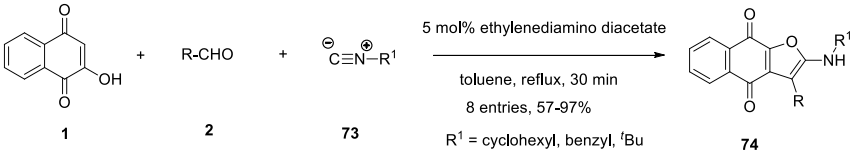


Figure 3.31: Synthesis of avicequinone B which can sensitize anoikis in human cancer cells.



R = Et, $CH_3(CH_2)_4CH_2$, cyclohexyl, C_6H_5, 3-furyl

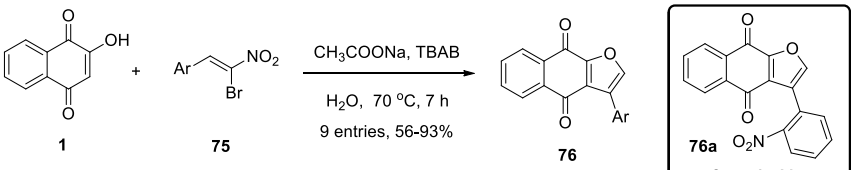
compound	R	R^1	Cancer cell lines GI_{50} (μM)		
			MCF7	MCF7/BUS	SK-Br-3
74a	$CH_3(CH_2)_4CH_2$	^{t}Bu	22.2 ± 1.2	9.22 ± 0.4	22.9 ± 2.0
74b	3-furyl	^{t}Bu	5.9 ± 0.4	2.8 ± 0.5	1.6 ± 0.4
74c	cyclohexyl	benzyl	7.6 ± 0.4	3.9 ± 0.07	5.8 ± 0.6

Figure 3.32: Synthesis of 2-amino-3-aryl/alkyl substituted avicequinone B derivatives containing anti-cancer activities.

growth inhibition) values revealed that compounds **74a**, **74b** and **74c** possess highest cytotoxicity among the eight synthesized compounds. The compound **74b** showed the most prominent antitumor efficacy against SK-Br-3 cell line. In 2018, Baiju *et al.* [93] synthesized a series of 3-aryl substituted avicequinone B derivatives (**76**) from the reaction of 2-hydroxy 1,4-naphthoquinone (**1**) and conjugated nitroalkenes (**75**) in water at 70 °C (Figure 3.33). Among all the compounds synthesized, compound **76a** showed highest anti-cancer activities against a number of cancer cells.

3.3.4 Synthesis of triphenylphosphonium-conjugated atovaquone derivatives

Atovaquone (**II**) (Figure 3.1) is regarded as a potent anti-malarial drug which was synthesized starting from 2-hydroxy-1,4-naphthaquinone [94]. Recently, in 2020, Cheng *et al.* [95] further derivatized atovaquone (**II**) and prepared four triphenylphosphonium-conjugated



Ar = 4-FC_6H_4, 4-BrC_6H_4, 4-$SMeC_6H_4$, 4-$OMeC_6H_4$, 3,4-$(OMe)_2C_6H_3$, 2-$NO_2C_6H_4$, 2-thienyl, 1-naphthyl

Figure 3.33: Synthesis of 3-aryl substituted avicequinone B derivatives containing anti-cancer activities.

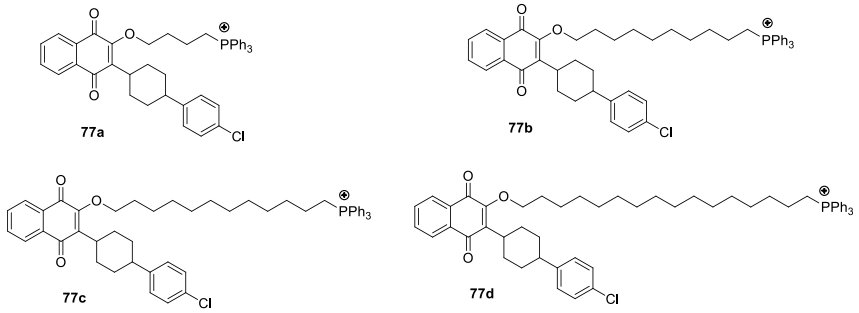


Figure 3.34: Derivatives of atovaquone having potent anti-cancer activity.

atovaquone derivatives (**77a**, **77b**, **77c** and **77d**), which showed excellent anticancer activities against pancreatic and other cancer cell lines (Figure 3.34). It was also reported that these synthesized compounds are more potent than the parent compound atovaquone (**II**). It was proposed that incorporation of the hydrophobic chain might help to stabilize the compound at the cytochrome pocket by effective hydrophobic interactions followed by accumulation into the cancer cells more effectively.

3.3.5 Synthesis of dunnione and related derivatives

Dunnione (**L**) and α-dunnione (**M**) were found to possess potent anti-cancer activity against breast and pancreatic cancer cell lines (Figure 3.2) [96]. Bian *et al.* [18] successfully synthesized dunnione and its analogs with high yields by following only three steps (*O*-allylation, Claisen rearrangement and cyclization). At first, by using 3,3-dimethyl allyl bromide (**78**), lawsone (**1**) or methoxy-substituted lawsone (**1a**) turned to *O*-alkylated product (**79**) which on Claisen rearrangement (heating at 120 °C) in toluene gave the corresponding 3,3-dimethyl allyl lawsone (**80**). Cyclization of the **80** at room temperature in the presence of $NbCl_5$ as catalyst afforded the desired

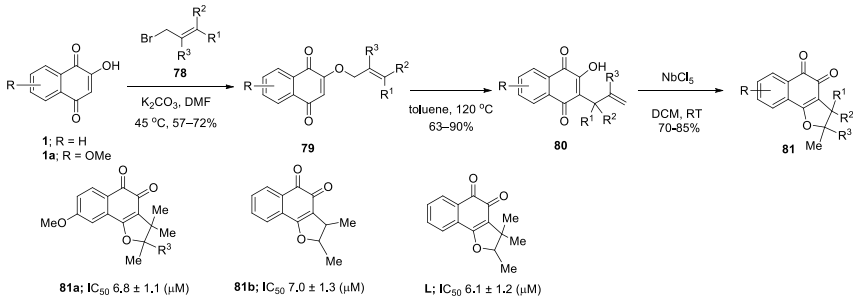


Figure 3.35: Synthesis of dunnione analogs containing anti-cancer activities.

product **81** (Figure 3.35). Anti-cancer activity study against lung cancinoma A549 cell line revealed that dunnione (**L**) and compound **81a**, **81b** possess significant anti-cancer efficacies. It was proposed that these compounds exerted their antitumor activity by redox cycling *via* NAD(P)H quinone oxidoreductase-1 mediated ROS production.

3.4 Conclusions

Lawsone or 2-hydroxy-1,4-naphthaquinone is a naturally occurring compound that exhibits a broad spectrum of biological activities. During the last decade, lawsone has gained tremendous attention and a huge number of methods have been reported for the synthesis of various heterocyclic or non-heterocyclic scaffolds using lawsone as one of the starting components. Importantly, many of these synthesized scaffolds exhibit a vast range of pharmacological activities including anti-carcinogenic activities against diverse cancer cell lines. Many naturally occurring compounds such as lapahol, nor-lapahol, α-lapachone, β-lapachone, nor-α-lapachone, nor-β-lapachone, avicequinone B, duninone, α-duninone, lapinone, lomatiol etc. were also synthesized in laboratory from lawsone under simple, catalyzed and greener conditions. Many of these compounds were found to possess significant anticancer activities. In this review, we summarized various synthetic and green protocols for lawsone derived scaffolds, and presented an overview on their anticancer activities on diverse cell lines along with cytotoxicity studies wherever reported.

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Aparna Das and Bimal Krishna Banik*

4 Advances in heterocycles as DNA intercalating cancer drugs

Abstract: The insertion of a molecule between the bases of DNA is known as intercalation. A molecule is able to interact with DNA in different ways. DNA intercalators are generally aromatic, planar, and polycyclic. In chemotherapeutic treatment, to suppress DNA replication in cancer cells, intercalators are used. In this article, we discuss the anticancer activity of 10 intensively studied DNA intercalators as drugs. The list includes proflavine, ethidium bromide, doxorubicin, dactinomycin, bleomycin, epirubicin, mitoxantrone, ellipticine, elinafide, and echinomycin. Considerable structural diversities are seen in these molecules. Besides, some examples of the metallo-intercalators are presented at the end of the chapter. These molecules have other crucial properties that are also useful in the treatment of cancers. The successes and limitations of these molecules are also presented.

Keywords: anticancer drugs; biological activity; DNA intercalators; heterocycles.

4.1 Introduction

The molecule deoxyribonucleic acid (DNA) exists like a double-stranded structure. It consists of two strands that coil across each other to create a double helix where genetic commands for the function, development, reproduction, and growth are carried. Thus this macromolecule is essential for all forms of life. In 1869, Friedrich Miescher isolated DNA first time. James Watson and Francis Crick identified the molecular structure of DNA.

As shown in Figure 4.1, four types of nucleotides are essential for every single strand of DNA [1, 2]. Nucleotides in DNA consist of phosphate, nucleobase, and deoxyribose sugar. The four different nucleotides corresponding to the four nucleobases are adenine (A), cytosine (C), guanine (G), and thymine (T) [3, 4]. Guanine and adenine are purine bases, at the same time thymine and cytosine are pyrimidines bases. The phosphodiester bonds formation and the creation of the DNA double helix's phosphate-deoxyribose backbone with the nucleobases directing inward, in other

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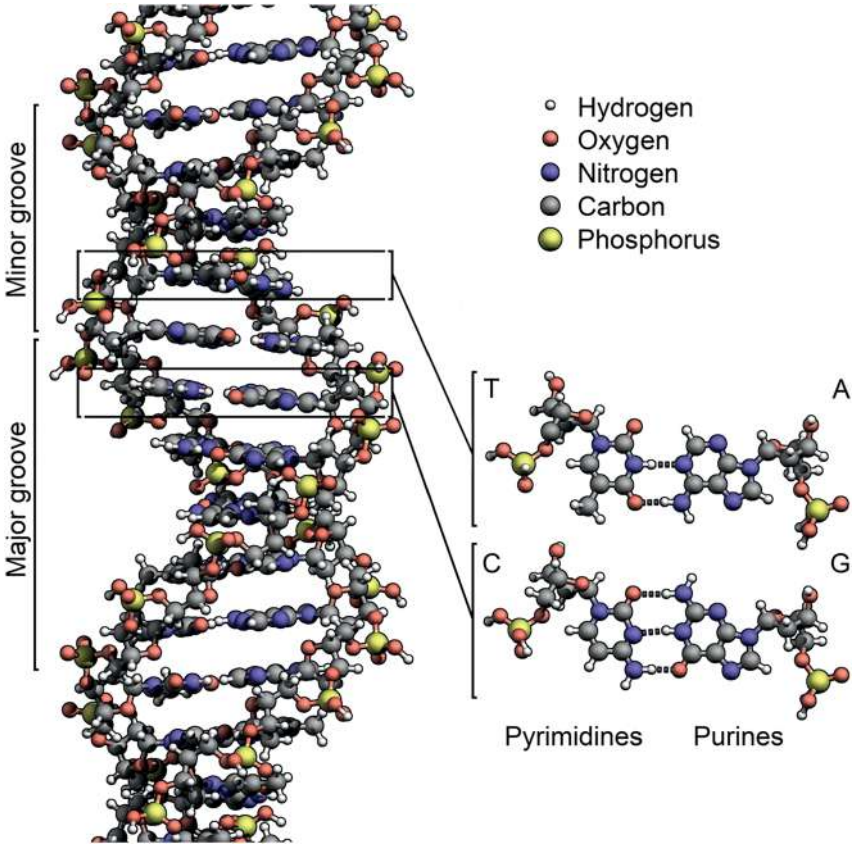

Figure 4.1: DNA double helix's structure. Adapted from Wikipedia.

words toward the opposing strand are caused by these nucleotides [5]. To create the base pairs nucleobases are linked among strands *via* hydrogen bonds. In detail, guanine connects with cytosine through three hydrogen bonds and adenine connects with thymine through two hydrogen bonds [6]. The dissimilarity among RNA and DNA is the sugar, RNA having a pentose sugar ribose instead of 2-deoxyribose [2].

In DNA, the linking of complementary bases *via* hydrogen bonds entails that the information carried inside each strand is redundant [7]. The strands stay separated from one another as the inter-strand (hydrogen) bonds are weaker than intra-strand (phosphodiester) bonds. To rebuild nucleotides on a newly synthesized partner strand, the nucleotides on a single strand can be employed.

DNA base pairing is shown in Figure 4.2. This organization of two nucleotides connecting unitedly across the double helix is addressed as Watson–Crick base pair. Hoogsteen base pairing is another base pairing, in that case, two hydrogen bonds

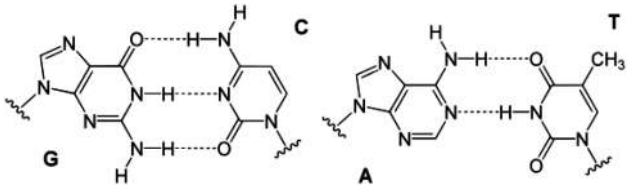

Figure 4.2: DNA base-pairing. Adapted from Wikipedia.

create among cytosine and guanine. By using a high temperature or mechanical force, the two strands of DNA in a double helix can be pulled apart like a zipper.

In DNA strands, the distinct terminals of a single strand are called the "5′ (five-prime) end" and "3′ (three-prime) end" and in that way, it possesses a directionality. Considering the base sequence of DNA's single strand, the right end of the sequence is the 3′ end at the same time the left end of the sequence is the 5′ end. In a DNA double helix, one strand being 3–5′, and the opposite strand 5–3′, in that way the strands of the double helix run anti-parallel. As DNA polymerase can synthesize DNA in only one way by adding nucleotides to the 3′ end of the strand, directionality is important during the synthesis of DNA. The same biological information is stored by both the strands of double-stranded DNA. Upon the separation of the two strands, replication occurs. A big region of DNA is non-coding, which implies that they are non-servers as patterns for protein sequences. In humans, it covers more than 98% portion of DNA. The genetic information is encoded by the sequence of four nucleobases along the backbone. For the synthesis of transcription RNA strands, DNA strands are used as a template.

DNA is coordinated into long structures named chromosomes inside eukaryotic cells. These chromosomes are duplicated in the process called replication, prior to cell division. In that way, it provides each daughter cell with a complete set of chromosomes. Plants, animals, protists, and fungi (eukaryotic organisms) keep most of their DNA inside the cell nucleus (nuclear DNA), and a few in chloroplasts (chloroplast DNA) or in the mitochondria (mitochondrial DNA) [8]. But archaea and bacteria (prokaryotes) keep their DNA only in the cytoplasm, in circular chromosomes. The chromatin proteins, histones, organize and compress DNA in the eukaryotic chromosomes. The compacting structures are important as it direct the interactions among the DNA and other proteins and also helps to control which portions of the DNA are transcribed.

A DNA sequence is addressed as a sense sequence if it is the same as that of a messenger RNA copy that is translated into protein. The opposite strand sequence is known as the antisense. In the same strand of DNA, both antisense and sense sequences stay in distinct portions. Even though antisense RNA sequences are produced in prokaryotes and eukaryotes, the purposes of this RNA are unclear [9]. The RNA–RNA base pairing, the antisense RNAs participate in gene expression regulation [10].

By having overlapping genes, a couple of DNA sequences in eukaryotes and prokaryotes, and a more number in viruses and plasmids, blur the differentiation among

antisense and sense strands [11]. A few DNA sequences do double work. For example, when read one strand in one direction, it will do encoding of one protein, and encoding of a second protein when read in the opposite direction along the other strand. In bacteria, this overlap is perhaps involved in gene transcription regulation [12]. The overlapped genes tend to heighten the number of information in viruses and are encoded inside the small viral genome [13].

DNA supercoiling is the process of twisting DNA like a rope. Once every 10.4 base pairs, a strand normally circles the axis of the double helix when it is in a relaxed state. Whereas the strands get more loosely or more tightly wound if DNA is twisted [14]. Twisting of the DNA is turned in the direction of the helix is known as positive supercoiling and the bases are more tightly together in that case. Twisting in the opposite direction makes the bases come apart very easily, this is negative supercoiling. In nature, enzymes called topoisomerases [15] cause most DNA slight negative supercoiling. These enzymes free the twisting stresses that occurred in the DNA strands during transcription and DNA replication [16]. The replication of DNA is shown in Figure 4.3.

DNA is used in several applications. In recombinant DNA technology, modern biochemistry, and biology do the intensive application of genetic engineering techniques. Recombinant DNA, the man-made DNA sequence, is made up of other DNA sequences. It can be converted into organisms in the form of plasmids or by employing a viral vector [17]. The genetically altered organisms developed are utilized to generate products like recombinant proteins which are used in agriculture [18, 19]. It is also used in medical research [20]. In forensic, DNA in semen, blood, hair, saliva, or skin discovered at a crime scene is used to key out a matching DNA of an individual. This action is named DNA fingerprinting or DNA profiling. To identify a matching DNA, it is a really authentic method [21]. This method is also applied in DNA paternity testing. DNA is also used in bioinformatics [22–24] and in nanotechnology [25–27].

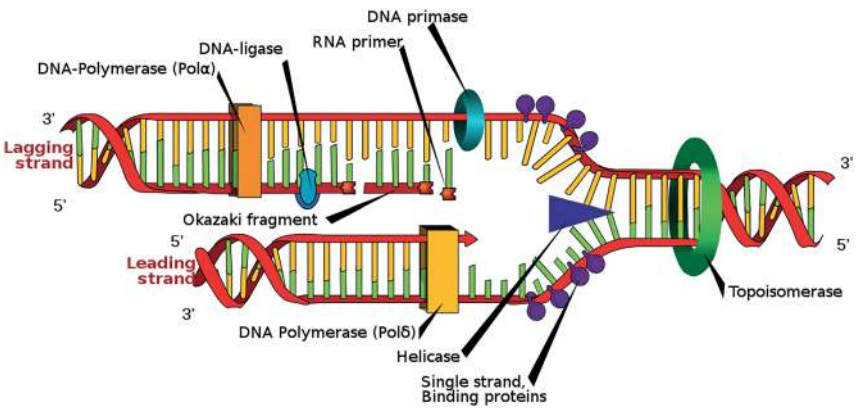

Figure 4.3: DNA replication. Adapted from Wikipedia.

In the synthesis of protein, messenger RNA has no direct involvement but transfer RNA (tRNA) is needed. The translation is the process by which mRNA conducts the synthesis of proteins with the help of tRNA (Figure 4.4).

The major and minor groove of DNA is shown in Figure 4.5. The grooves are unequally sized. The width of the minor groove is nearly 12 Å and the width of the major groove is nearly 22 Å [28]. B-DNA is the most usual double-helical structure observed in nature, and this double helix is right-handed and per turn contains 10–10.5 base pairs [29].

DNA intercalation is the process of insertion of molecules among the DNA's planar bases (Figure 4.6). Intercalation is mainly used in analyzing DNA and form the basis of certain sorts of poisoning. Chemicals can insert itself between the bases at the center of the DNA strand and can cause a frameshift mutation. Substances that insert itself into the DNA of a cell and link to it and cause damage to the DNA are called DNA intercalators. In the case of treatment for cancer, the intercalators can destroy cancer cells by altering DNA and blocking the division.

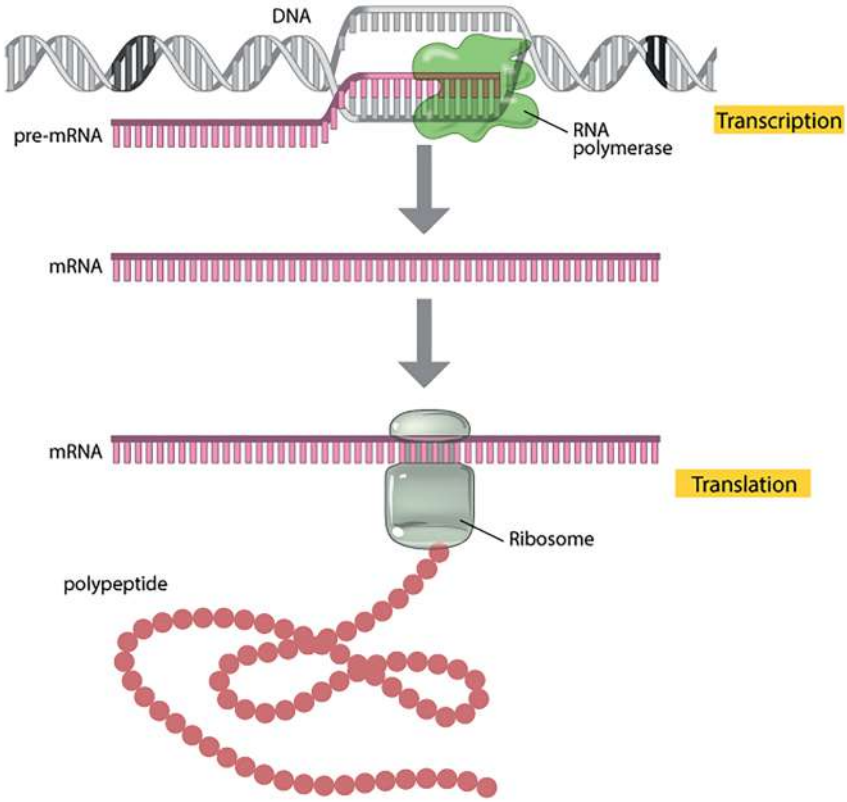

Figure 4.4: Transcription and translation. Adapted from Nature Education. (https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393/).

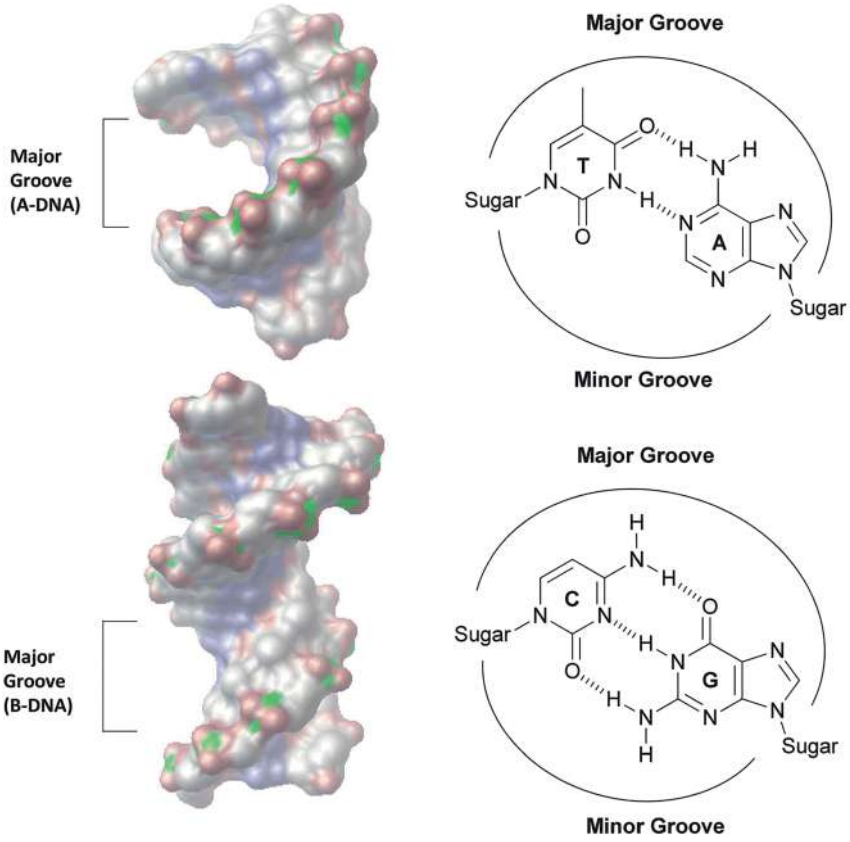

Figure 4.5: Major and minor groove of DNA. Adapted with permission from [30].

Ligands (molecules) can act with DNA by intercalating, electrostatically binding, or covalently binding [31]. When ligands of a suitable chemical nature and size can befit themselves into DNA's base pairs, then the intercalation happens. The ligands are normally planar, polycyclic, and aromatic. The widely employed DNA intercalating agents are berberine, proflavine, ethidium bromide, doxorubicin, thalidomide, and daunomycin. Most of these intercalating agents are employed for chemotherapeutic therapy to suppress the replication of DNA in cancer cells which are growing rapidly. For example, dactinomycin is used to treat Wilm's tumor, rhabdomyosarcoma, and Ewing's Sarcoma, and the agents daunorubicin and doxorubicin are used to treat Hodgkin's lymphoma.

By unwinding, the DNA should open the space between the base pairs for an intercalator to fit into the base pairs. Depending on the intercalator, the stages of unwinding differs. For example, proflavine unwinds DNA by nearly 17° on the other hand ethidium cation unwinds it by nearly 26°. The unwinding and related process

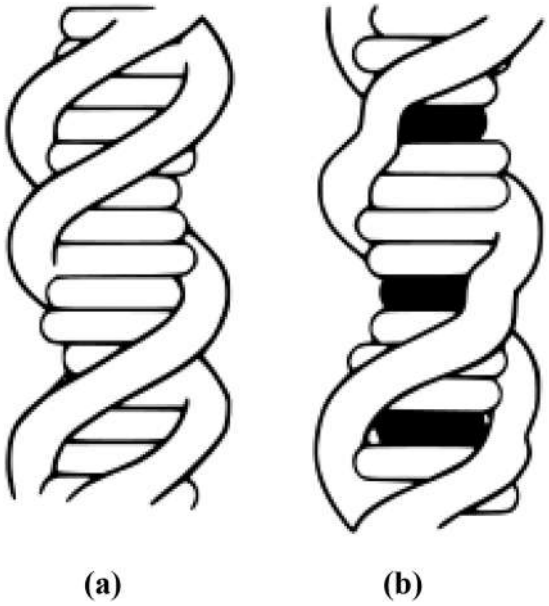

Figure 4.6: DNA intercalation. (a): unaltered DNA strand. (b): intercalated DNA strand. Adapted from Wikipedia.

make the DNA base pairs to go apart and in that way an opening of about 0.34 nm forms. It causes structural modifications to the DNA strand. The modifications include base pair twisting or the DNA strand lengthening. These types of modifications in the structure cause functional modifications. Suppression of transcription, replication, and DNA repair works takes place due to this.

Studies showed that during the intercalation process, to the surface of the polyanionic DNA the cationic intercalator is pulled electrostatically, in an aqueous isotonic solution. The ligand displaces a cation that stayed in the condensation cloud of such cations which surrounds DNA. Then with the outer surface of DNA a weak electrostatic association forms. The ligand then disperses through the DNA surface and may fall into the hydrophobic environment formed among the base pairs. Transiently it may open to create an intercalation region.

Another important class of intercalators is metallo-intercalators which are complexes of polycyclic aromatic ligands with a metal cation. Generally, employed metal ions are iridium (III), ruthenium (II), and rhodium (III). The planar structure of terpyridine and dipyridine is ideal for intercalation, thus commonly used as ligands to attach to the metal ion [32].

The second leading cause of death is cancer. In worldwide, it has an impact on even all living beings. Most of the currently available anticancer drugs have lots of side effects. In the article, we discuss the anticancer activity of 10 intensively studied heterocyclic DNA intercalators. The list includes proflavine, ethidium bromide, doxorubicin, dactinomycin, bleomycin, epirubicin, mitoxantrone, ellipticine, elinafide, and

echinomycin. Besides, some examples of the metallo-intercalators are presented at the end of the chapter.

4.2 Proflavine

Proflavine (proflavin or diaminoacridine or 3,6-diaminoacridine) is an acridine derivative [33]. The molecular structure is shown in Figure 4.7. It possesses various pharmacological activities. For instance, it is used as a potent anticancer agent, antibacterial agent, antifungal agent, antiparasitic agent, and antiviral agent [34–36]. This compound modifies bacterial DNA and that results in the lysis of bacteria. It affects host DNA because of its intercalating property and it can trigger to make cancer in the skin and other malignancies. To denature the host DNA, reactive oxygen species freed by proflavine play a critical role. It is reported that Proflavine can accumulate in cell nuclei by penetrating beyond epidermal and dermal structures. It is taken up by several categories of cells in human cell culture. It finds a broad range of applications in industrial, cutting-edge research, clinical, and therapeutic.

Proflavine acts by intercalation, thereby disrupting DNA synthesis thus causing high levels of mutation in the copied DNA strands. Proflavine causes base pair-insertions or base pair-deletions and no substitutions in that way it differs from other mutagenic components. Also, it causes double-stranded breaks in DNA in the presence of light [37].

One of the most usual childhood malignancies is Osteosarcoma. The effect of proflavine on the human osteosarcoma cell was investigated by Zhang et al. [38]. The study evidenced that *via* the activation of apoptotic cascades proflavin halted the osteosarcoma cell's growth. Furthermore, it was disclosed that autophagy synergistically contributes to the proflavin-suppressed osteosarcoma cell's growth.

Through extensive computational methods, the intercalation mechanism of the proflavine into DNA was reported by analyzing the molecular kinetics and thermodynamics [39]. The results showed that through the major groove side proflavine's de-intercalation and intercalation pathways proceeds. At the same time, intercalation is typically ruled by the minor groove's pre-intercalative bound state which is found to be stable. Figure 4.8 shows the collective variables and initial proflavine–DNA configurations used for the study.

Considering proflavine derivatives, Janovec et al. reported the synthesis of proflavine–dithiazolidinone derivatives and their interaction with DNA together with the

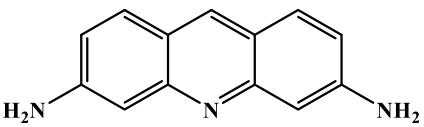

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Figure 4.7: Molecular structure of proflavine.

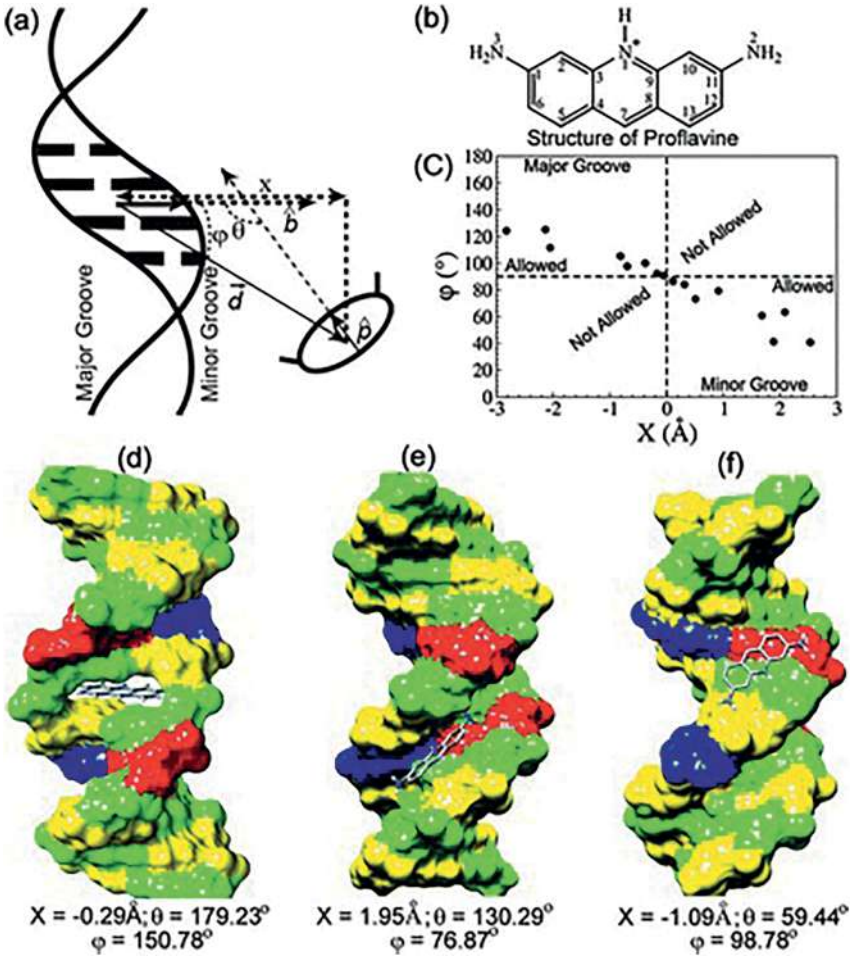

Figure 4.8: Schematics of the collective variables and initial proflavine–DNA representations. Adapted with permission from [39].

cytotoxic activity [40]. The compounds dialkyl acridin-3,6-diyl dithioureas and methyl bromoacetate were used for the synthesis of these proflavine derivatives. The synthesis pathway is shown in Figure 4.9. Using ultra violet–visible, fluorescence, and CD spectroscopic methods, the compound's binding affinity with calf plasmid as well as thymus DNA was studied. The cytotoxic activities (*in vitro*) of compounds against human cancerous cells and murine leukemia cells were also investigated. The proflavine-dithiazolidinone derivative with propyl chain showed the highest activity.

Kozurkova et al. investigated the preparation and cytotoxic activity of proflavine diureas [41]. The schematic of the synthesis is shown in Figure 4.10.

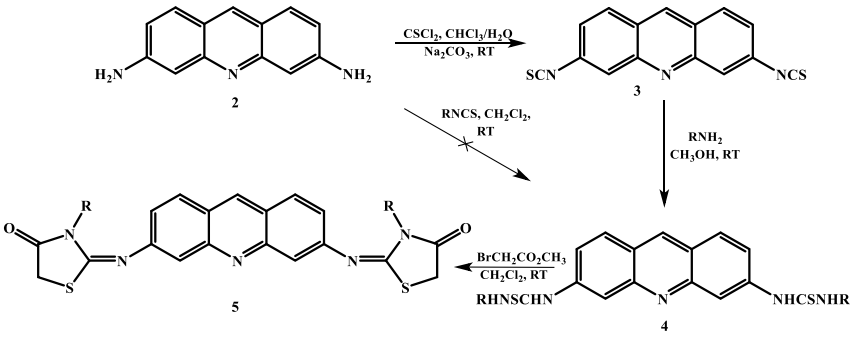

Figure 4.9: Synthetic route of proflavine derivatives.

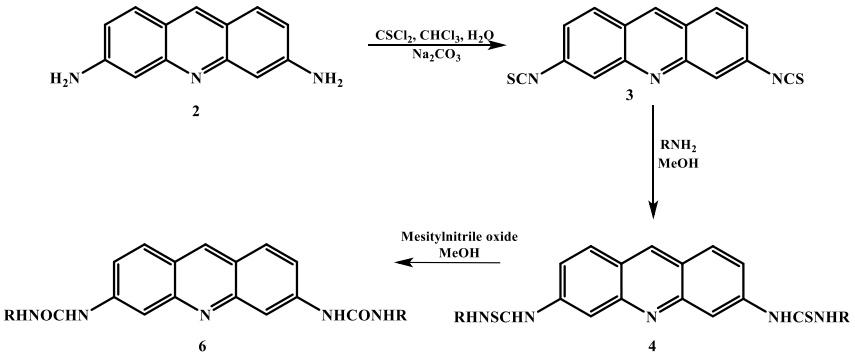

Figure 4.10: Synthesis of diureas.

The compound 3,6-diisothiocyanatoacridine (**3**) was prepared from proflavine hemisulfate (**2**) which is available commercially. The intermediate dithioureas (**4**) were made from aliphatic amines and 3,6-diisothiocyanatoacridine. Using mesitylnitrile oxide in methanol the conversion of dithioureas (**4**) into corresponding diureas (**6**) was obtained. Using methanolic HCl the corresponding hydrochlorides were prepared. The synthesized compounds exhibited potent cytotoxic properties against HCT-116 and HeLa cells.

A series of 3,6-di-substituted acridines' cytotoxicity, as well as photo-enhanced cytotoxicity, were reported [42]. The neutral proflavine **2** was the starting material for the targeted compounds. Proflavine was acylated in an alternate way with various benzoyl chlorides and anhydrides to produce the desired amides (Figure 4.11).

Transformed primary cultures of human keratinocytes and Chinese hamster ovary cells were used to test the cytotoxicity as well as photo-enhanced cytotoxicity of the compounds. Two derivatives showed specific cytotoxicity on Chinese hamster ovary cells.

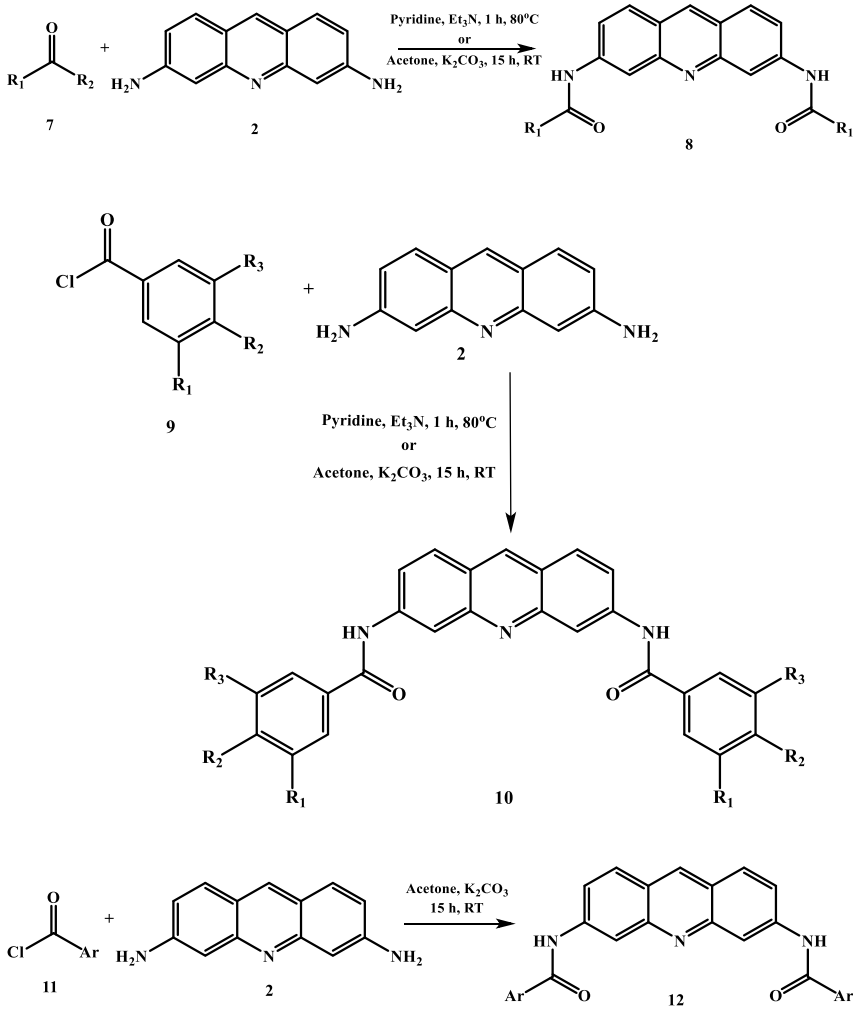

Figure 4.11: Synthesis of compounds the targeted compounds.

Plsikova et al. investigated the preparation of a series of 3,6-bis(3-alkylguanidino) acridines and their activity as intercalating agents [43]. The synthetic pathway is shown in Figure 4.12. The compounds 3,6-bis(thiourea)acridines were made using proflavine and 3,6-diisothiocyanatoacridine **3**. These thioureas were used for the preparation of carbodiimides. The reaction of carbodiimides with ammonium hydroxide produced guanidines. Later column chromatography on silica gel was used to purify guanidines and eventually, the bis(guanidino)acridines **13** were obtained as hydrochlorides salts.

The synthesized compounds depicted a strong binding activity towards DNA. Fluorescence, circular and linear dichroism, ultra violet–visible spectroscopy, viscosimetric, and DNA melting methods pointed that the synthesized compounds can

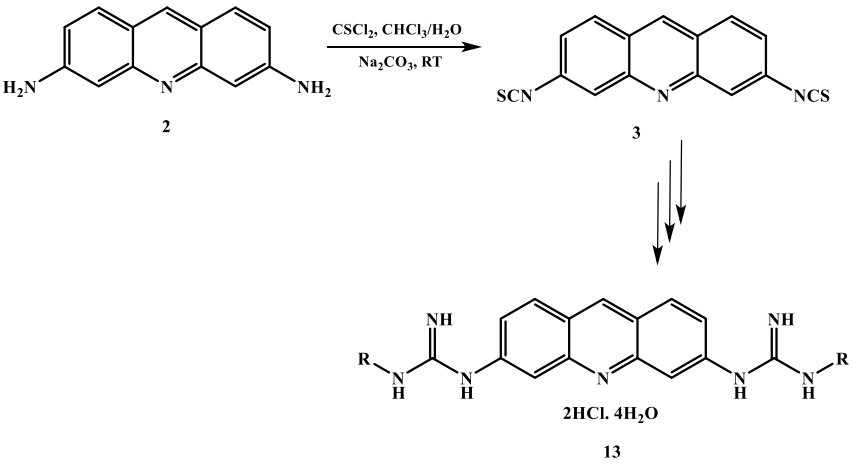

Figure 4.12: Synthesis of bis(guanidino)acridines.

work as efficient DNA-intercalating agents. Different methods such as phosphatidylserine externalization, cell cycle distribution, caspase-3 activation, changes in mitochondrial membrane potential were used to evaluate the biological activities of the compounds. The hexyl derivative of the compound showed the most cytotoxic activity. For individual derivatives, distinct forms of cell penetration were detected.

4.3 Ethidium bromide

Ethidium bromide, also known as homidium bromide, is a DNA intercalating agent which resembles a DNA base pair. Ethidium bromide can intercalate into DNA strands very easily owing to its unique structure. The molecular structure is shown in Figure 4.13. The cause of ethidium bromide's intense fluorescence following binding with DNA is the hydrophobic environment established between the base pairs. This intercalating agent is commonly used as a fluorescent tag in techniques. For example in molecular biology laboratories to detect nucleic acids it is used in agarose gel electrophoresis [44]. As single-stranded RNA normally folds back onto itself and in that way it allows local base pairing for intercalation, it can also be detected. During exposure to UV light, ethidium bromide will glow with an orange color, and after sticking with DNA the color will intensify. For the treatment of trypanosomiasis in cattle it is also utilized in veterinary medicine [45]. Ethidium bromide is a mutagen, depending on the area of exposure and the organism exposed.

In the early years, this compound was screened for antitumor activity in mice and rats against different ascitic and solid tumors [46]. The study showed that the compound was active against Flexner–Jobling carcinoma solid tumors, mammary

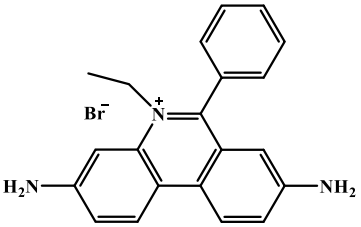

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Figure 4.13: Molecular structure of ethidium bromide.

adenocarcinoma, and sarcoma cancer cell lines. At the same time, the compound was inactive against leukemia L-1210 solid and Ehrlich solid carcinoma. Nishiwaki et al. was also reported the antitumor activity of ethidium bromide and related compounds [47].

Recently, Galindo–Murillo et al. investigated the ethidium bromide interactions with DNA using molecular dynamics simulations [48]. The study presented the theoretical analysis of the ethidium's interaction with two distinct double-stranded DNA models. It was reported that the ethidium ligand connects majorly intercalated between or stacked on the terminal base pairs of the DNA. And with the inner base pairs, little to no interaction (Figure 4.14). As the intercalation at the terminal CpG steps is comparatively speedy, the resultant DNA rigidification, unwinding, and enhanced stability of the internal base-pair steps suppress additional intercalation.

Banerjee et al. examined the binding potential of ethidium bromide and its structural analogue, propidium iodide to chromatin, and the accompanying modification in geometry [49]. The analysis reported mainly the binding energetics and the connection of these molecules with chromatosome, chromatin, core histones, and chromosomal DNA. At the chromatosome level, the molecules induced chromatin compression and release of DNA. Vardevanyan et al. reported, with DNA the interaction of Hoechst 33258 and ethidium bromide as well as the interaction of methylene blue and ethidium bromide [50].

Scaria et al. investigated the ethidium bromide's bonding to a DNA triple helix [51]. The fluorescence energy transfer, as well as the quenching analysis, demonstrated the ethidium's intercalation in both triplexes as well as duplex structures. Interaction with ethidium heads to an initial decrement in viscosity for both the triplex and duplex complexes, accompanied by an enhance.

4.4 Doxorubicin

Doxorubicin (Adriamycin) is a chemotherapy medication (Figure 4.15). Mostly applied for the treatment of Hodgkin's lymphoma, leukemias, breast cancer, ovarian cancer,

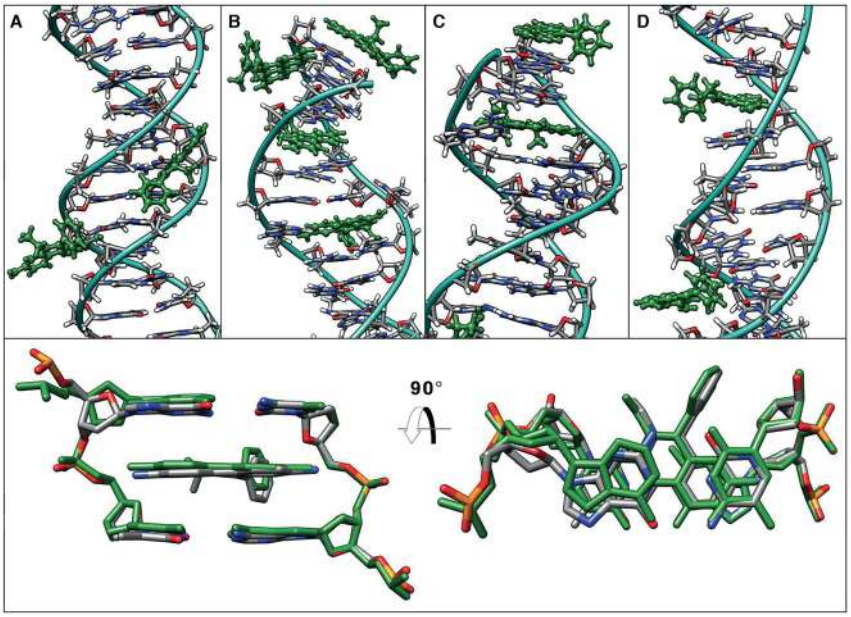

Figure 4.14: Selected binding modes of the ethidium ligand with the GAAC sequence (Top panel). Overlay of X-ray experimental structure and a 10 μs average structure from the intercalation event from panel B (Bottom panel). Adapted with permission from [48].

stomach cancer, soft tissue sarcoma, thyroid cancer, multiple myeloma, and lungs cancer. Many combination therapies, for example, combination adriamycin and cyclophosphamide are also widely used. This medication was originally synthesized from the bacterium *Streptomyces peucetius* [52].

Through the major groove of DNA double helix, doxorubicin intercalates and stops the activity of DNA-associated enzymes by stabilizing the DNA-enzyme complex. Basically, it behaves as a topoisomerase toxicant. An array of cytotoxic issues happens in alignment with antiproliferation when doxorubicin binds to several molecular

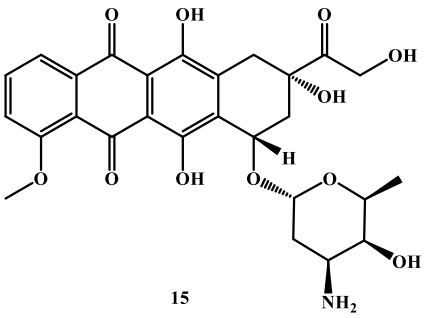

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Figure 4.15: Molecular structure of doxorubicin.

objects like topoisomerase enzymes I and II. It results in the damage of DNA [53]. When the effort to fix the cuts in DNA goes wrong as well as the cellular growth is suppressed at the G1 phase and G2 phase, the apoptosis pathway is triggered. This compound is also known to intercalate itself into the DNA, together with the suppression of both DNA and RNA polymerase. In the end discontinuation of DNA replication and RNA, transcription occurs. Another important action of doxorubicin is the generation of free radicals. This makes advanced DNA damage, suppression in the production of the macromolecule, an increase in alkylation, and DNA separation/unwinding [54, 55]. Studies also showed that doxorubicin can also intercalate with mitochondrial DNA [56].

Even though doxorubicin is employed for cancer treatment in a widespread way, it is extremely hydrophilic, has a poor half-life, and produces severe adverse effects such as myelosuppression, nephrotoxicity, and cumulative cardiotoxicity. Various methods have been reported to make better delivery of the drug. For example, by using chemical alteration in a parent drug [57], by connecting the drug to longer propagating particles such as metal nanoparticles, polymeric nanoparticles, and other nanoparticles [58–61], or by encapsulation in safe sheaths such as niosomes, hydrogels/organogels, liposomes, dendrimers, or nanomicelle [62–66].

Several derivatives of doxorubicin were synthesized extensively to upgrade the anticancer activity and lipophilicity of the drug. For example, Kruger et al. investigated the preparation and stability of maleimide derivatives of doxorubicin [67]. This study was important for the preparation of chemoimmunoconjugates. The derivatives were obtained by attaching maleimide groups to doxorubicin. To thiolated carrier proteins, the synthesized derivatives connect in a selective manner. Four maleimide derivatives of doxorubicin were synthesized (Figure 4.16).

Chhikara et al. investigated the preparation and anticancer activities of the fatty acyl amide derivatives of doxorubicin [68]. In the presence of *N*,*N*-diisopropylethylamine (DIPEA), and 1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), doxorubicin reacted with fatty acids and produced the monosubstituted fatty acyl-doxorubicin conjugates (Figure 4.17).

The study depicted that with the increment in the length of the chain of fatty acyl moiety, the lipophilicity of the compound was increased. The biological activity of the compounds was screened *in vitro* against human colon adenocarcinoma cell lines, ovarian adenocarcinoma, breast adenocarcinoma, and leukemia cell lines. The study suggested that the existence of a free amino group is necessitated for the anticancer activity of this drug. Among the synthesized derivatives the most efficient one was the dodecanoyl–doxorubicin derivative.

The same research team reported the preparation, cellular uptake studies, and biological activities of doxorubicin succinate's lipophilic derivatives [69]. To increase the cellular uptake, cellular retention, and lipophilicity, a number of doxorubicin derivatives were made through coupling various tetradecanol or fatty amines with doxorubicin-14-hemisuccinate.

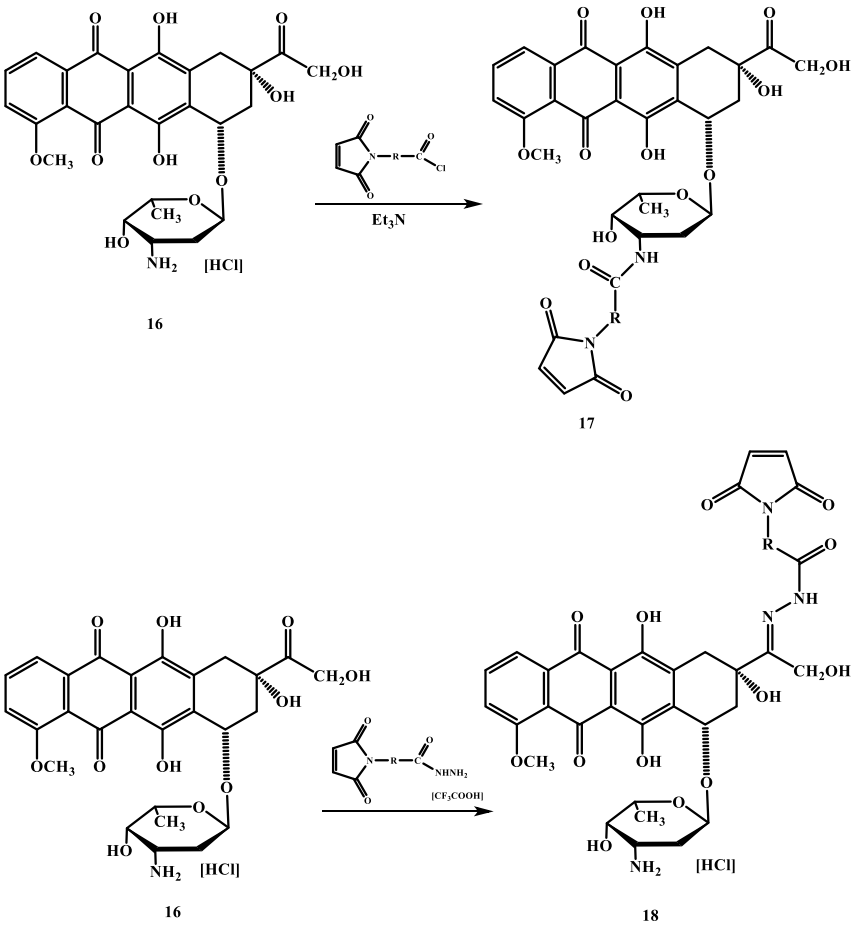

Figure 4.16: Preparation of maleimide derivatives.

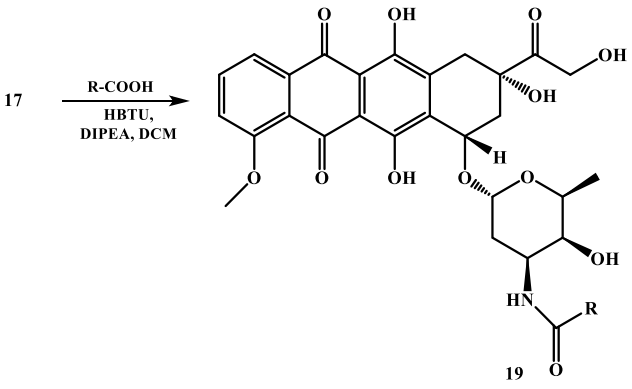

Figure 4.17: Preparation of fatty acyl derivatives of doxorubicin.

Doxorubicin can be modified at the aglycone unit or a daunosamine amino unit, for example, doxorubicin derivatives can be prepared by modifying the C-3′ location of the daunosamine moiety with the formamidine unit. Marczak et al. investigated the preparation of doxorubicin derivatives bearing a formamidine unit at the 3′ location with piperidine, pyrrolidine, morpholine, hexamethyleneimine, and *N*-methylpiperazine group [70]. The chemical structures of the formamidine derivatives of doxorubicin are depicted in Figure 4.18.

The compounds were tested against ovarian cancer cell lines and all the derivatives were effective against ovarian cancer cells. All the compounds were substantially better cytotoxic to the human cancer cell than doxorubicin. The survival curves of the ovarian cancer cells exposed to doxorubicin and its derivatives are presented in Figure 4.19. The study showed that the compounds accelerated majorly the caspase-3 mediated apoptotic pathway of cell death. Other than a substantial enhance in the activity of caspase-3, morphological alterations in cancer cells, and fragmentation of DNA was also found much greater in these formamidine derivatives of doxorubicin.

Recently, amide derivatives of doxorubicin were reported [71]. The compounds were obtained by the interaction of the amino group with docosahexaenoic acids and α-linolenic, also the double-substituted derivatives were prepared via ester and amide linkages. The synthetic procedure is shown in Figure 4.20.

All the compounds were tested against human metastatic colon cancer cells, primary colon cancer cells, and metastatic prostate cancer cells. The compounds showed moderate cytotoxicity for the normal cell line and great cytotoxicity against the cancer cell lines. The study also showed that all conjugates display higher selectivity but lower cytotoxicity than doxorubicin. Within the compounds, compared with conjugates made only by the amide linkage, the conjugates made by the ester and amide linkages were more assuring.

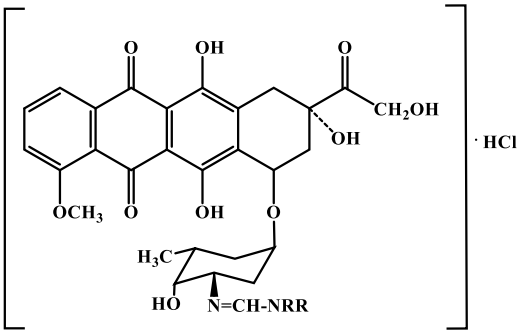

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Figure 4.18: Molecular structure of formamidine derivatives of doxorubicin.

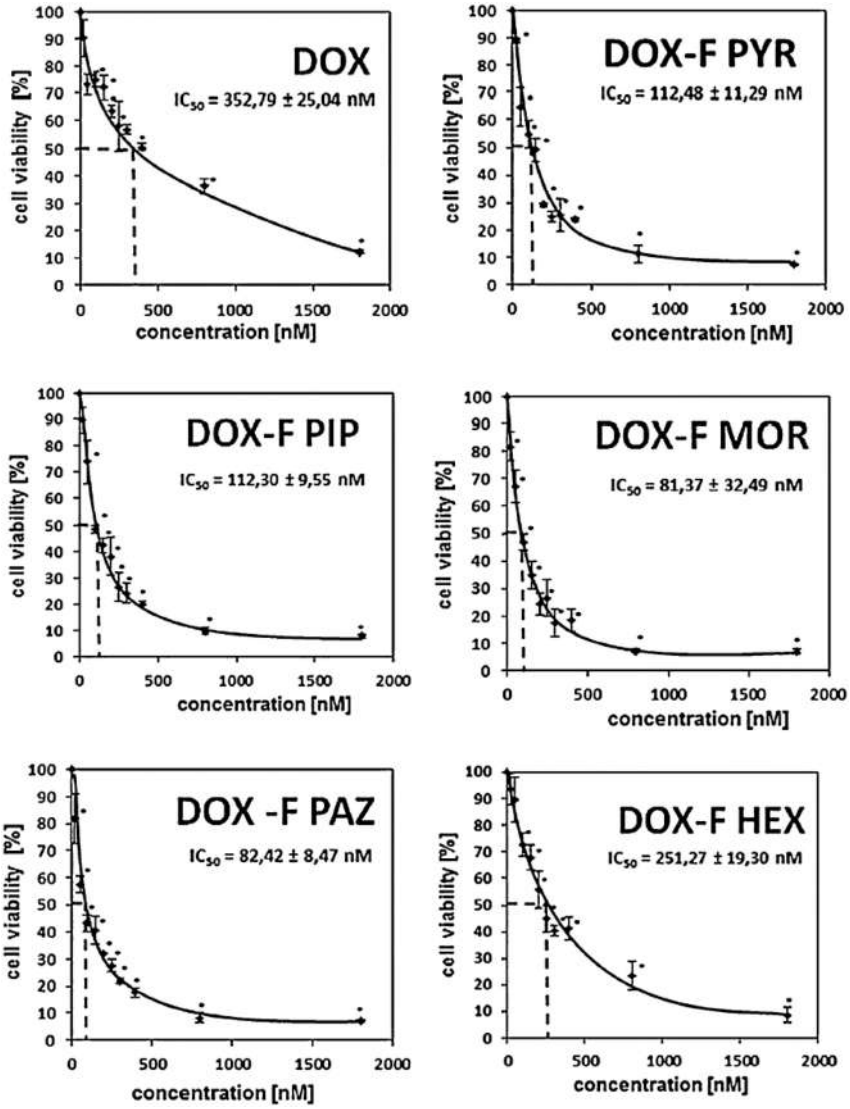

Figure 4.19: Dose-response curves and the IC_{50} parameters. Adapted with permission from [70].

Activity analysis of the (6-maleimidocaproyl)hydrazone derivatives (Figure 4.21) was also reported [72]. It is an albumin-binding prodrug. The compound has acid-sensitive characteristics that demonstrate high antitumor activity in murine tumor models.

Meo et al. reported polyaspartamide–doxorubicin as a possible anticancer prodrug [73]. Synthetic routes are shown in Figures 4.22 and 4.23.

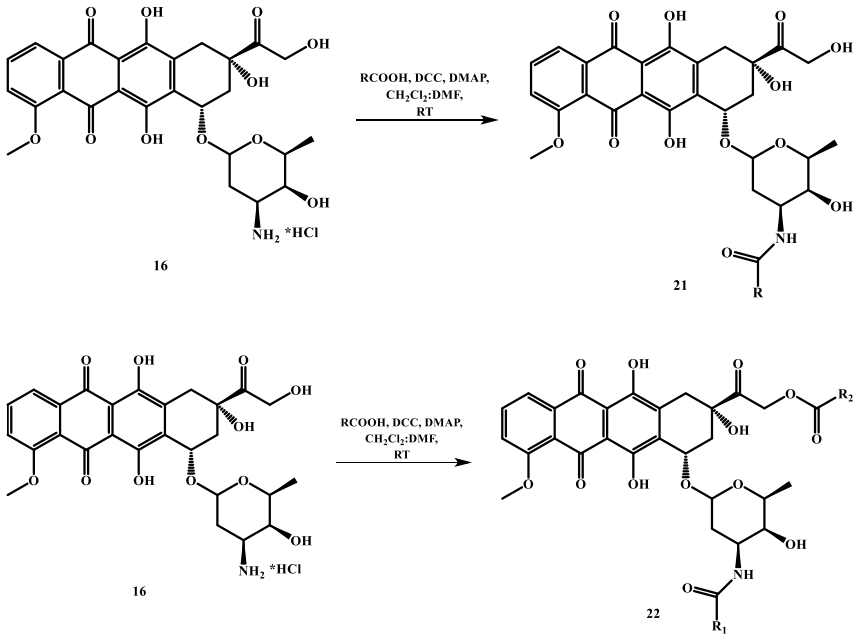

Figure 4.20: Synthesis of doxorubicin conjugates.

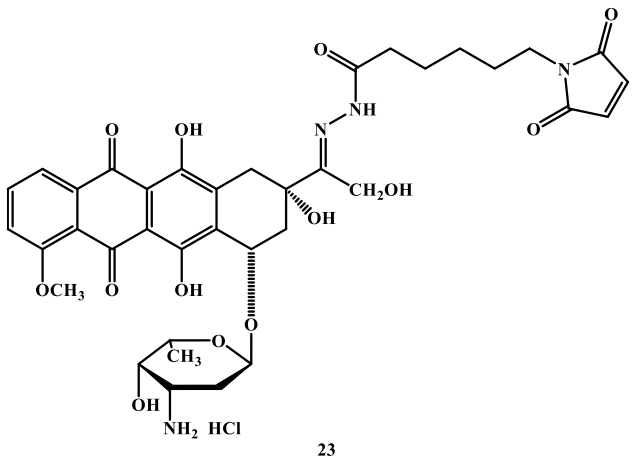

Figure 4.21: Molecular structure of hydrazone derivative.

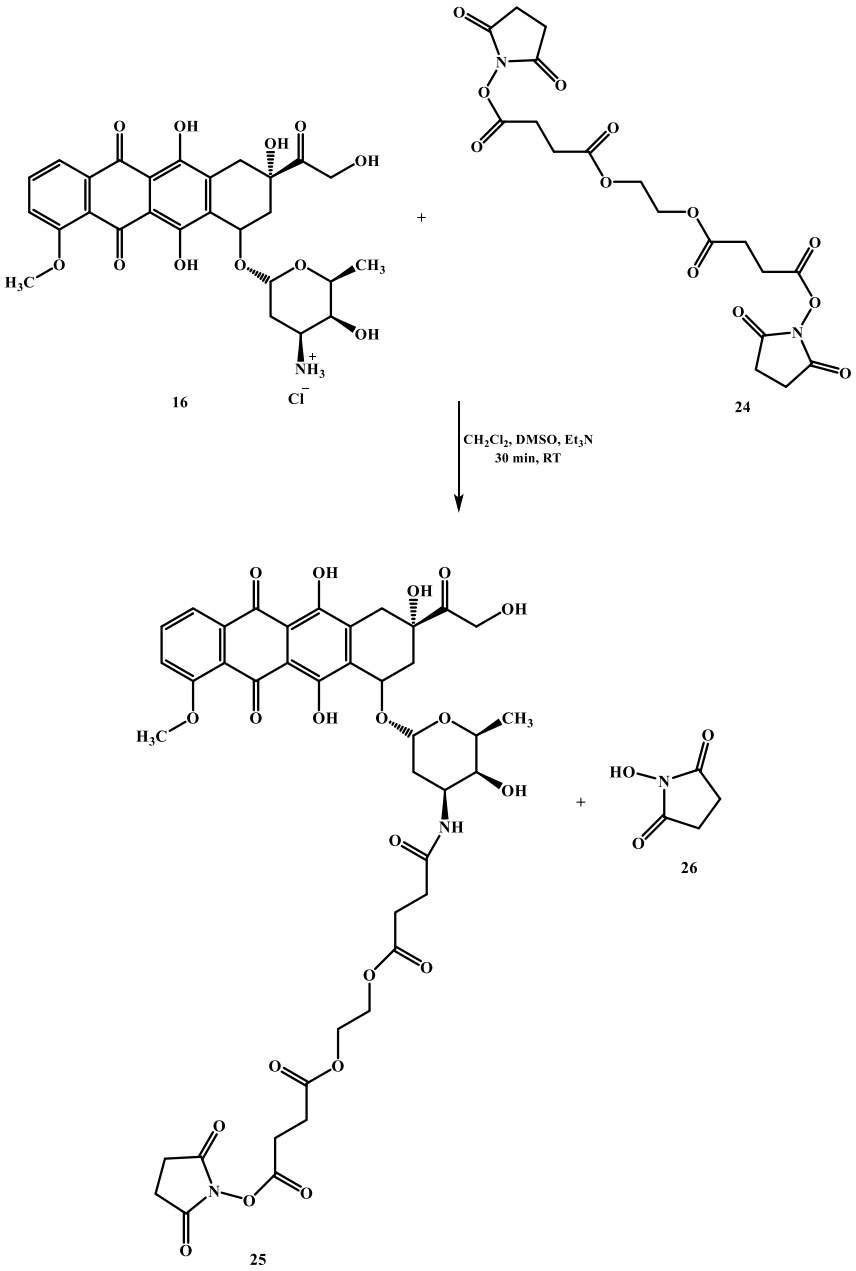

Figure 4.22: Synthetic route of DOXO-NHS using ethylene glycol-bis(succinic acid *N*-hydroxysuccinimide ester) and doxorubicin.

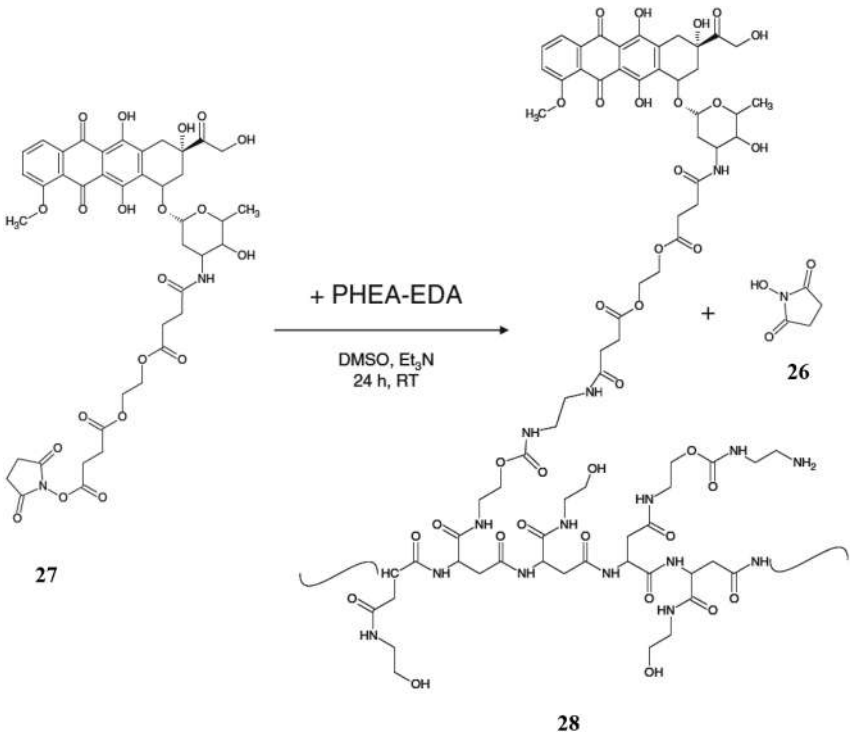

Figure 4.23: Synthetic route of PHEA-EDA-DOXO using DOXO-NHS and PHEA-EDA. Adapted with permission from [73].

4.5 Dactinomycin

Actinomycin D, also known as dactinomycin, is one of the most important members of the actinomycin family. It belongs to the group of polypeptide antitumor antibiotics which are extracted from soil bacteria (*Streptomyces*). It is used as a chemotherapy drug for the treatment of many cancers, including rhabdomyosarcoma, Wilm's tumor, certain kinds of ovarian cancer, and testicular cancer. It is given intravenously and the common side effects are bone marrow suppression, infections, future cancers, muscle pains, vomiting, hair loss, mouth ulcers, allergic reactions, liver problems, and death of tissue at the injection site.

Dactinomycin bears a phenoxazine chromophore connected to two cyclic depsipeptides carrying five amino acid residues (Figure 4.24). As this compound acts both as a minor groove binding agent and a DNA intercalator, it can be counted as a hybrid compound. This compound lacks a positive charge, in that way it is different from other intercalating drugs. However, its large dipole moment arises from a non-symmetrical distribution of polar group compensating this. As it intercalates through

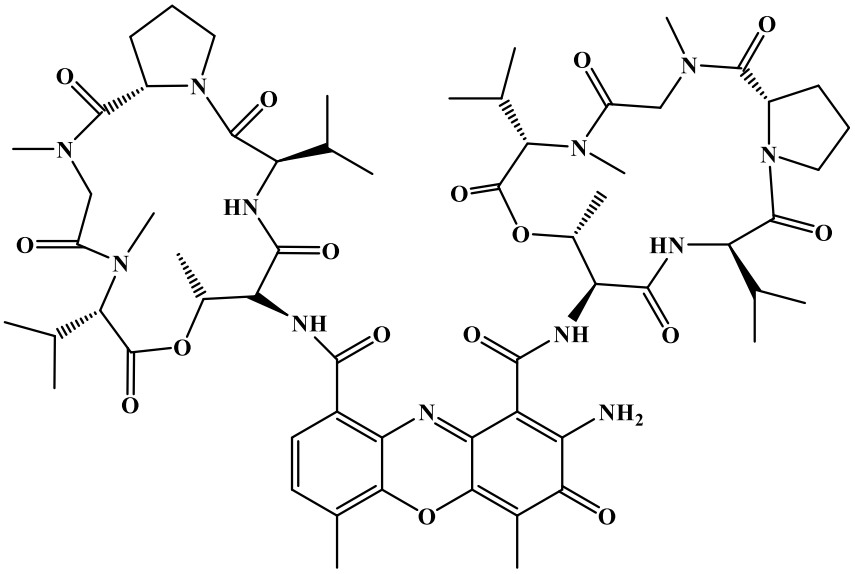

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Figure 4.24: Molecular structure of dactinomycin.

the DNA double helix's minor groove, thus preventing DNA double helix's unwinding. It blocks transcription by stopping DNA-dependent RNA polymerase.

The effects of vinorelbine and dactinomycin on DNA and soluble chromatin employing were compared [74]. Spectroscopy methods like UV/vis, fluorescence, and circular dichroism were applied for the study. The study showed that the absorbance at 210 and 260 nm was diminished (Figure 4.25) and quenching of drugs with chromatin chromospheres and DNA produced the decrease in the fluorescence emission intensity. Circular dichroism data demonstrated that structural modifications in both negative and positive extremes of chromatin and DNA were produced by the action of drugs. The observation revealed that from chromatin the displacement or release of histone proteins occurred due to the binding with dactinomycin. On the other hand, vinorelbine led the chromatin into compaction. The study revealed that compared to chromatin dactinomycin shows a more affinity towards DNA. On the contrary, vinorelbine recognized the chromatin with more affinity compared to DNA.

The gestational trophoblastic disorder is a range of disease phenomenon and the abnormal trophoblastic proliferation was characterized by this. Gestational trophoblastic neoplasia is a lesion with the possibility for metastasis and local invasions such as gestational choriocarcinomas, placental site trophoblastic tumors, and invasive moles [75]. The consequences of aggregated chemotherapy treatment applying dactinomycin and methotrexate in the controlling of women having low-risk gestational trophoblastic neoplasia were examined [76]. The study showed that low-risk

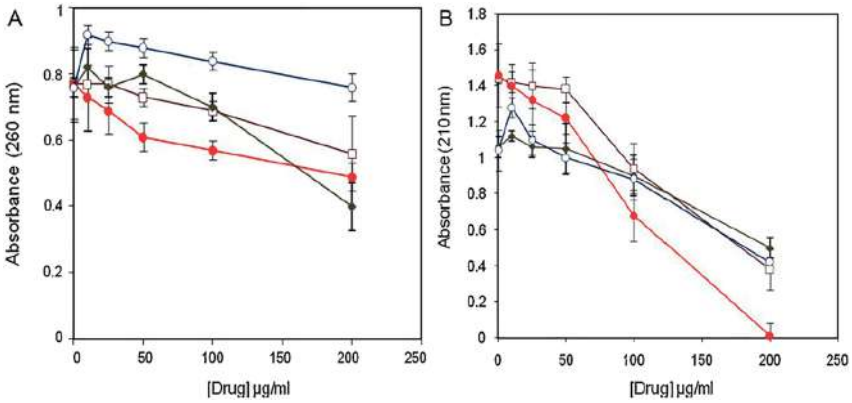

Figure 4.25: Ultra violet absorbance variations of chromatin and DNA in the absence and presence of various amounts of vinorelbine and dactinomycin at 260 (A) and 210 nm (B). Adapted with permission from [74].

gestational trophoblastic neoplasia is constantly and quickly cured with combined dactinomycin–methotrexate and also low toxicity was observed.

Recently, Beziat et al. reported the activity of actinomycin against acute myeloid leukemia with NPM1 Mutations [77]. In acute myeloid leukemia, the NPM1 mutations were frequent. NPM1 mutations were normally connected with a favorable prognosis and chemosensitivity. At the same time, according to co-mutational events outcomes varied, and still after achieving complete response many patients were relapsed. Wild-type NPM1 was mostly situated in the nucleolus. It played a critical part in protein creation, regulation of ribosome biogenesis, and tumor suppression through TP53 activation. A complete response was reported in NPM1-mutated acute myeloid leukemia patients with dactinomycin.

4.6 Bleomycin

Bleomycin is used as an anticancer agent. It is mainly employed for the treatment of cancer such as non-Hodgkin's lymphoma, Hodgkin's lymphoma, ovarian cancer, cervical cancer, and testicular cancer. Mostly applied with other cancer drugs and functions by forbidding the synthesis of DNA. The main adverse effects are fever, a severe kind of anaphylaxis, vomiting, rash, weight loss, and lungs inflammation. This medication can be given intravenously, intramuscularly, or subcutaneously. Sometimes to stop the reoccurrence of fluid around the lung because of cancer, it is applied inside the chest.

By using a bithiazole ring system bleomycin intercalate (Figure 4.26). Nitrogens from the pyrimidine ring, primary amines, and amide chelate ferrous ions [78, 79]. Also,

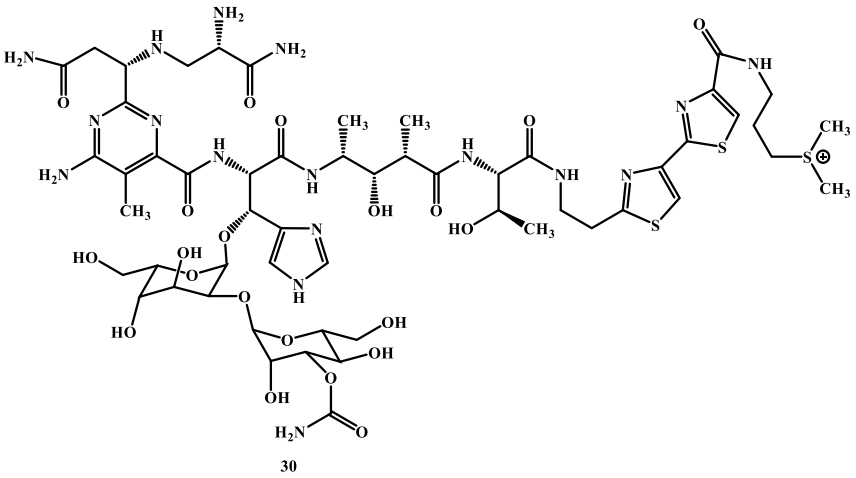

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Figure 4.26: Molecular structure of Bleomycin.

interaction with oxygen atoms leads to a reactive oxygen species and ferric ion [80, 81]. It leads to the chain cutting and formation of radicals. This medication forbids DNA ligase from fixing the breakage. The mechanism of bleomycin-induced mitochondrial dysregulation in diverse tumors is considered to be oxidative stress-mediated DNA alterations [82–85].

Many anticancer drugs, including bleomycin, have the incapability to get over the cell membrane accounts in an effective manner and this limits the clinical efficaciousness of the drug. One of the options to get over the physical barrier and to increase the intrinsic cell membrane permeability is to increase the intracytoplasmic concentration. That process potentiates drug cytotoxicity [86–89]. Studies showed that the usage of low-intensity ultrasound enhanced the antitumor cytotoxicity of drugs *in vitro* [90–92]. It is anticipated that the transient cell membrane permeabilization and cavitation by acoustic pressure may help to increase the drug's intracytoplasmic concentration and because of that cytotoxicity enhances [93]. It was reported that the usage of ultrasound at low intensity against subcutaneously planted tumors in mice following the bleomycin application inhibits the rate of growth of tumors compared to tumors addressed with only bleomycin or untreated tumors [94]. The optimal ultrasonic conditions for improvement of cytotoxicity of bleomycin were reported by Larkin et al. [95]. In that study, ultrasound parameter optimization for bleomycin application was attempted, and an efficient antitumor activity was presented in solid tumors of human as well as murine cells (Figures 4.27 and 4.28). The same results were observed during bleomycin–electrochemotherapy.

Even though different types of cancers affect women, cervical cancer is the fourth most common cancer that strikes women. Cervical cancer is normally connected with human papillomavirus infection. This virus integrates into the genome of the host cell

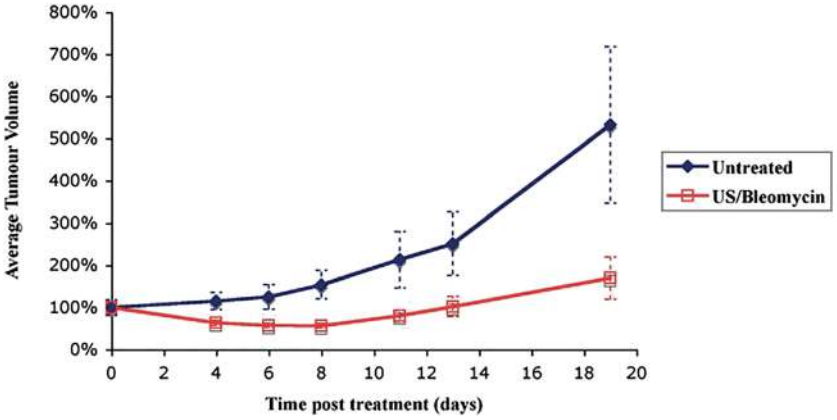


Figure 4.27: Growth curves for tumors of the human esophageal carcinoma cells in athymic nude mice: After treated with ultrasound-enhanced bleomycin and untreated controls. Adapted with permission from [95].

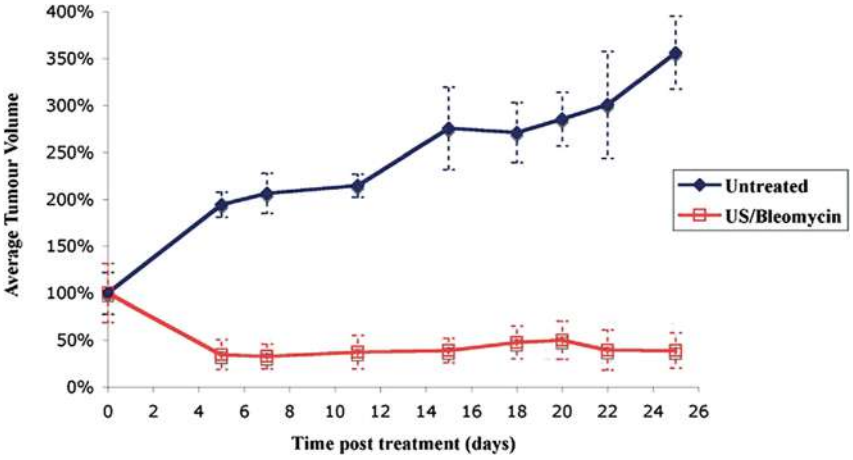


Figure 4.28: Growth curves for tumors of the human renal carcinoma cell line in athymic nude mice: After treated with ultrasound-enhanced bleomycin and untreated controls. Adapted with permission from [95].

and that heads to its participation in various regulatory gene connections. In the foundation and advancement of cervical cancer, external as well as internal elements are involved. Different techniques are reported for the treatment of human papilloma virus-positive cervical cancer, for example, chemotherapy, radiotherapy, and surgery. However, most of these techniques have negative impacts.

The authorized anticancer drugs used for the treatment of cervical cancer are bevacizumab, cisplatin, topotecan, and bleomycin. But these medications lack specific targets and that makes damage to a normal cell, injury to nervous tissue, myelotoxicity, renal failure, and diarrhea [96–98]. Many of the anticancer medications produce free radicals in high-level or metabolic intermediates that make oxidative damage to normal cells' DNA and it heads to chemoresistance *via* anti-apoptosis mechanisms.

Even though bleomycin is a good choice for the treatment of cervical cancer, bleomycin monotherapy can make different adverse effects like hyperpigmentation, immune system damage, pulmonary fibrosis, and pneumonitis. Most of these side effects are arbitrated by redox status disruptions [99, 100]. Combined chemotherapy can overcome the disadvantages of monotherapy as it is using more than one medication. It shows significant promise in cancer therapy. Combined cancer therapies using drugs and phytochemicals have gained much attention as the phytochemical interactions and drugs can either increase or diminish therapeutic effects.

Also, it is reported that cancer risk can be reduced by dietary supplements. It can decrease chemotherapy-associated side effects and prolong cancer patient survival times [101, 102]. Synthetic drugs' toxic adverse effects can also be eliminated by dietary phytochemicals [103–105].

When bleomycin was used with phytochemicals in combinational therapies, the phytochemicals significantly enhanced the efficiency of the bleomycin and cut down the adverse effects [85, 106].

In human cervical cancer cells, the synergistic anticancer activity of bleomycin hydrochloride dietary and tea polyphenols were reported by Alshatwi et al. [68]. Tea polyphenols are effective chemo-preventive and antioxidative agents. Cervical cancer cells were handled with different amounts of tea polyphenols, bleomycin, and tea polyphenols in combination with bleomycin. The effects on poly-caspase activity, cell growth, early apoptosis, intracellular reactive oxygen species, and the expression of caspase-9, caspase-8, and caspase-3, Bcl-2, and p53 were examined. The study disclosed that the cancer cells were less sensitive to growth suppression by tea polyphenols treatment in comparison with both bleomycin and the combined treatment. The study also demonstrated that compared with mono-agent therapy the combination therapy enhanced the percentage of apoptotic nuclei. In the cells applied with the combination therapy, the proportion of early and late apoptotic/secondary necrotic cells was more than mono-agent treated cells. The combination therapy synergistically accelerated apoptosis *via* the activation of caspase-9, caspase-8, and caspase-3, p53 overexpression, and upregulation of Bcl-2 (Figure 4.29).

4.7 Epirubicin

Epirubicin (EPR) is an anthracycline drug mainly used for cancer treatment. This medication is primarily used to treat ovarian cancer, gastric cancer, lung cancer, and

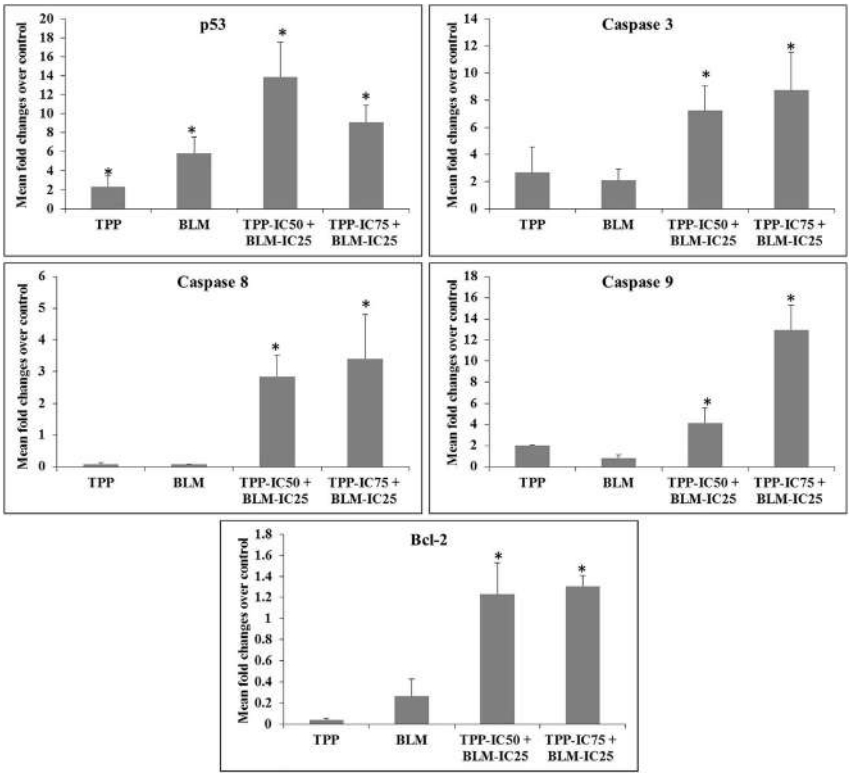
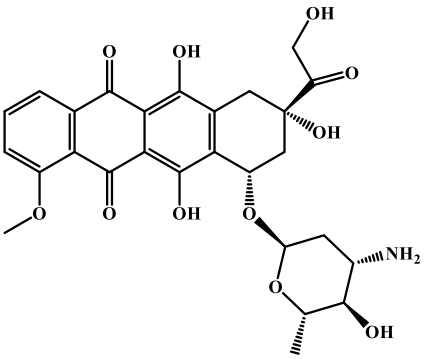


Figure 4.29: RT-PCR apoptotic marker genes investigations from the cervical cancer cell lines. Adapted with permission from [107].

lymphomas. In patients who underwent surgery to remove the tumor, epirubicin is used to handle breast cancer in combination with other drugs. It acts by intercalating DNA strands which leads to the formation of a complex that suppresses RNA as well as DNA synthesis. Other than that, using topoisomerase II epirubicin triggers DNA segmentation and finally leads to cell death. It also produces free radicals which induce DNA and cell damage. In the cytotoxic effects of the compound, binding to plasma proteins and cell membranes are involved. As epirubicin has fewer side effects, in some chemotherapy regimens it is preferred over doxorubicin. The distinct spatial orientation of the OH group at the 4′ carbon of the sugar might be the reason for the shortened toxicity and quicker elimination of epirubicin (Figure 4.30).

The effects of 8-hydroxydaidzein (8HD) and epirubicin on the yield of reactive oxygen species were evaluated [108]. The isoflavone, 8-hydroxydaidzein, is basically extracted from fermented soy germ koji [109–111]. Also from daidzein in rat and human livers, it can be converted [112]. 8-Hydroxydaidzein displays several biological properties such as cardioprotective, radical-scavenging, antioxidative, tyrosine-inhibitory



31 **Figure 4.30:** Molecular structure of epirubicin.

activities, antiproliferative, skin-whitening activity, melanogenesis-inhibitory, and antimutagenic activities, [109, 111, 113–119]. The study showed that 8-hydroxydaidzein raised the cytotoxicity of epirubicin and produced a synergistic result [108]. Epirubicin and/or 8-hydroxydaidzein application enhanced the levels of superoxide and hydrogen peroxide. At the same time, the combination therapy significantly reduced multidrug resistance protein's mRNA expression levels. 8-Hydroxydaidzein significantly intensified epirubicin intracellular accumulation in human colon adenocarcinoma cells. Moreover, 8-hydroxydaidzein and epirubicin markedly raised the mRNA expressions of Bax, p53, caspase-9, caspase-8, and caspase-3.

Several studies were reported about the epirubicin conjugates. For instance, Pasut et al. synthesized poly(ethylene glycol)-epirubicin conjugates [120] and Kmiecik et al. reported the epirubicin and methotrexate conjugates as potential anticancer drugs [121].

Due to the magnetically accumulated anticancer drugs in cancer cells, the usage of magnetic nanoparticles as a delivery system has gained much attention. By the use of magnetic mesoporous silica nanoparticles, the anticancer efficiency of epirubicin was improved [122]. The anticancer properties of epirubicin-loaded magnetic mesoporous silica nanoparticles were assessed in a C-26 colon carcinoma system. The cellular uptake analysis showed that in comparison with free epirubicin, the geometric mean fluorescence intensity of cells applied with epirubicin-loaded magnetic mesoporous silica nanoparticles in presence of an external magnetic field was increased. Also, therapy with drug-loaded magnetic mesoporous silica nanoparticles with the assistance of external magnetic gradient had greatly higher prohibition efficiency for tumor growth than the free epirubicin applied mice. The directed drug delivery via external magnet-attraction utilizing epirubicin-loaded magnetic mesoporous silica nanoparticles led to higher tumor cell uptake and it heads to the efficient cancer cell elimination.

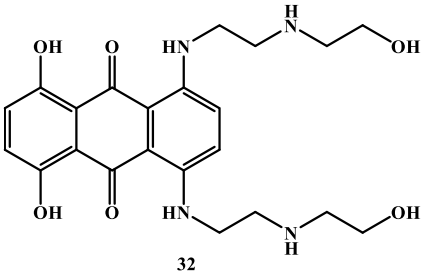


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Figure 4.31: Molecular structure of mitoxantrone.

4.8 Mitoxantrone

It is an anthracenedione antineoplastic agent (Figure 4.31). Mitoxantrone is applied for the treatment of certain kinds of cancer such as non-Hodgkin's lymphoma, acute myeloid leukemia, and metastatic breast cancer. This drug is a type II topoisomerase inhibitor. It interrupts the synthesis of DNA and repair in both cancer cells as well as in normal cells by intercalation [96, 97]. It has immunosuppressive effects. To treat metastatic hormone-refractory prostate cancer, the combination of prednisone and mitoxantrone is used as second-line therapy.

Mitoxantrone also acts with RNA helices and by that means elicits its medicinal activity. Tiwari et al. investigated the spectra analysis, electronic structure, and nano-range activities of mitoxantrone with RNA helices [123]. Various thermodynamic and electronic properties as well as Raman and infrared spectra of mitoxantrone were presented (Figure 4.32). By applying modified second-order perturbation theory, the stacking models of mitoxantrone with RNA helices were analyzed. The study

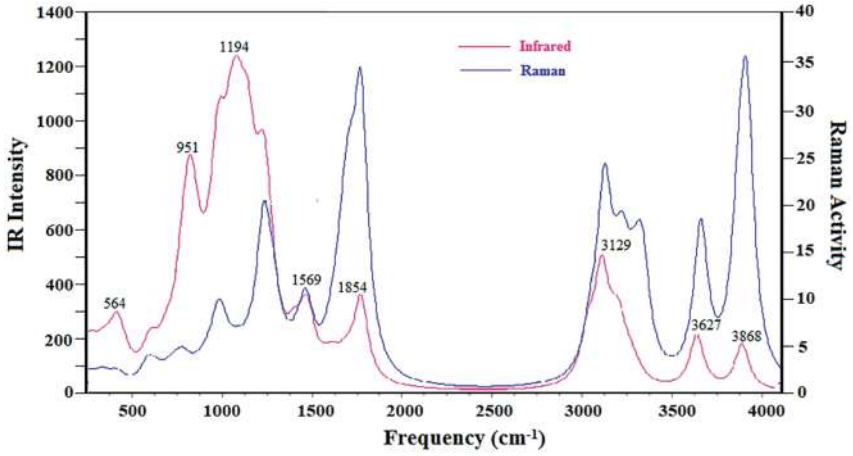

Figure 4.32: Raman and Infrared spectra of mitoxantrone drug. Adapted with permission from [123].

demonstrated that *via* intercalation mitoxantrone chooses to link in a guanine-cytosine-rich region of RNA base pairs.

Combination chemotherapy mitoxantrone with other drugs is believed as a good choice for the treatment of cancer [124, 125]. Recently, the combination treatment of mitoxantrone with naphthalimide derivative demonstrated stronger inhibitory activity on colorectal cancer in comparison with the treatment with mono-agent [126]. After the combination treatment downregulation of IDH2 caused reactive oxygen species creation was observed and it led to the accumulation of MIT. Finally, autophagic cell death occurred.

The *in vitro* delivery of mitoxantrone to HeLa cancer cells by employing polyethylene glycol liposomes functionalized with the novel cell-penetrating peptide gH625 was reported [127]. The study demonstrated that the distribution of doxorubicin to the cancer cell's cytoplasm was enhanced by this hydrophobic peptide.

The *in vitro* genotoxicity of mitoxantrone was compared with idarubicin [128]. The study showed that the effect produced by idarubicin was more marked compared to mitoxantrone. The cells exposed with mitoxantrone were capable to repair damage to their DNA in 30 min incubation, at the same time the lymphocytes treated with idarubicin required 180 min. Due to the effect of idarubicin methylation of the DNA bases, direct strand breaks, and oxidation was observed. Mitoxantrone induced alteration of the bases and strand breaks together with oxidation. Furthermore, much lesser genotoxicity was observed with mitoxantrone compared to idarubicin.

4.9 Ellipticine

Ellipticine is the prototype of intercalators. This drug is based on the pyridocarbazole system and shows a wide range of anticancer activity [129]. The chemical structure of Ellipticine is depicted in Figure 4.33. It is an alkaloid extracted from the leaves of *Apocynaceae plants*. It exists both as a monocation and as a neutral species at physiological pH values. This monocation is important for the intercalation of DNA, which heads to inhibition of RNA polymerase. Ellipticine also inhibits other DNA-associated enzymes such as RNA methylase, topoisomerase II, and DNA polymerase.

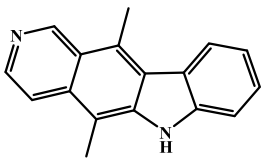

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Figure 4.33: Molecular structure of ellipticine.

This drug links powerfully and rests parallel to the base pairs in its intercalated state [130, 131]. This resulted in an increase in the DNA's superhelical density [132]. In a direct manner the intercalated ellipticine links to the enzyme which is involved in the replication of DNA (topoisomerase II) [133]. Then it suppresses the enzyme and that resulted in the potent antitumor activity [131].

Many synthetic methods have been reported for the preparation of ellipticine and its derivatives [134–136]. Ellipticine quinone is the key intermediate in ellipticine synthesis [137–139] and it exhibited antitumor activity [140]. Nishiyama et al. reported the preparation of ellipticine quinone based on the creation of a carbazole-1,4-quinone applying a tandem ring-closing metathesis and dehydrogenation [141]. Besides, the ellipticine quinone analogs possessing replacement at the 9- and/or 8-positions were also synthesized. The ellipticine quinone synthesis is demonstrated in Figure 4.34 and the synthesized new ellipticine quinone analogs are shown in Figure 4.35. The antiproliferative activity of the synthesized products and natural compounds were analyzed towards HL-60 and HCT-116 cell lines. The analysis displayed that the 9-nitroellipticine quinone was more active than calothrixin B.

Mousset et al. reported the preparation of 1,4-benzoxazine analogues of ellipticine [142]. For the synthesis, they used a basic synthetic method that depended on an anionic ring annulation as the important transformation. The final step is shown in Figure 4.36. The successive protection/deprotection steps produced phenol. This compound was then

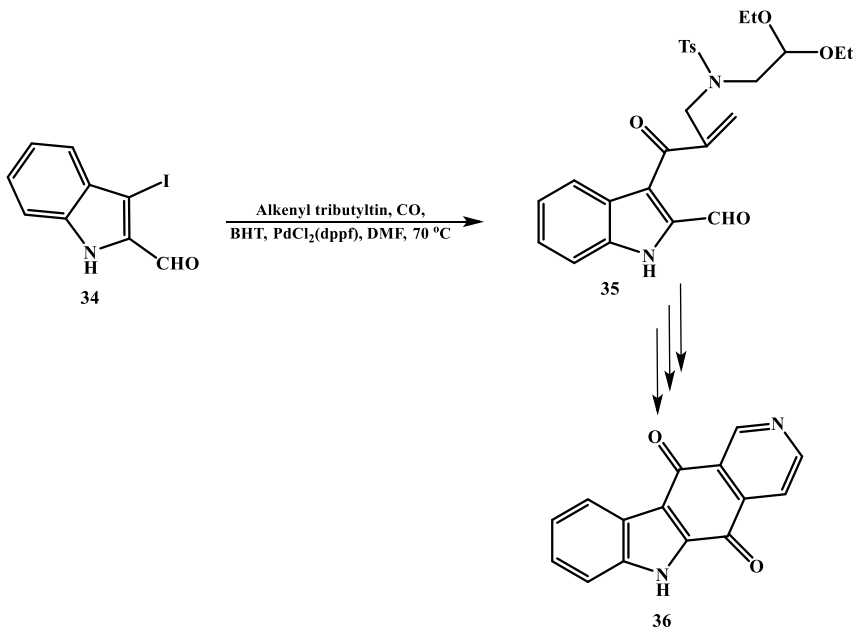

Figure 4.34: Synthesis of ellipticine quinone.

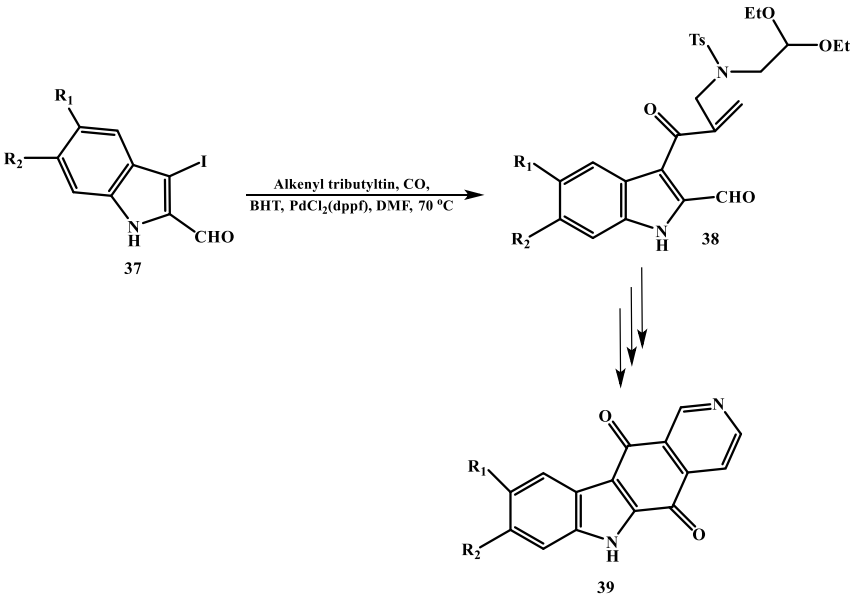

Figure 4.35: Synthesis of ellipticine quinones.

transformed into triflate **30** after reacting with N-phenyltrifluoromethanesulfonimide. To get the desired analogs, the compound was then put forward to palladium-induced cross-coupling reaction.

Antiproliferative activities of the synthesized compounds were tested against human colon carcinoma and murine leukemia cell lines. At micromolar concentration, the synthesized derivatives were detected to have moderate activity.

Chabane et al. investigated the preparation of 2-cyanothiazolocarbazoles analogues of ellipticine [143]. The preparation routes are depicted in Figures 4.37 and 4.38. At room temperature, the aminocarbazole was condensed with 4,5-dichloro-1,2,3,-dithiazolium chloride in dichloromethane, pursued by the pyridine addition, to produce the imino-1,2,3-dithiazolocarbazole. The desired product **48** was obtained from imino-1,2,3-dithiazolocarbazole (Figure 4.37).

From various 3-aminocarbazoles **49**, the demethylated analogues of ellipticine were synthesized through the appropriate imino-1,2,3-dithiazoles **50**. The minor products, linear thiazolocarbazoles **52** and their angular counterparts (**51**), the major products, were obtained after thermolysis (Figure 4.38).

4.10 Elinafide

The bis-intercalator, elinafide was prepared using naphthalimide pharmacophore [144]. It has anticancer activity [145] and has shown anti-neoplastic activeness in breast

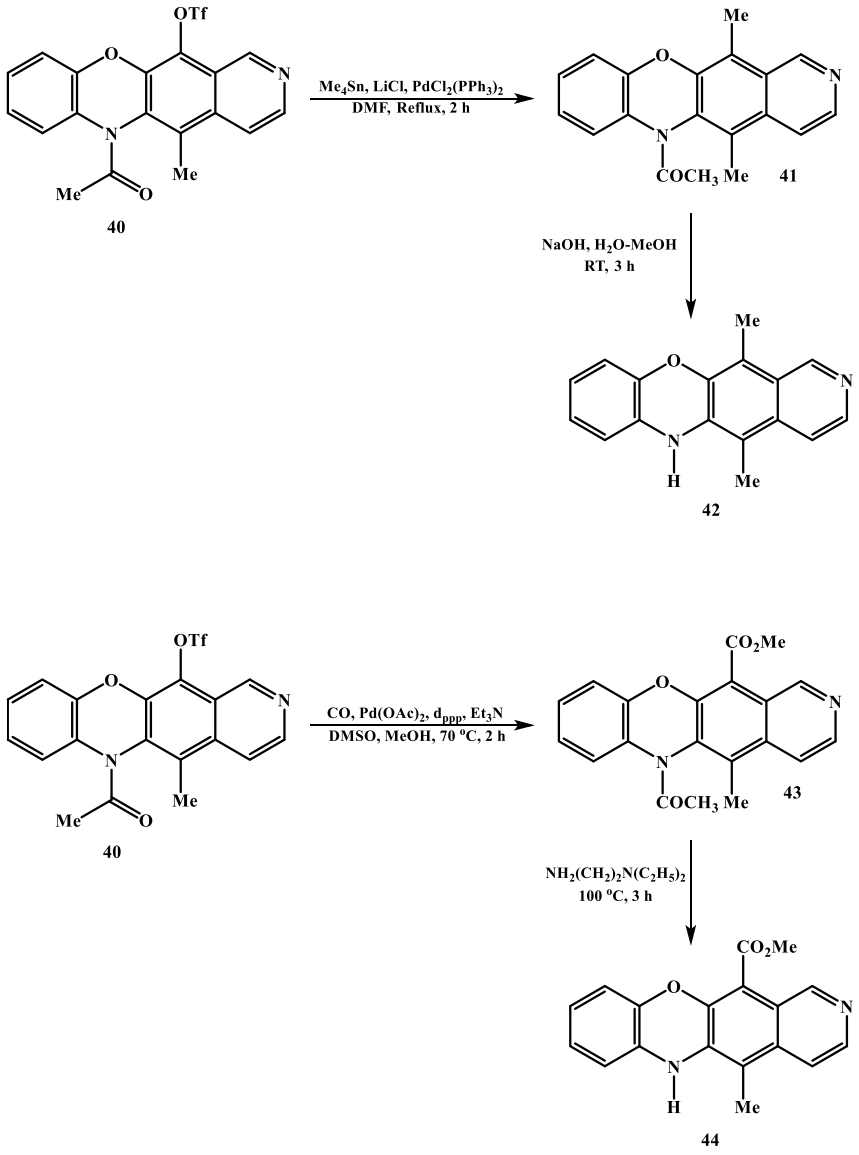

Figure 4.36: Synthesis of ellipticine analogues.

cancer, mesothelioma, and ovarian cancer. The chemical structure of Elinafide is depicted in Figure 4.39.

Studies demonstrated that elinafide and its nitro-substituted and amino-substituted analogues (Figure 4.40) bisintercalate into DNA [146]. The interaction takes place *via* the major groove and is sequence-specific.

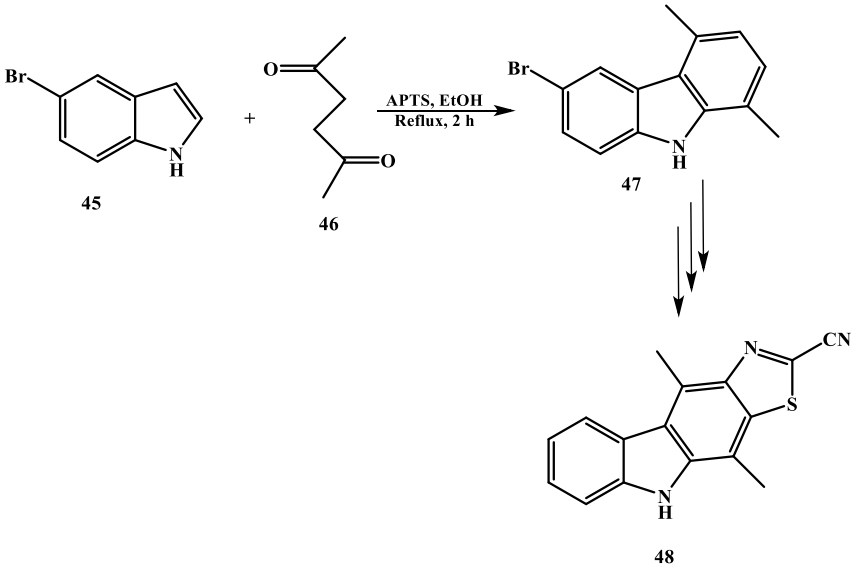

Figure 4.37: Synthesis of ellipticine analogues.

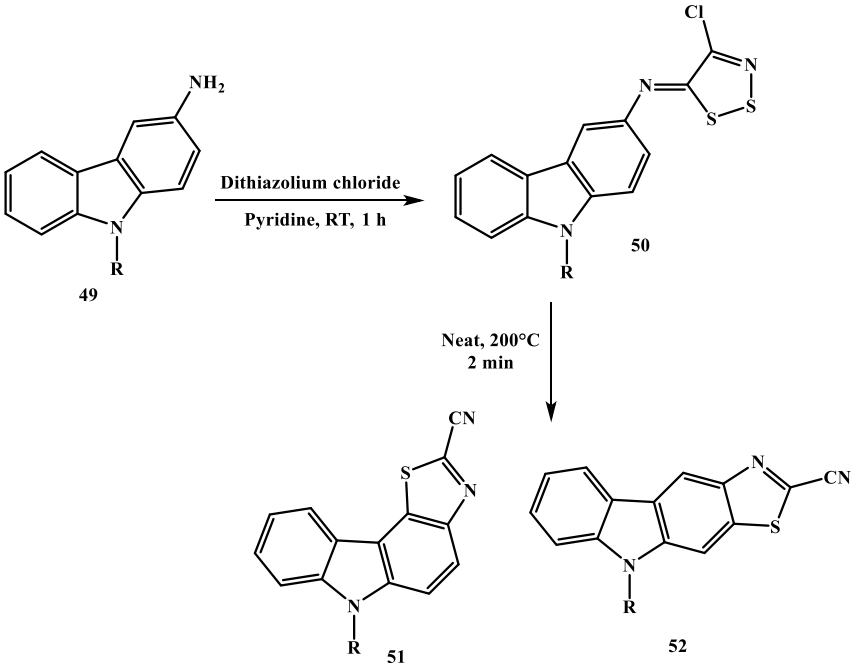

Figure 4.38: Synthesis of ellipticine analogues.

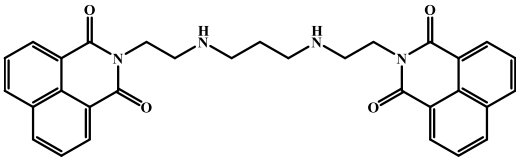

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Figure 4.39: Molecular structure of elinafide.

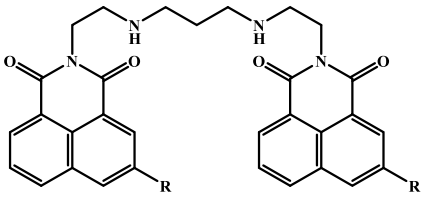

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Figure 4.40: Nitro- and amino-substituted analogues of elinafide.

Synthesis, DNA binding properties, anticancer activity, and molecular patterning of analogues of elinafide and amonafide, containing aromatic heterocycles were reported [147]. By the insertion of a thiophene ring or π-excedent furan fused to the naphthalimide moiety has modified the structure of amonafide and elinafide. The synthesized compounds showed an interesting antitumor profile, against human colon carcinoma cells, some compounds were more than 2 fold active than elinafide. The theoretical analysis demonstrated that the prepared compounds are able to form stable DNA complexes.

4.11 Echinomycin

It is an antitumor antibiotic, mostly extracted from *S. echinatus*. Echinomycin has two quinoxaline chromophores connected to a cyclic octadepsipeptide ring, having a thioacetal cross-bridge (Figure 4.41). This compound has gone to various clinical examines due to its powerful antitumor activity, [148]. However, it showed high toxicity in some cases. Echinomycin is a very active suppresser of the bonding of hypoxia-inducible factor 1 to DNA. Hypoxia-inducible factor 1 is a transcription factor that governs genes needed in processes crucial for tumor metastasis and progression, including migration, invasion, and angiogenesis.

With CG selectivity, both the echinomycin quinoxaline rings bisintercalate into DNA. But the inner portion of the depsipeptide forms hydrogen bonds with the DNA bases of the minor groove area of the two base pairs constituted among the chromophores. By hydrophobic interactions, the complex is stabilized. In stabilizing the complex, the direct molecular recognition among the echinomycin and DNA, mediated by van der Waals contacts and hydrogen bonding has a crucial role.

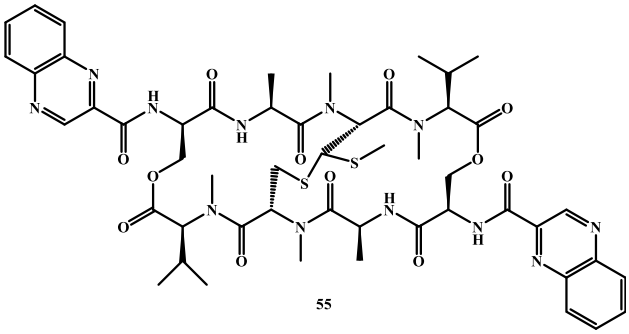


Figure 4.41: Molecular structure of echinomycin.

The preparation and anticancer activity of quinoxaline antibiotics of echinomycin analogs were reported (Figure 4.42) [149]. The new compounds were having the methylenedithioether bridge which was obtained by the introduction of methylene moiety between the –S–S– bond. Some compounds showed notable cytotoxicities against various human cancer cells.

Acute myeloid leukemia (AML) is generally chemo-resistant and frequently relapses adopting chemotherapy-induced remission. Considering the possible role of cancer stem cells in relapse, aiming the leukemia-originating cell in acute myeloid leukemia may give bettered outcomes adopting remission induction. At the same time, because of the overlap in their self-renewal process with normal hematopoietic stem cells, therapeutical aiming of the leukemia-initiating cell may have a contrary effect on long-term hematopoietic recovery. Wang et al. applied a mouse model of relapsed acute myeloid leukemia to investigate whether the hypoxiainducible factor 1 inhibitor echinomycin can be employed for the treatment of relapsed acute myeloid leukemia without impacting the host hematopoietic stem cells [150]. The study showed that these drugs healed 40–60% of mice transplanted with relapsed acute myeloid leukemia. Furthermore, echinomycin seemed to totally remove leukemia-initiating cells in mice

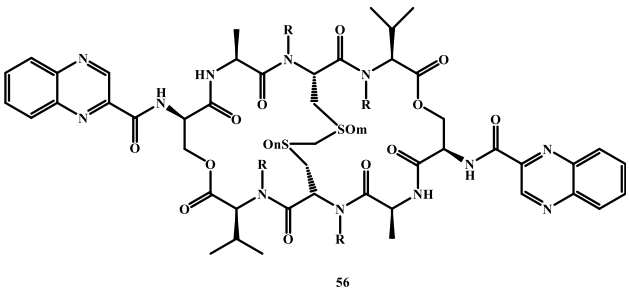


Figure 4.42: Molecular structure of echinomycin analogs.

with complete remission, as no leukemia could be spread *in vivo* following serial transplantation. Thus, small-dose echinomycin in a selective manner targeted leukemia-originating cells and spares normal hematopoiesis.

Park et al. reported that the relevant factors in echinomycin-induced apoptosis are the caspase-3 and ERK activation in the last apoptosis pathway and the release of *cytochrome* c [151]. Dose-dependent apoptotic cell death in HT-29 cells induced by ehinomycin is shown in Figure 4.43. A high dose echinomycin treatment-induced necrosis. Approximately 50% of counted cells seemed to be apoptotic after 24 h of echinomycin (2 μg/mL) exposure.

A schematic of the signal transduction cascade that liaises echinomycin-accelerated cell death in HT-29 cells is shown in Figure 4.44.

4.11.1 DNA-binding metallo-intercalators

Metallo-intercalators are mostly positively charged aromatic, planar, and polycyclic compounds. These intercalators can separate the double helix of DNA without replacing or expelling the original nitrogenous bases. It can also insert itself between DNA base pairs [152]. At the same time, at the site of intercalation, the hydrogen bonds between the nitrogenous bases remain intact [152–154]. Hydrophobic, van der Waals, entropic, and electrostatic interactions together with π-stacking between the nitrogenous bases of DNA and the aromatic regions of the intercalator stabilize the intercalation. Because of the capability to interact with specific DNA base pairs, metallo-intercalators possess lots of therapeutic applications.

After entering through the groove (mostly minor groove), these intercalators π-stack with DNA base pairs which are unbroken. Intercalation produces a very low strain in the DNA duplex compared to insertion. Intercalators increase the major groove's width [152]. The melting temperature of the DNA duplex lifts as the metal compound's intercalation among the DNA base pairs stabilizes the double helix. In the

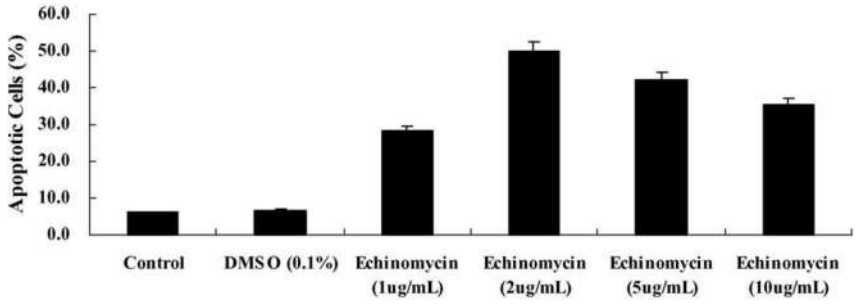


Figure 4.43: Echinomycin-induced cell death. The cancer cell lines were treated with diverse amounts of echinomycin. Adapted with permission from [151].

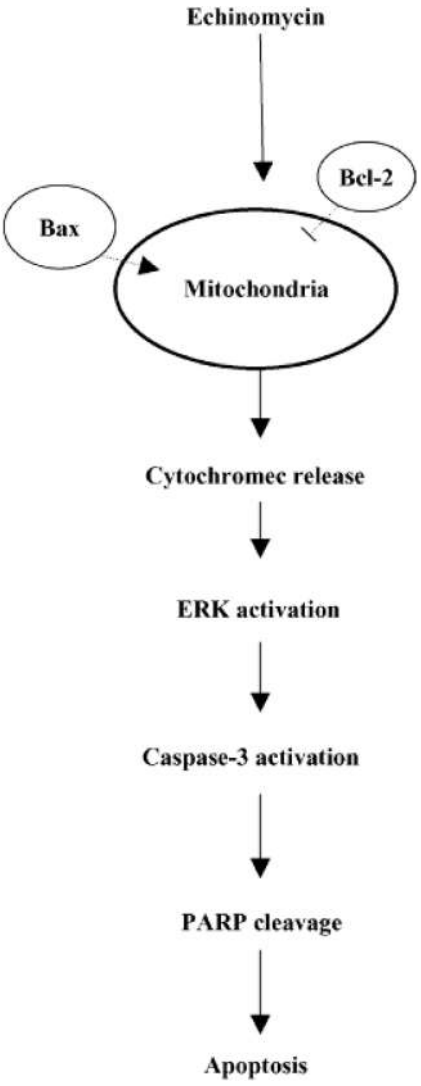


Figure 4.44: Signaling pathway in echinomycin-induced cell death. Adapted with permission from [151].

case of intercalators based on metal compounds, binding to DNA is a reversible process and completely counts on the characteristics of the intercalating compound. Intercalators based on metal compounds exhibit distinct depths of insertion as they have various metal centers, coordination geometries, overall sizes, and oxidation states [154]. For instance, compared to tetrahedral or octahedral complexes, square planar complexes get through thicker into the DNA base pairs [154]. Because of the electrostatic attraction to the sugar-phosphate backbone (negatively charged), the positive charges on these intercalators fortify binding towards DNA [155].

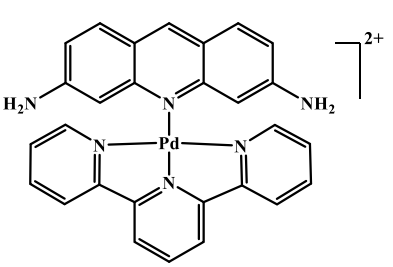
Intercalators based on metal complexes have diversity in structure as well as photooxidative effects and thus used in various therapeutic applications. These intercalators fight with diseased cells within the body by aiming at particular mismatched DNA base pairs. It also has the power to alter the ligands to bind to the metal center admits for an eminent level of particularity in the binding interactions among the DNA sequence and the intercalator [156–158].

Synthesis of metallo-intercalator is a multistep process. For example, in ruthenium-based intercalators, the synthesis mainly comprises of intercalative ligands preparation and then pairing them to a specific ancillary ligand coordinated ruthenium metal complex. The *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ and *cis*-$[Ru(bpy)_2Cl_2]$, which can be synthesized into $Ru(phen)_2(bfipH)](ClO_4)_2$, $[Ru(bpy)_2(paip)]^2$, $[Ru(bpy)_2(maip)]^{2+}$, and $[Ru(bpy)_2(bfipH)](ClO_4)_2$, are the examples for ruthenium complexes which are employed as precursors for metallo-intercalators [159, 160].

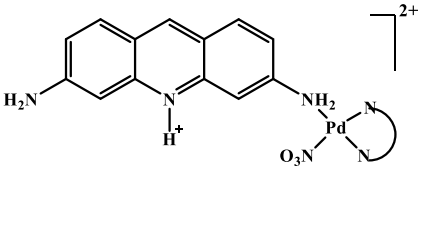
It was also reported that metal complexes with proflavine ligands also exhibit potential cytotoxic activity. For example, Polyanskaya et al. investigated the preparation, anticancer activity of palladium (II)-proflavine complexes [161]. The complex $[Pd(terpy)(proflavine)](NO_3)(HSO_4)\cdot 3H_2O$, (**57**) was formed by the reaction of proflavine with $[Pd(terpy)(NO_3)](NO_3)$ in water. In this case, the proflavine was connected to the Pd(II) through the N10 atom as shown in Figure 4.45. The proposed structure for $[Pd(en)(proflavineH))(NO_3)](SO_4)$, complex is shown in Figure 4.45.

In the solid-state, the complex $[Pd(proflavineH)Cl_2](SO_4)_{0.5}\cdot H_2O$ seemed to be polymeric with a 1:1 mol ratio of proflavine:Pd(II). When placed in DMSO, two unique forms were originated as shown in Figure 4.46. The complexes were tested against ovarian cancer cells and two types of breast cancer cells and the result demonstrated that compounds **57** and **59** had important activity.

In the preparation of metal complexes of drugs, the selection of the metal ion majorly relies on their DNA binding capabilities and metabolic actions. For example, because of the significant binding activities, Pt(II) has several applications. Another choice is Cu(II), which is an extremely important biocompatible ion. To maintain a living organism's metabolic activities Cu(II) has several functions in redox reactions



57



58

Figure 4.45: Structure of palladium complexes with proflavine.

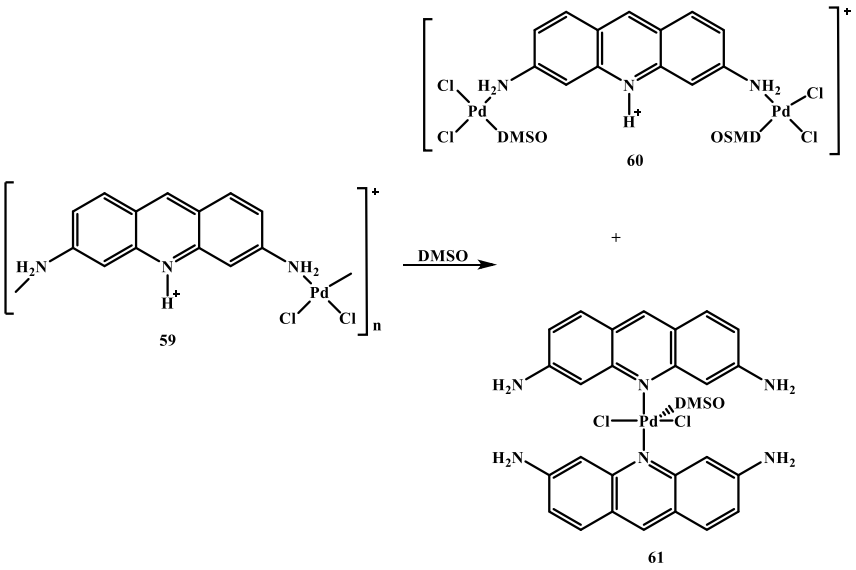


Figure 4.46: Decomposition of the complex.

and metabolic actions [162]. The synthesis of epirubicin complex based on Cu(II) as a drug candidate for usage in chemotherapeutic experiments was reported [163]. The proposed molecular structure of the metal complex is depicted in Figure 4.47.

The structural characterizations of the compound were performed using methods like FT-IR, ultraviolet–visible, ICP-OES, and LC-MS/MS. The MTT analysis was executed on the L929 fibroblast cells for the antiproliferative studies. A comparison of the activity of the metal complex and EPR was also performed. The results pointed the metal complex might be a new promising drug candidate. The in-silico DNA–ligand interaction works demonstrated that the metal complex and EPR, are groove binders (Figure 4.48). At the same time, to maintain the stability of the compound-DNA complexes, the H-bond interactions have acted an important role.

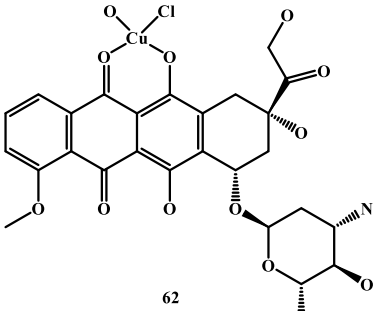


Figure 4.47: The proposed molecular structure.

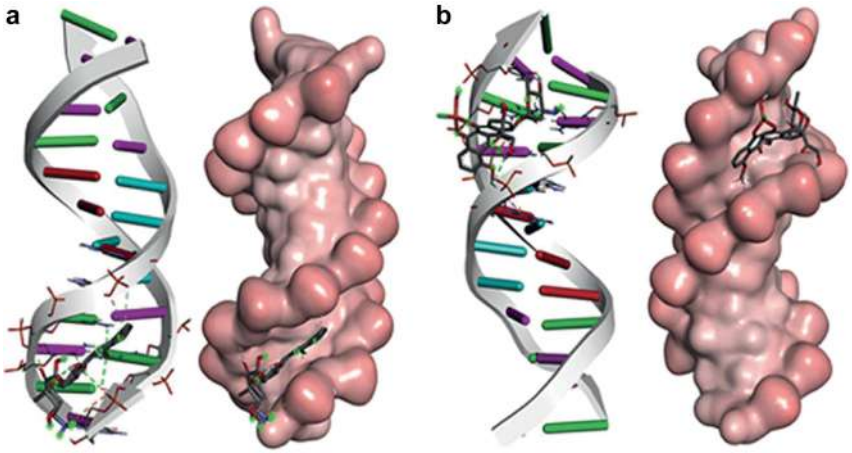


Figure 4.48: Molecular docked structure of EPR (a) and metal complex (b) connected with DNA. Adapted with permission from [163].

4.12 Conclusions

Worldwide, cancer is noted as a major medical problem. It is the second leading cause of death. Because of the urgency in the anticancer drugs with fewer side effects, significant studies are going on in this field. In the article, we discussed the anticancer activity of 10 intensively studied heterocyclic DNA intercalators. Mostly we presented the synthesis and anticancer activities of proflavine, ethidium bromide, doxorubicin, dactinomycin, bleomycin, epirubicin, mitoxantrone, ellipticine, elinafide, and echinomycin. Besides, some examples of the metallo-intercalators are presented at the end of the chapter. It is really important to point out that the above molecules demonstrate DNA intercalator properties although they may work through different other mechanisms against diverse cancers. Appropriate enzyme inhibition seems to be a major pathway along with the mechanism through apoptosis. These molecules are structurally diverse and therefore, no generalizations or assumptions on their preference as anticancer agents can be made. However, a number of other chemical modifications on these parent drugs are possible to increase their activity and decrease toxicity. Ultrasonic and magnetic exposures of some of these agents may become helpful in treating cancers. Moreover, a combination therapy works better in most instances revealing the dual roles of these drugs. We anticipate that more studies will appear, based on this current contribution, for the synthesis of novel anticancer drugs with fewer side effects.

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5 Recent advances of heterocycle based anticancer hybrids

Abstract: Cancer is one of the major causes of death across the world. Cancer is a broad word that encompasses a wide range of illnesses that can affect any part of the body. Cancer research has increased understanding of molecular biology and cellular biology, resulting in new cancer therapies. Despite of adverse effects, surgery, radiation, and anticancer medicines are the modern cancer treatments. Keeping in mind the excellent anticancer activity exhibited by various heterocyclics, various medicines with heterocyclic moiety have been developed to identify particular target regions. The chapter aims to discuss new discoveries in the field of anticancer pharmaceuticals comprising the thiazole, pyrazole, oxazole, and triazole rings over the last five years. The proposed anticancer drugs have a lot of future significance due to their high potency.

Keywords: anti-cancer; heterocycles; oxazole; pyrazole; thiazole; triazole and structural activity relationships.

5.1 Introduction

Heterocyclic compounds are abundant in nature and are essential to life; they play an important part in the metabolism of all living cells [1]. The following are some examples of heterocyclic compounds: the essential amino acids tryptophan, histidine, and proline; the vitamins and coenzyme precursors pyridoxine, thiamine, riboflavine, biotin, and folic acid; the vitamins B_{12} and E families of vitamins; the photosynthetic pigment chlorophyll; the oxygen transporting pigment hemoglobin, and its breakdown products, the bile pigments; the biological hormones and so many other biological molecules [2]. There are several pharmacologically active heterocyclic compounds in the market, many of which are currently used in clinical practice. Antibiotics like cephalosporin and penicillin, alkaloids like ellipticine, vinblastine, reserpine, and morphine, and cardiac glycosides like digitalis are the examples of natural compounds containing heterocyclic rings [3].

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The majority of synthetic heterocyclics, on the other hand, have found widespread application as anticancer agents [4], analgesics, analeptics, hypnotics, and vasopressor modifiers, as well as insecticides, pesticides, rodenticides, weedkillers, and antioxidants [5, 6]. Their increasing application in the synthesis of exclusively functionalized non-heterocyclic structures is another consequence of their diverse chemical reactivity, which includes the potential breakdown of the heterocyclic ring [7, 8]. Because of the obvious applications of chemicals formed from heterocyclic rings in pharmacology, health, agriculture, and other sectors, heterocyclic chemistry is a significant and developing area of chemistry. Furthermore, during the last two decades, drug discovery has undergone significant changes, with an increase in biological screening of putative drug candidates, resulting in an ever-growing demand for innovative drug-like molecules [9–12].

Cancer is one of the leading causes of death in the contemporary era, with almost 3.5 billion people expected to be impacted by the disease by 2050 [13]. Cancer is a pathological condition defined by uncontrolled and increased cell division among certain tissue types, resulting in cysts, lesions, and tumors [14]. Heterocyclic compounds act as key structural component of anticancer drugs used for mankind. Some of the FDA-approved anticancer medicines with heterocyclic rings are listed below Figure 5.1.

Considering remarkable anticancer activity exhibited by various heterocyclic conjugates, the chapter particularly focusses on recent developments made in the field

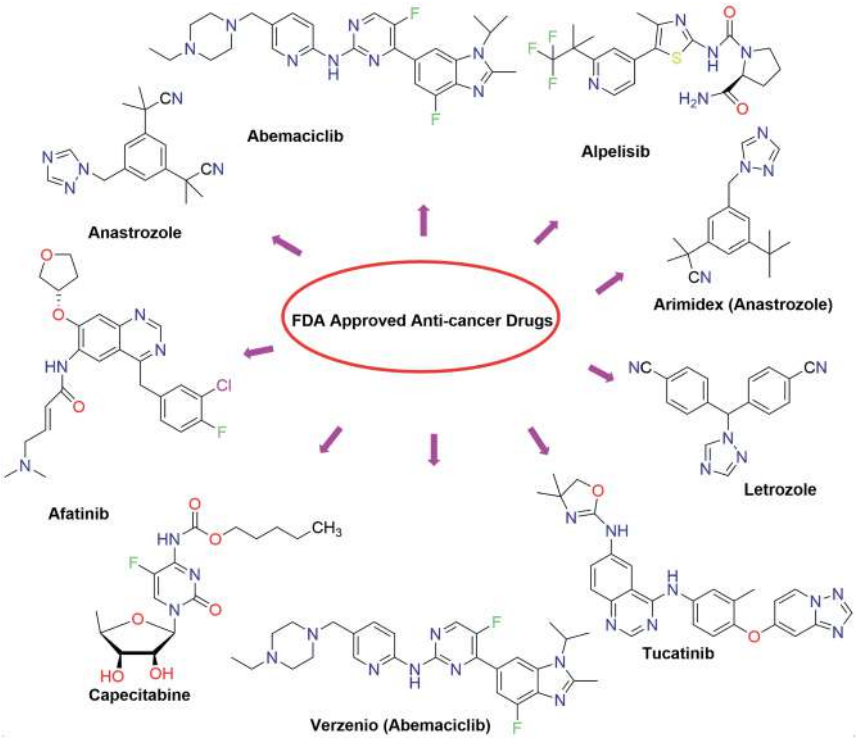


Figure 5.1: Some FDA-approved anticancer medicines with heterocyclic rings are listed below.

of anticancer therapeutic agents comprising the thiazole, pyrazole, oxazole, and triazole rings over the last five years.

5.2 Anticancer activity discovered in recently created hybrids

5.2.1 Few oxadiazole ring containing drugs

Oxadiazole is a five membered heterocyclic moiety containing two nitrogen atoms and one oxygen in addition to two carbons [15]. Oxadiazole derivatives have reported to show wide range of profile such as antiviral, antibacterial, antitumor, antioxidant, antitubercular, antiAlzheimer activity, antidepressant, antiinflammatory, CNS stimulants and other important biological therapeutic properties. Various drugs containing oxadiazole ring have been approved by FDA [16]. Raltegravir, an oxadiazole derivative is HIV integrase inhibitor used to treat HIV/AIDs as combination with other medication. Nesapidil is an oxadiazole based drug which is used to treat abnormal heart rhythms. Furamizole is antibacterial drug also containing oxadiazole ring. Tiodazosin is another drug having oxadiazole embedded in it used to treat stress and hypertension. Zebotentan is an anticancer drug which is endothelin A receptor antagonist. Continuous efforts have been made by various medicinal chemists to further explore anticancer activity of oxadiazole derivatives [17].

Amides and sulfonamide derivatives having oxadiazole ring were synthesized and *in vitro* screening against prostate cancer cell lines was performed by Mochana and team. All derived compounds (Figure 5.2) showed markable anticancer activity whereas

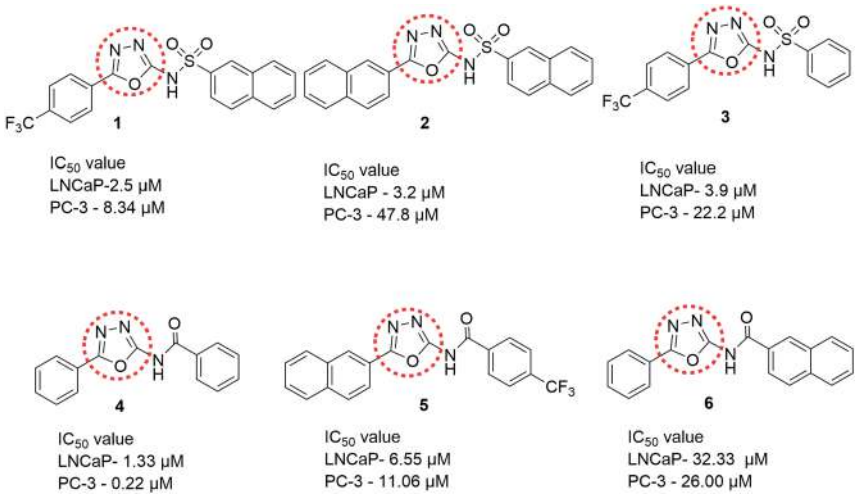
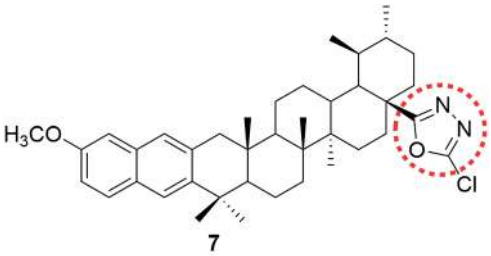


Figure 5.2: Anticancer hybrids based on amides and sulfonamide derivatives having oxadiazole ring.

compound **4** showed maximum potency by showing induced apoptosis and shifting the cells to the sub G0/G1 and S phase [18].

Gu and coworkers developed oxadiazole–ursolic acid hybrids and evaluated cytotoxicity against MDA-MB-231, HeLa, and SMMC-7721. The various hybrids reported (Figure 5.3) showed appreciable anticancer activity against investigated cell lines. Compound **7** was found to show appreciable activity against all three cell lines by cycle arrest of MDA-MB-231 cells at G0/G1 phase. Substituents resulting in greater polarity like introduction of alkyl chains at R^2 position with hydroxyl group, aliphatic or aromatic amines, or heterocycles having larger polarity on ursolic derivatives showed appreciable cytotoxic effect. Moreover, presence of carbonyl groups helped to reduce its cytotoxicity [19].

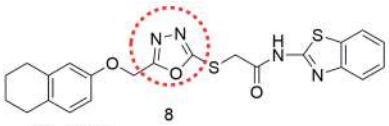
Altıntop et al. prepared a series of oxadiazole based Akt and FAK inhibitors and evaluated their cytotoxicity through *in vivo* studies by MTT assay approach. Also,



7

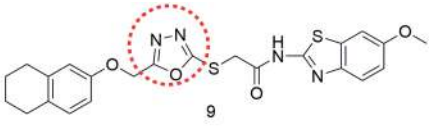
IC_{50} Values
MDA-MB-231- 8.35 ± 0.25 μM
HeLa 7.11 ± 0.13 μM
SMMC-7721- 20.24 ± 0.43 μM

Figure 5.3: Oxadiazole–ursolic acid hybrids showing anticancer activity.



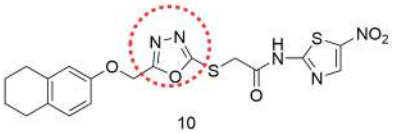
8

IC_{50} value
C6 cell line- 89.33 ± 14.47 μM
A549 cell line- 91.67 ± 7.64 μM
NIH/3T3 cell line- 66.67 ± 12.58 μM



9

IC_{50} value
C6 cell line- 4.63 ± 0.85 μM
A549 cell line- 39.33 ± 4.04 μM
NIH/3T3 cell line- 283.33 ± 5.00 μM



10

IC_{50} value
C6 cell line- 44.50 ± 3.53 μM
A549 cell line- 138.33 ± 45.37 μM
NIH/3T3 cell line- > 403.00 μM

Figure 5.4: Oxadiazole based Akt and FAK inhibitors.

hydrogen bonding with various receptors was examined through *in silico* studies. Among the derived compounds (Figure 5.4), compounds **8–10** were found to be lead derivatives with remarkable anticancer activity against C6 cell line, A549 cell line (Akt inhibition), and NIH/3T3 cell lines (FAK inhibition). Compounds **8** and **9** showed greater anticancer activity than cisplatin therefore, have found their way towards clinical trials studies. It was revealed that substitution of chlorine at 6th position of benzothiazole decreased its activity whereas nitro substituent at the 5th position of the thiazole ring increased the efficiency of hybrids [20].

Increasing anticancer drugs resistance and lesser selectivity against cancer cells, fascinated another group of workers to build a series of benzimidazole–oxadiazole derivatives and evaluated their anticancer potency and cytotoxicity in A549 and HepG2 cell lines by using the MTT assay. Compounds **11–13** were reported to show potent activity against HepG2 cell lines by inducing cell death by apoptosis (Figure 5.5). However, these compounds did not inhibit topoisomerase I enzyme [21].

Another set of oxadiazole based compounds were designed by Hamdy and team workers. The designed compounds were subjected to *in vitro* studies to evaluate their pro-apoptotic Bcl-2 inhibition. The studies revealed that compounds with oxadiazole directly bound to aryl ring have greater potency potential. Compound **14** (Figure 5.6) was found to display highest anticancer activity which was demonstrated through *in silico* studies in which Bcl-2 ELISA binding was evaluated [22].

Figure 5.7 gives a brief study about structural activity relationship (SAR) studies of thiazole based anticancer derivatives.

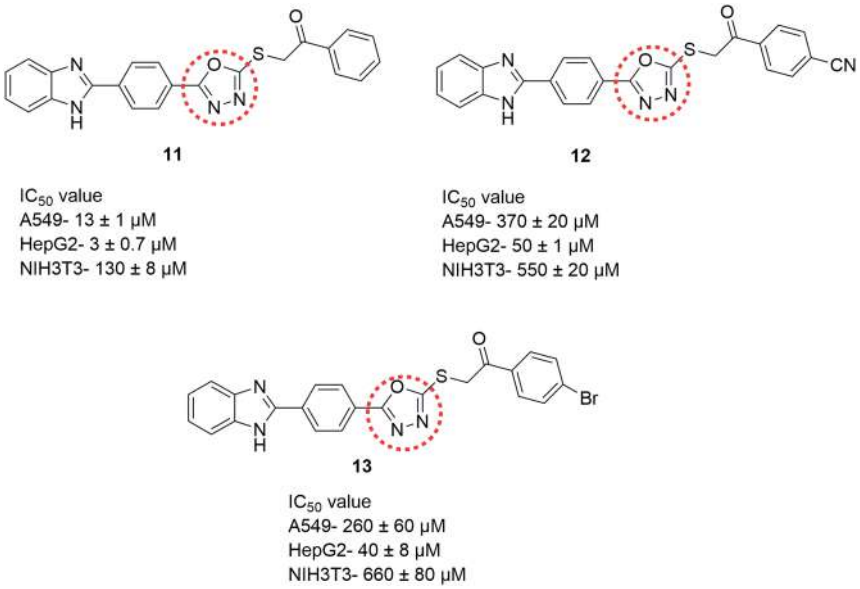
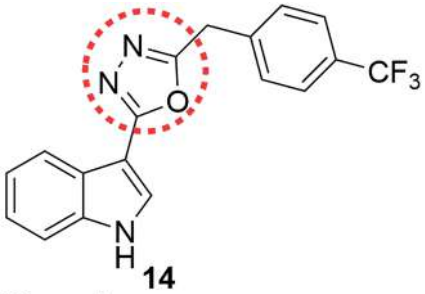


Figure 5.5: Benzimidazole–oxadiazole derivatives.



14

IC_{50} value
MDA-MB-231- 0.52 ± 0.15 mM
HeLa - 0.88 ± 0.16 mM
KG1a - 0.73± 0.09 mM
Jurkat - > 100 mM

Figure 5.6: Oxadiazole based other anticancer hybrids.

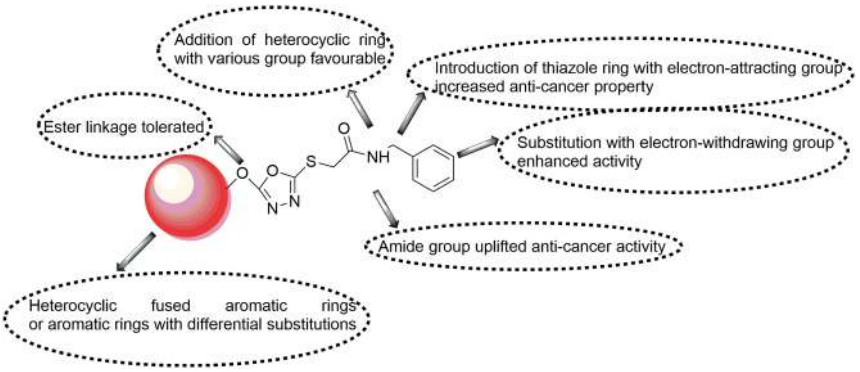


Figure 5.7: Structural activity relationship (SAR) studies of thiazole based anticancer derivatives.

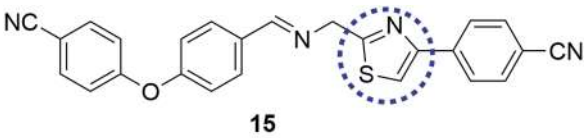
5.2.2 Recently developed anticancer agents based on thiazole scaffold

Thiazole is a heterocyclic chemical molecule having the molecular formula C_3H_3NS and a five-membered molecular ring structure [23–25]. It has an electron-donating (-S-) as well as an electron-accepting (C=N) group. It is a heterocyclic molecule that is stable in nature. Thiazole is aromatic because the lone pair of electrons from the sulfur atom delocalize, completing the required six p electrons to satisfy Huckel's rule. A variety of new compounds with a broad spectrum of pharmacological properties such as anti-bacterial, antioxidant, antifungal, diuretic, antitubercular, antiinflammatory, and anticancer effects have resulted from diverse changes of the thiazole ring at various structural locations [26, 27]. Pramipexole containing a 2-amino-thiazole moiety fused

to a cyclohexane ring, which is an isostere to dopamine's catechol ring, showed dopamine D2 agonist action. Riluzole, a recently approved neuroprotective medication for the treatment of amyotrophic lateral sclerosis, likewise bears aminothiazole moiety. In addition, Alanine et al. have created a benzothiazole analog as a strong adenosine A2AR antagonist for Parkinson's disease treatment that is currently in phase II clinical studies [28]. Several thiazole analogs were created in the search for more efficient adenosine A2AR antagonists. Amino thiazole analogs have also been identified as potential dopamine receptor agonists. Neuroprotective drugs such as tetrahydrobenzo thiazoles [29], benzothiazoles [30], and phenolic thiazoles [31] have been studied extensively. Furthermore, the glutamate receptor antagonist activity of ethynyl thiazole [32] analogs and pyrimidyl thiazole [33] analogs was found to be the best for the management of anxiety disorders. Aside from butyl thiazole analogs [34], imidazolo thiazole analogs [35], 2-amino thiazole analogs [36], and triazole linked thiazole derivatives [37], the anticonvulsant activity of riluzole derivatives [38], thiazolesemicarbazides [39], thiazolepyridons [40], and thiazole carboxamide [41] derivatives seemed promising candidates for further development.

Considering tremendous biological profile of thiazole scaffold, medicinal chemists developed various compounds by fusing thiazole with various rings to build potent anticancer derivatives. Altıntop et al. developed thiosemicarbazone based thiazole derivatives, studied their anticancer activity and cytoxicity on A549 human lung adenocarcinoma and C6 rat glioma cell lines. Compound **15** (Figure 5.8) was reported to be show maximum potency. Tyr272 residue at active binding site of Akt was found to undergo π–π stacking interaction with compound **15**. Addition of hydroxyl group on 4th position of thiazole resulted in upgraded anticancer activity [42].

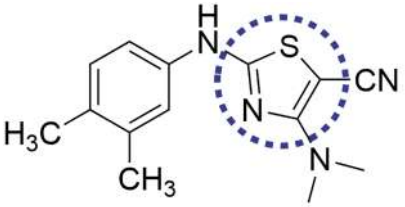
Another group of authors developed various thiazole scaffold containing hybrids. The synthesized compounds were studied through *in vitro* studies against Bcl-2-Jurkat, A-431 cancerous cell lines and ARPE-19 cell lines. Compound **16** exhibited magnificent potency comparable to doxorubicin (Figure 5.9) by undergoing hydrophobic and hydrogen bonding interactions with glycine 104 in Bcl-2 protein (Xp G-score = –4.151 kcal/mol) [43].



15

IC_{50} value-
A549 Cell Line- 12 ± 1.73 µg/mL
C6 Cell Line- 3.83 ± 0.76 µg/mL
NIH/3T3 Cell Line - > 50 µg/mL

Figure 5.8: Thiosemicarbazone based thiazole containing anticancer derivatives.



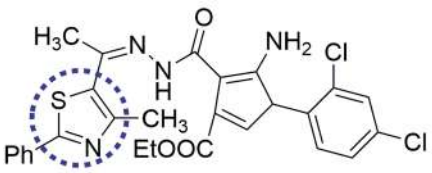
16

IC_{50} value-
Jurkat Bcl-2 - 34.77 µM
A-431 - 34.31 µM

Figure 5.9: Thiazole based anticancer derivatives.

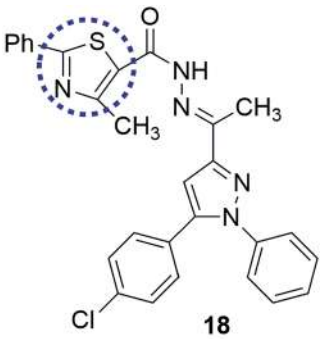
Melha and team applied green chemistry approach to build up thiazole-based derivatives and evaluated their anticancer inhibition through *in vitro* studies conducted against HCT-116, HepG-2 and MCF-7 cell lines. Substitution of chlorine atom at 4th and 2nd position at aryl ring enhanced anticancer activity of synthesized compounds. Moreover, SAR studies reported that substitution at one aryl ring reduced cytotoxicity whereas substitution on both the aryl rings completely nullified cytotoxicity. Compound **17** and **18** (Figure 5.10) was assessed to show maximum anticancer inhibition on studied cell lines [44].

Shokrollahi and coworkers synthesized 4,5,6,7- tetrahydrobenzo[d]thiazole-based schiff-bases and monitored their cytotoxicity studies on MCF-7 and HepG2 cell lines by an MTT-based assay. The interactions of prepared Schiff base were examined and



17

IC_{50} value
HepG-2 - 7.4 ± 0.2 µg/mL
HCT-116 - 11.8 ± 0.5 µg/mL
MCF-7 - 3.77 ± 0.2 µg/mL



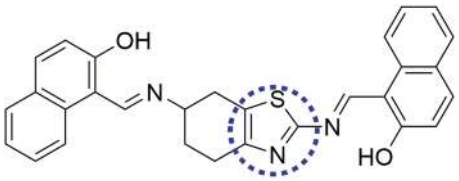
18

IC_{50} value
HepG-2 - 4.24 ± 0.3 µg/mL
HCT-116 - 7.35 ± 0.4 µg/mL
MCF-7 - 2.99 ± 0.2 µg/mL

Figure 5.10: Thiazole based anticancer derivatives synthesized by using green chemistry approach.

compound **19** (Figure 5.11) was found to bind effectively with human serum albumin by undergoing hydrophobic and hydrogen bonding interactions [45].

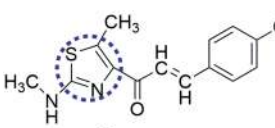
Thiazole-based-chalcones and 4-heteroarylthiazoles were discovered and their anticancer activity as studied by Farghaly and team. Compounds **20–23** were found to show activity greater than doxorubicin on HepG-2, A549 and MCF-7 cell lines (Figure 5.12). Compounds **20–23** were found to show apoptosis induction potential by fitting efficiently in the ATP binding site of CDK1 enzyme. Thiazole bonded with pyrimidines was found to show equivalent activity like chalcones. However, substitution in the pyrimidine nucleus in the S-alkylated derivatives decreased its anticancer activity [46].



19

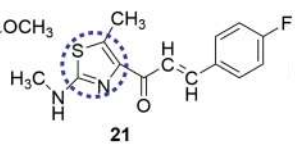
IC_{50} value-
MCF-7 - 7.75 ± 3.70 μM
HepG2 - 34.52 ± 2.01 μM

Figure 5.11: Thiazole-based Schiff-bases exhibiting anticancer activity.



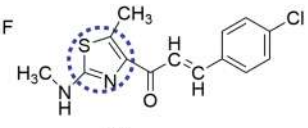
20

IC_{50} value
HepG-2 - 1.56 ± 0.07 μM
A549 - 1.39 ± 0.061 μM
MCF-7 - 1.97 ± 0.09 μM



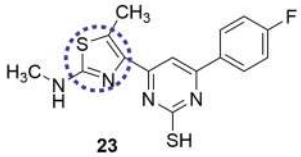
21

IC_{50} value
HepG-2 - 3.79 ± 0.21 μM
A549 - 3.17 ± 0.151 μM
MCF-7 - 4.14 ± 0.35 μM



22

IC_{50} value
HepG-2 - 2.29 ± 0.11 μM
A549 - 2.01 ± 0.1 μM
MCF-7- 2.38 ± 0.14 μM



23

IC_{50} value
HepG-2 - 3.5 ± 0.19 μM
A549 - 2.37 ± 0.12 μM
MCF-7 - 4.11 ± 0.3 μM

Figure 5.12: Thiazole-based-chalcones and 4-heteroarylthiazoles hybrids showing anticancer activity.

Figure 5.13 gives brief elucidation of structural activity relationship of thiazole based anticancer hybrids.

5.2.3 Recently developed pyrrole based anticancer agents

Heterocyclic compounds containing nitrogen and their derivatives have been recognized as medicinal agents [47, 48]. Pyrazole, with two nitrogen atoms and an aromatic property, is a five-membered ring structure that enables a wide range of functionality and stereochemical complexity. Various pyrazole derivatives and their many physiological and pharmacological effects have been the subject of numerous investigations in the last decade. Part of the goal of these ongoing researches was to uncover the vast variety of drug-like characteristics of pyrazole derivatives, as well as their structure–activity correlations, so that they might be used to their maximum potential [49, 50]. Pyrazole containing marketed drugs include celecoxib which inhibits cyclocooxygenase (COX-2) and therefore shows antiinflammatory properties; rimonabant is a cannabinoid receptor that is used to treat obesity; fomepizole inhibits alcohol dehydrogenase; and sildenafil inhibits phosphodiesterase-2, tepoxalin is used as nonsteroidal antiinflammatory drug, Betazole acts as histamine H2 receptors [51].

Pyrazole carbothioamides based compounds were prepared to build a new series of anticancer derivatives. The derived compounds were evaluated by assessing their topoisomerase IIa inhibition. Also, cytotoxicity against MCF-7, NCI-H460, HeLa cancer cell lines and HEK-293T normal cell line were revealed in the study. Compound **24** was reported to be highly efficient by showing highest percentage of topoisomerase IIa inhibition (Figure 5.14). Further, molecular docking studies data also supported highest binding of compound **24** with targeted ligand by maximum docking score of –8.24 [52].

A series of trisubstituted derivatives containing pyrazole moiety were synthesized and screened against hepatocellular carcinoma, lung carcinoma and prostatic cancer cell lines. The reported compounds **25–26** were found to show appreciable anticancer activity (Figure 5.15) [53].

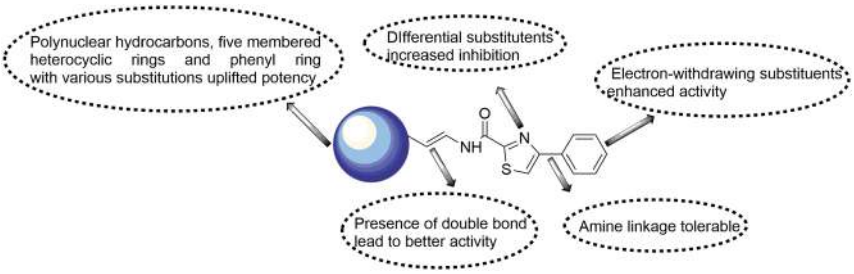
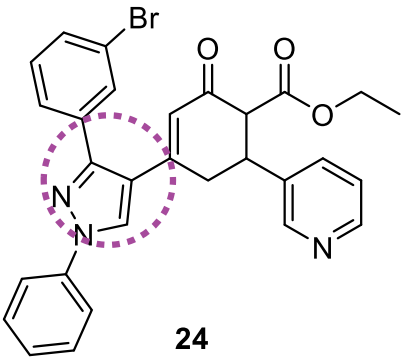


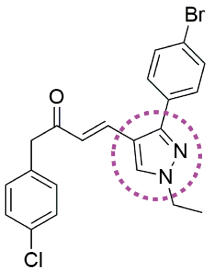
Figure 5.13: Structural activity relationship of thiazole based anticancer hybrids.



24

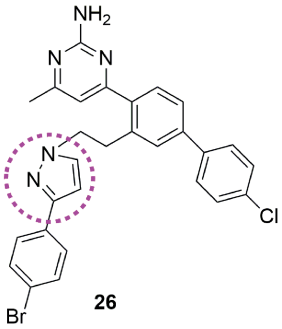
IC_{50} value
HEK-293T- 124.35 ± 3.74μM
MCF-7- 14.31 ± 0.90 μM
NCI-H460- 14.54 μM
HeLa - 7.01 ± 0.60 μM

Figure 5.14: Carbothioamides fused pyrazole hybrids exhibiting anticancer activity.



25

IC_{50} value
HepG2- 9.13 μM
MCF-7 - 29.35 μM
A549- 21.11 μM
PC3- 15.22 μM



26

IC_{50} value
HepG2- 9.78 μM
MCF-7 - 16.52 μM
A549- 6.52 μM
PC3- 9.13 μM

Figure 5.15: Pyrazole based anticancer derivatives.

Mukarram and team prepared a series of α,α-difluoro-β-hydroxycarbonyl pyrazole derivatives. The synthesized derivatives were screened by studying their COX inhibition on leukemia, breast cancer and lung cancer cell lines with methotrexate as standard drug. Compounds **27–29** were found to show maximum COX inhibition (Figure 5.16). Further, structural activity relationship studies revealed that acid and hydrazide derivatives with chlorine and fluorine on aromatic ring resulted to produce more potent anticancer derivatives as compared to ester derivatives [54].

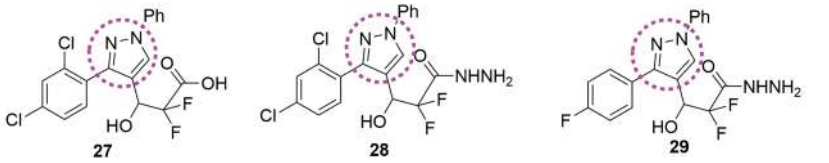


Figure 5.16: Difluoro-β-hydroxycarbonyl pyrazole derivatives showing anticancer activity.

A series of indole-based pyrazole containing derivatives were developed by Wang et al. The compounds were assessed on four cancer cell lines by using MTT assay. Compound **30** and compound **31** was found to be three times more potent than 5-fluorouracil on HGC-27, PC-3 cell lines and EC-109 cell lines respectively (Figure 5.17) [55].

Ran and co-workers developed pyrrole containing derivatives and studied their anticancer inhibition against mantle cell lymphoma cell lines. Compound **32** was reported to have highly potent as compared to brutinib standard drug (Figure 5.18). Further, it was confirmed that substitution with chloroacetyl group resulted in remarkable activity [56].

Sayed and team clubbed thiazole and pyrazole rings to prepare a series of anticancer derivatives. Few compounds were screened against human liver carcinoma cell line and were found to show significant activity comparable to doxorubicin standard drug. 4-pyridyl substitution resulted in greater activity than 4-phenyl thiazole-based derivatives. Further, presence of electron-donating groups on the phenyl ring in the aryl hydrazo-pyrazolone moiety resulted in greater anticancer activity. Compound **33**

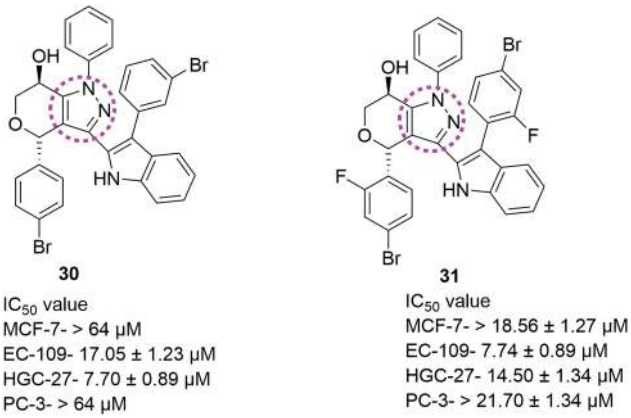


Figure 5.17: Anticancer hybrids containing indole and pyrazole fusion.

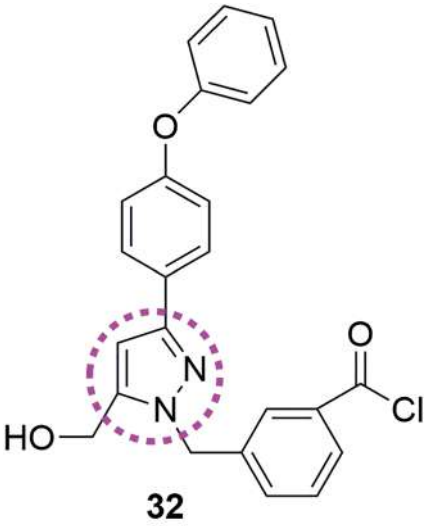


Figure 5.18: Pyrazole based anticancer hybrids.

was revealed to be emerging potent EGFR inhibitor which was confirmed by molecular docking studies (Figure 5.19) [57].

Another effort towards synthesis of anticancer agents was done by Thomas and coworkers by preparing two pyrazole compounds. The newly obtained compounds maximum showed inhibition on human microsomal prostaglandin E synthase 1 having affinity values of −6.0 and −6.4 kcal/mol by undergoing weak covalent interactions with the active site of substrate [58].

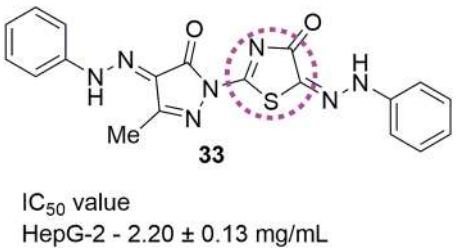


Figure 5.19: Thiazole and pyrazole fused anticancer agents.

Figure 5.20 includes brief structural activity relationship studies of pyrazole based anticancer compounds.

5.2.4 Newly fabricated triazole ring containing anticancer derivatives

The triazole nucleus is a frequent and fundamental characteristic of a number of natural products and therapeutic medicines containing three nitrogen atoms in five membered ring and it is one of the most significant and well-known heterocycles. Triazoles and their derivatives have established themselves as pharmacologically relevant scaffolds due to their wide and strong action [59–63]. Triazole and its derivatives have been the subject of much investigation, demonstrating the pharmacological significance of this heterocyclic nucleus. Triazole containing compounds are known to exhibit important biological activities like anticancer, antimigraine, antioxidant, antibacterial, antiseptic, antianxiety, antidepressant, and antihistaminic activity [64, 65]. Itraconazole and voriconazole are two of the many FDA approved antifungal drug containing triazole ring, moreover, triazole based compounds have also been found to act as potential agent against coronavirus [66].

Plethora of triazole based derivatives was prepared to explore potential of these three nitrogens containing five membered moieties. One such effort was made by Gregorić and team to build up pyrimidine integrated triazole containing hybrids using Sonogashira cross-coupling approach. Compound **34** emerged out as promising candidate by showing Wee-1 kinase inhibition and terminating sphingolipid signaling which is mediated by acid ceramidase and sphingosine kinase (Figure 5.21). Also, the compound was found to show negligible mitochondrial toxicity. The activity of derived compounds was examined on lung adenocarcinoma hepatocellular carcinoma, ductal pancreatic adenocarcinoma, cervical carcinoma, and metastatic colorectal adenocarcinoma [67].

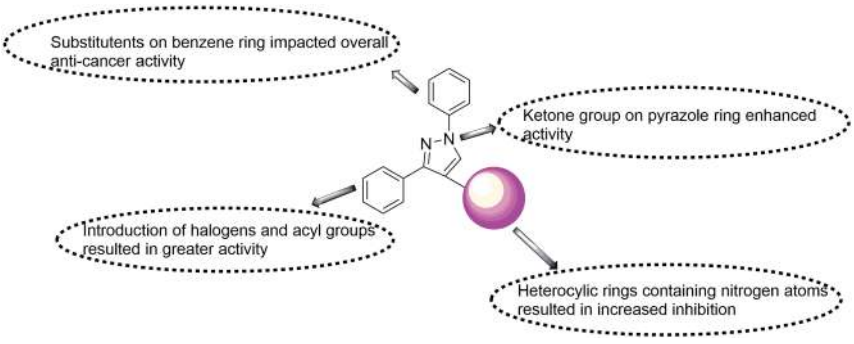
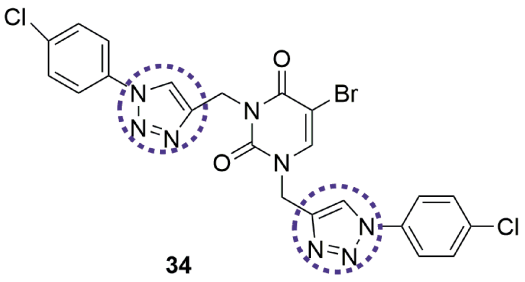


Figure 5.20: Structural activity relationship (SAR) studies of pyrazole based anticancer compounds.



34

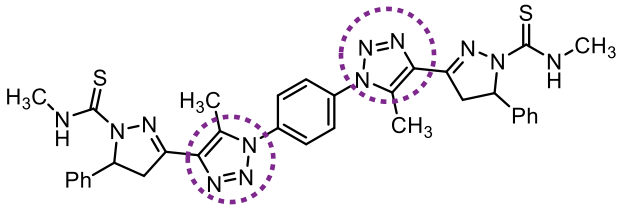
IC_{50} value
HEP-G2- 3.62 μM

Figure 5.21: Anticancer agents based on pyrimidine fused triazole compounds.

Singh et al. designed isatin–coumarin fused triazole containing hybrids. The developed hybrids were investigated on human cancer cell lines. SAR studies revealed compound **35** with no substitution at isatin and containing one carbon bridge between triazole and isatin which emerged out as most influential antitumor agent by tubulin inhibition (Figure 5.22). Further, presence of electron-withdrawing group on isatin significantly increased cytotoxicity against investigated cell lines [68].

A series of VEGFR TK inhibitors having triazole ring were synthesized by Abd-Rabou and team. The reported derivatives were evaluated as antitumor agents against breast cancer, colorectal cancer and human hepatocellular carcinoma cell lines. Compound **36** was found to be show broad range of anticancer activity against all three cell lines (Figure 5.23). Molecular docking studies also supported the results obtained from *in vitro* studies [69].

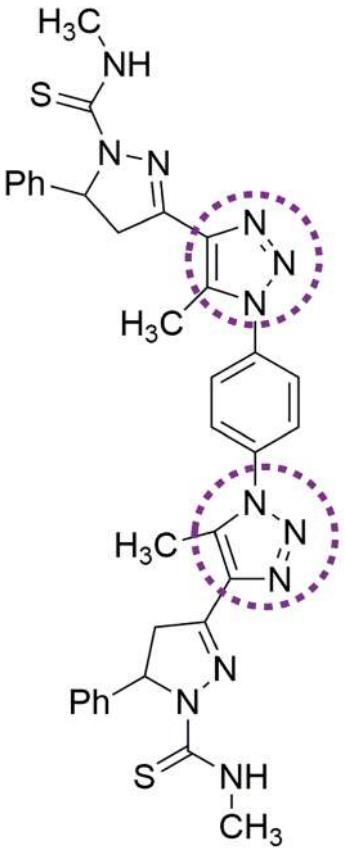
Chekir and coworkers used click approach to develop triazole tethered coumarinopyrazole conjugates and biological evaluation as anticholinesterase, anti-5-lipoxygenase, antityrosinase, and anticancer agents was studied. Compound **37** having three chlorine groups emerged out as new potential in the discovery of



36

IC_{50} value
MCF-7- 32.26μg/mL
HepG2- > 100 μg/mL
HCT-116 - > 100 μg/mL

Figure 5.22: Isatin–coumarin fused triazole containing anticancer hybrids.



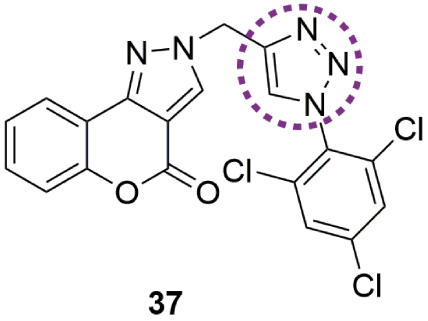
36

IC_{50} value
MCF-7- 32.26µg/mL
HepG2- > 100 µg/mL
HCT-116 - > 100 µg/mL

Figure 5.23: Triazole based anticancer agent.

anticancer agents (Figure 5.24). Furthermore, the data revealed electron-attracting group as important enhancer of anticancer activity as compared to electron-releasing groups. Also, clubbing of triazole scaffold through methylene linker improved anticancer activity [70].

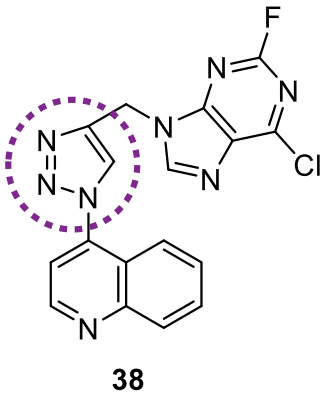
Another synthesis of quinoline/coumarin coupled triazole conjugates was carried out and *in vitro* studies were performed against gastric cancer cell lines. The studies demonstrated compound **38** to be most promising conjugate prepared. Compound **38** was found to inhibit cell invasion by inhibiting TCF b1 in the gastric cell lines (Figure 5.25). During SAR studies, it was revealed that replacing purinyl ring with thymine ring



37

IC_{50} value
HCT-116- 14.2 ± 0.5 μM
MCF-7- 25.3 ± 1.5 μM

Figure 5.24: Anticancer coumarinopyrazole conjugates.



38

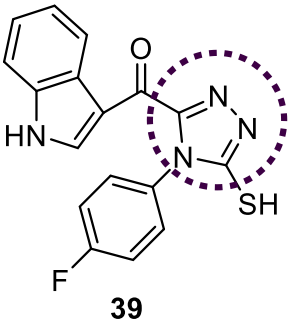
IC_{50}
MGC-803- 1.48 ± 0.17 μM
SGC-7901- 2.28 ± 0.36 μM

Figure 5.25: Quinoline/coumarin coupled triazole conjugates showing anticancer activity.

diminished anticancer activity of prepared derivatives. Fluorine and chlorine substitution on purinyl ring resulted in significant enhancement in anticancer activity [71].

Naaz and team clubbed a new set of 1,2,4-triazole based Topsentin conjugates. Compound **39** emerged out a lead compound by regulating tubulin polymerization and therefore act as tubulin inhibitor. Compound **39** was found to be most active hybrid formed during *in vitro* tubulin polymerization studies (Figure 5.26). Molecular docking studies also supported results from *in vitro* studies [72].

Figure 5.27 gives brief report of structural activity relationship (SAR) studies of triazole containing anticancer derivatives.



Docking score -5.18 kcal/mol

Figure 5.26: Anticancer derivative based on 1,2,4-triazole clubbing with Topsentin.

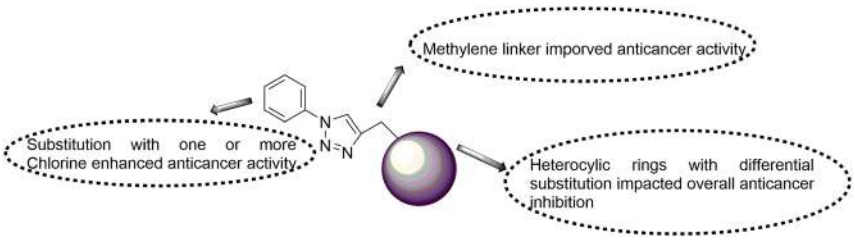


Figure 5.27: Structural activity relationship (SAR) studies of triazole based anticancer agents.

5.3 Conclusions

Heterocyclic compounds are known to possess a large number of therapeutic properties like anticancer, antimalarial, antiparasite, antifungal, antibacterial, antitubercular, antiviral, antidepressant, and antiinflammatory activities. Cancer is one of the leading causes of death in the contemporary era and hence challenging field of research. A plethora of heterocyclic moieties based anticancer derivatives have been explored recently keeping in mind the brighter potential of heterocyclic compounds. A number of FDA approved marketed drugs also contain heterocyclic scaffolds. This chapter deals with recent five years progress towards developing of new heterocyclic ring based anticancer activity. Few of the newly designed derivatives have shown much greater anticancer inhibition compared to standard anticancer drugs. Therefore, research towards exploring more anticancer agents based on heterocyclic rings can prove to be rewarding in future.

Abbreviations

HIV/AIDs	human immunodeficiency virus infection and acquired immune deficiency syndrome
MDA-MB-231	metastatic mammary adenocarcinoma1
HeLa	cervical cancer cell line
SMMC-7721	human hepatocarcinoma cell line
Akt	serine/threonine protein kinase
FAK	Focal adhesion kinase
A549	adenocarcinomic human alveolar basal epithelial cells
HepG2	human liver cancer cell line
Bcl-2	B-cell lymphoma 2
A2AR	human adenosine A2a receptor
Tyr	tyrosine
Bcl-2-Jurkat	human cell line
A-431	epidermoid carcinoma
HCT-116	human colorectal carcinoma cell line
MCF-7	Michigan Cancer Foundation
CDK1	cyclin-dependent kinase 1
COX-2	cyclooxygenase 2
NCI-H460	nonsmall cell lung cancer cell line
HEK-293T	human embryonic kidney 293 cells
HGC-27	gastric cancer cell line
PC-3 cell lines	prostate cancer cell line
EC-109 cell lines	esophageal squamous cell carcinoma cell line
VEGFR	vascular endothelial growth factor receptor

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Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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6 Fused pyrrolo-pyridines and pyrrolo-(iso) quinoline as anticancer agents

Abstract: This work emphasizes the synthesis strategies and antiproliferative related properties of fused pyrrolo-pyridine (including indolizine and azaindoles) and pyrrolo-(iso)quinoline derivatives recently reported in literature.

Keywords: anticancer; azaindole; indolizine; pyrroloisoquinoline; pyrrolopyridine; pyrroloquinoline.

6.1 Introduction

According to World Health Organization, the new cases of cancer are estimated to be continuously increasing [1], therefore, considerable efforts are focused on the design and development of new antiproliferative drugs with improved efficacy, reduced toxicity and less prone to develop multidrug resistance (MDR). Among the strategies to achieve greater structural diversity, the fusion of diverse types of bioactive rings occupies a leading place in the field of medicinal chemistry [1–7].

Thus, fused *N*-heterocyclic compounds are important structural motifs that gained huge attention during last decades due to their diverse properties and applications. But, probably they are the most known in medicinal chemistry, being commonly present in many natural and synthetic bioactive agents [2, 8, 9].

Pyrrole is a five membered *N*-heterocyclic aromatic ring with multiple pharmacophores that due to its versatility and simplicity provides an excellent base for the generation of molecules with biological activity (anticancer, antimicrobial, antiviral, antimalarial, antitubercular, anti-inflammatory, antihypertensive, antiulcer, antidepressant, enzyme inhibitor, etc.) [10, 11].

The fusion of pyrrole ring to other different *N*-heterocycle rings results in novel scaffolds that are an important source of bioactive molecules. Therefore, the literature is plenty of pyrrolo fused heterocycles with interesting biological activities. This

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chapter focuses on the recent syntheses of fused pyrrolo-pyridines (including indolizines) and pyrrolo-(iso)quinolines that exhibited important anticancer properties.

6.2 Fused pyrrolo-pyridines

Pyridine ring, present in many natural compounds (including genetic material), is considered a privileged scaffold in anticancer agents due to its role in many biological processes, but also in cancer pathological pathways [12–14].

The fusion of five-membered pyrrole to a six-membered pyridine ring can result in several chemical pyrrolo-pyridine scaffolds (four of them also known as azaindoles), but also in indolizines that have the nitrogen atom bridged (Figure 6.1). We found six of the pyrrolo-pyridine isomers being present in the structures of molecules that have been reported to display anticancer potency, and their syntheses and biological activities are described below.

6.2.1 Indolizines

The occurrence of the aromatic indolizines in natural compounds is not very common, but there are many reduced derivatives of indolizine being part of natural compounds (probably the most important being Vinca alkaloids). Indolizines have a broad spectrum of pharmaceutical importance, being considered a target for both synthetic and medicinal chemists [15–19]. Therefore, many strategies to build the indolizine system of biological active indolizine derivatives (e.g., 1,3-dipolar cycloaddition, intramolecular aldol cyclization, Chichibabin cyclization, domino Knoevenagel condensation, other multicomponent coupling strategies, etc.) have been employed and are continuously improving [15–22]. We describe here the synthesis and biological data of the recent reported fully aromatized indolizine derivatives that showed significant antitumor potency.

Kim et al. [23] used molecular modeling to perform a virtual screening to identify indolizine compounds with tubulin polymerization inhibition properties by binding to

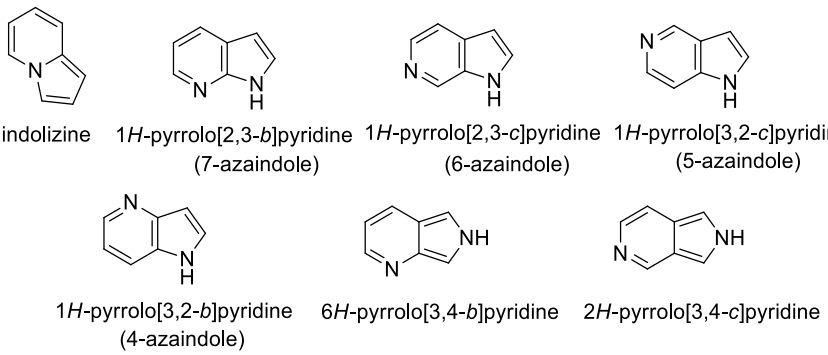


Figure 6.1: Pyrrolo-pyridine scaffolds.

the colchicine site of tubulin. From the 34 virtually selected compounds, indolizines (**1**) and (**2**) (Figure 6.2), showed a strong potential for the inhibition of tubulin polymerization *in vitro* with IC_{50} values of 5.0 and 20.0 μM, respectively. These indolizines also showed antiproliferative activity against the HL-60 cancer cell line, with IC_{50} values of 1.4 and 0.6 μM, respectively. According to molecular docking study, indolizines (**1**) and (**2**) are able to directly interact with the colchicine binding site of tubulin.

The above study inspired a series of reports of new indolizines that showed similar properties (anticancer *via* interactions with tubulin mechanisms).

Thus, Abuhaie et al. [24] reported a number of indolizine derivatives that aim to polymerize tubulin into microtubules. Assembly of the indolizine unit of compounds (**6a–b**) was achieved by [3 + 2] cycloaddition reaction of the ethyl propiolate to the corresponding ylide, followed by the oxidative aromatization of intermediates under atmospheric conditions. Ylides were obtained *in situ* by the triethylamine treatment of pyridinium salts synthesized from the reaction of substituted pyridines (**3a–b**) with 10-(chloroacetyl)-10*H*-phenothiazine (**4**) (Figure 6.3). Compounds have been tested for their anticancer activity against 60 tumor cell lines and as microtubule-targeting agents. At 10^{-4} M concentration, compounds (**6a–b**) inhibited tubulin polymerization by 31 and 42%, respectively. The anticancer screening showed that compound (**6a**) was more active than the (**6b**) derivative, with GI_{50} values ranging from 0.67 to 8.22 μM against the 60 lines examined. The best activity was registered against melanoma (SK-MEL-2) cell line (GI_{50} = 0.67 μM).

The same group realized a structure-anticancer activity relationship (SAR) study on three series of new indolizine derivatives, two of them (with general structure

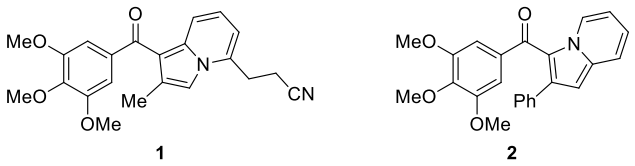


Figure 6.2: Structures of bioactive indolizines (**1**) and (**2**).

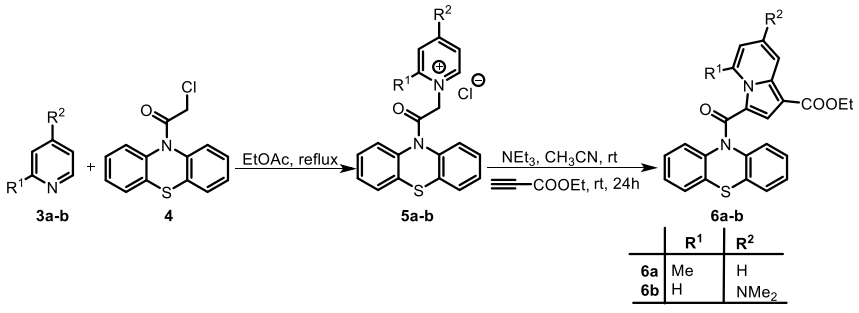
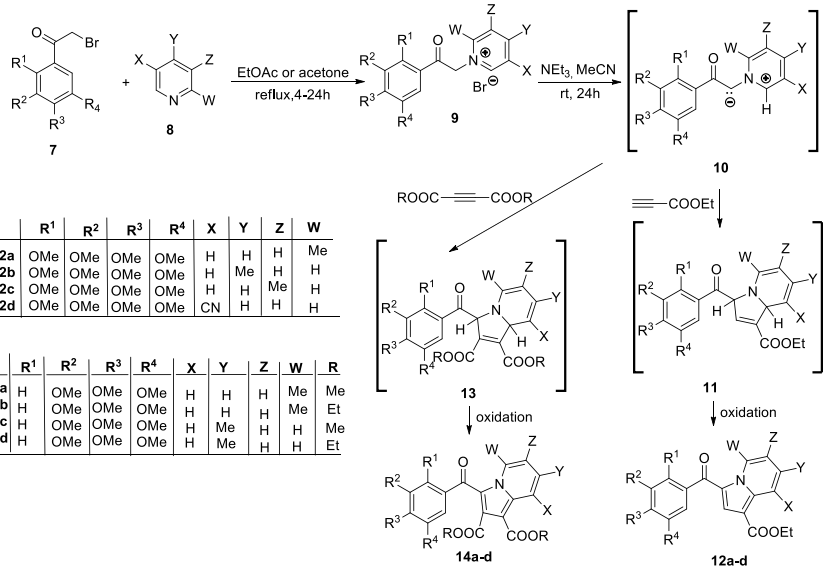


Figure 6.3: Synthesis of indolizine derivatives (**6a–b**).

showed in Figure 6.4) proving an excellent potential as anticancer agents [25]. The synthesis of indolizines (**12**) and (**14**) was achieved by [3 + 2] cycloaddition reaction of cycloimmonium ylides (**10**) (*in situ* generated by Et_3N treatment of pyridinium salts (**9**)), to ethyl propiolate, dimethyl or diethyl acetylenedicarboxylate, followed by subsequent aromatization of obtained dihydropyrrolopyridine intermediates (**11**) and (**13**), respectively (Figure 6.4).

New indolizine derivatives were screened for their interaction with tubulin and cytotoxic effects. Indolizines (**12a**) and (**12d**) were the most active as anticancer agents showing the greatest antiproliferative impact on melanoma MDA-MB-435 cell line (IC_{50} = 30 nM for both compounds). The indolizines having an ester function at C-1 (**12a–d**) showed also the highest tubulin polymerization inhibitory activity. Presence of the second ester groups at C-2 of indolizine ring, or replacement of ester group at C-1 with a cyano group, led to an important decrease in the inhibitory activity.

Lucescu et al. [26] described a novel family of 3-aroylindolizines with a dimethoxytriazine unit at position 1 (Figure 6.5). Compounds have been synthesized also *via* [3 + 2] cyaloaddition mechanism using 2-ethynyl-4,6-dimethoxy-1,3,5-triazine as dipolarophyle, and tested *in vitro* for their potential to inhibit tubulin polymerization and cancer cell's growth. Compound (**18**) provided GI_{50} values of 0.92 μM against breast cancer cells MDA-MB-231/ATCC and of 0.87 μM against CNS cancer cells SNB-75. SAR study realized in the series of compounds type (**18**), showed that replacing Br with Me, Cl or OMe groups at the *para*-position of phenyl unit was critical and reduced biological potency. Also, substitution on the indolizine ring at positions 5, 6 or 7, led to diminution of the inhibitory properties.



	R^1	R^2	R^3	R^4	X	Y	Z	W
12a	OMe	OMe	OMe	OMe	H	H	H	Me
12b	OMe	OMe	OMe	OMe	H	Me	H	H
12c	OMe	OMe	OMe	OMe	H	H	Me	H
12d	OMe	OMe	OMe	OMe	CN	H	H	H

	R^1	R^2	R^3	R^4	X	Y	Z	W	R
14a	H	OMe	OMe	OMe	H	H	H	Me	Me
14b	H	OMe	OMe	OMe	H	H	H	Me	Et
14c	H	OMe	OMe	OMe	H	Me	H	H	Me
14d	H	OMe	OMe	OMe	H	Me	H	H	Et

Figure 6.4: Synthesis of indolizine derivatives (**12**) and (**14**).

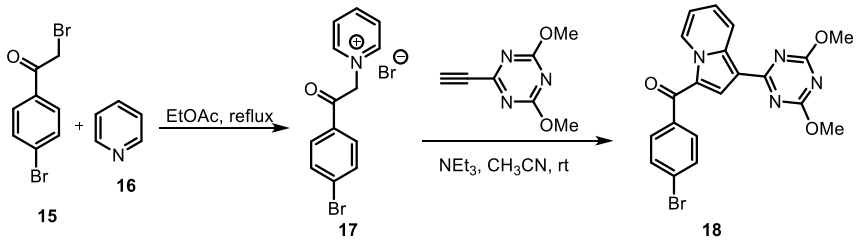
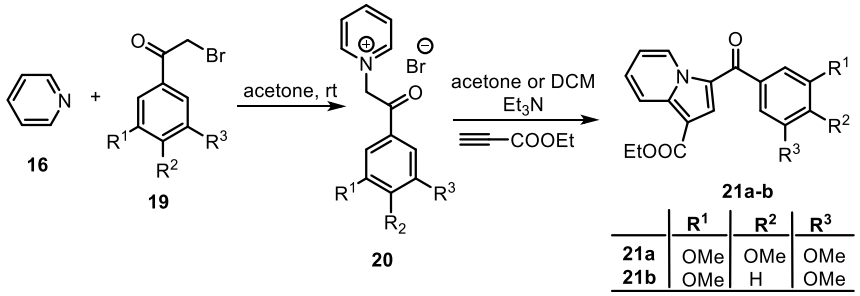


Figure 6.5: Synthesis of indolizine derivative (**18**).

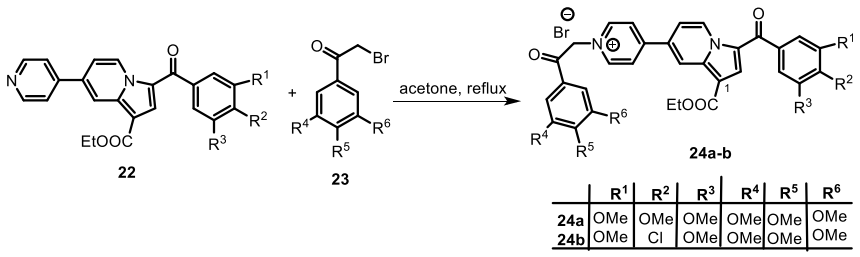
Other series of indolizines were reported by Sardaru et al. [27] as analogs of Phenstatin (a well-known inhibitor of tubulin polymerization) [28, 29]. Thus, compounds (**21**) and (**24**) (Figures 6.6 and 6.7) have been synthesized and tested for both, their anticancer activity against National Cancer Institute (NCI) panel of 60 human cancer cell lines, and capacity to inhibit tubulin polymerization. The synthesis of the indolizines (**21**) was carried out in two steps including a 1,3-dipolar cycloaddition step (Figure 6.6).

For synthesis of compounds (**24**), monoindolizine (**22**) (synthesized by a similar pathway as compounds (**21**), from 4,4′-bipyridine) was reacted with reactive halide derivative (**23**) (Figure 6.7).



	R^1	R^2	R^3
21a	OMe	OMe	OMe
21b	OMe	H	OMe

Figure 6.6: Synthesis of indolizine derivatives (**21**).



	R^1	R^2	R^3	R^4	R^5	R^6
24a	OMe	OMe	OMe	OMe	OMe	OMe
24b	OMe	Cl	OMe	OMe	OMe	OMe

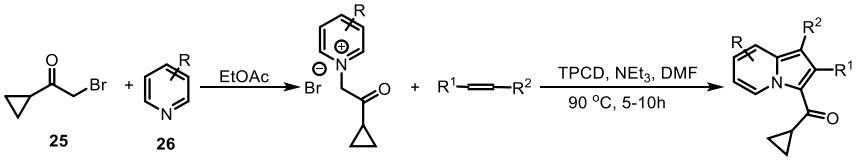
Figure 6.7: Synthesis of indolizine derivatives (**24**).

Compounds (**21a–b**) and (**24a–b**) showed the widest range of growth inhibitory activity against cancer cell lines representing leukemia, melanoma and cancer of lung, colon, central nervous system, ovary, kidney, breast, and prostate. Compound (**21a**) showed cytotoxic effect on all these lines, the best activity being recorded against melanoma MDA-MB-435 cell line. Compound (**21a**) exhibited GI_{50} values in the range of 10–100 nM against 43 cell lines, the most notable being on those belonging to leukemia, skin cancer, and kidney cancer. Compounds (**24a–b**) provided values of GI_{50} of micromolar order against different cell lines. In the series of compounds (**24**), the best GI_{50} value was obtained in the case of compound (**24b**), against leukemia SR cell line (0.33 μM). *In vitro* assay revealed tubulin polymerization inhibition by all active compounds, while docking to the colchicine binding site of tubulin showed a good match of the active compounds.

Shen et al. [30] reported new indolizine and annulated indolizine derivatives that bear a cyclopropylcarbonyl group. Synthesis of compounds (**28**) is based on the [3 + 2] cycloaddition mechanism as presented in previous cases (Figure 6.8).

This series of 3-cyclopropylcarbonylindolizines (**28**) was investigated for the antiproliferative activity against the human hepatocellular liver carcinoma (Hep-G2) cell line using MTT technique and for the epidermal growth factor receptor (EGFR) kinase inhibitory properties. Comparing with 5-fluorouracil used as positive control reference, the most active compounds (**28a–d**) (Figure 6.8) provided IC_{50} values of 0.48, 0.39, 0.29 and 0.20 μg/mL, respectively. In addition, compound (**28d**) exhibited excellent EGFR kinase inhibitory activity (IC_{50} = 0.085 μM) similar to the one shown by the reference anticancer drug Iressa (IC_{50} = 0.033 μM). Molecular docking supported the experimental data that suggest a binding mode of compound (**28d**) to EGFR kinase.

Moon et al. [31] reported the synthesis of a new family of indolizine derivatives from pyrrole-2-carboxaldehydes and a variety of active methylene compounds. The procedure for the construction of pyridine unit of indolizines consisted in a domino Knoevenagel condensation/intramolecular aldol condensation (Figure 6.9). Remarkable anticancer activity was observed in case of most of the indolizine derivatives, as well as



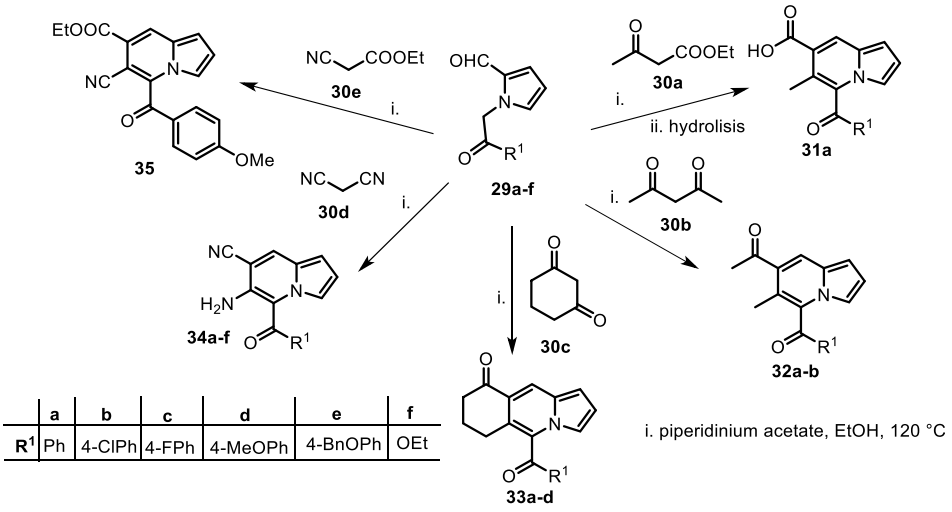
	R	R^1	R^2
28a	H	H	CN
28b	OMe	1,2-*N*-phenylmaleimide	
28c	7-Me	COOEt	COOEt
28d	5-Me, 8-Br	H	CN

Figure 6.8: Synthesis of indolizine derivatives (**28a–d**).

inhibition of transcriptional activity of β-catenin and activation of p53 in human lung adenocarcinoma A549 cells. The death of 60% of the A549 cells was observed after the three days treatment with compound (**34d**) at 3 mM. A decrease in activity was revealed when cyano group was replaced with the ethyl ester group (**35**). The presence of cyano and 4-methoxyphenyl groups as substituents of indolizine appears to be essential for the inhibitory action.

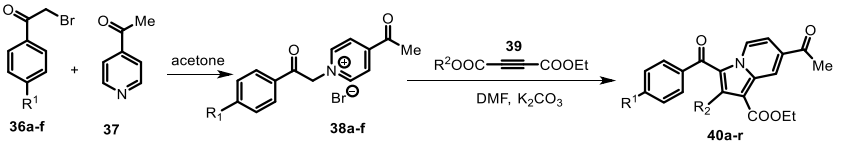
Sandeep et al. [32] reported synthesis of a new class of 7-acetyl-2-substituted 3-(substituted benzoyl) indolizine-1-carboxylates using 1,3-dipolar cycloaddition as key step (Figure 6.10). Compounds (**40a–r**) were screened for their *in vitro* anticancer activity against human cervix cancer cell line SiHa using Adriamycin as reference.

Compounds (**40a**), (**40q**) and (**40r**) exhibited good antiproliferative activity at 10, 20, 40 and 80 µg/mL with the lowest cell growth activity against human cervix SiHa cell line of –63.1, 22.4 and –54.8%, respectively, when compared with doxorubicin (–74.1%) used as positive control.



	a	b	c	d	e	f
R^1	Ph	4-ClPh	4-FPh	4-MeOPh	4-BnOPh	OEt

Figure 6.9: Synthesis of indolizine derivatives (**31–35**).



	40a	40b	40c	40d	40e	40f	40g	40h	40i
R^1	H	H	F	F	F	F	Cl	Cl	Cl
R^2	H	COOEt	H	Me	Et	COOEt	H	Me	COOEt

	40j	40k	40l	40m	40n	40o	40p	40q	40r
R^1	Br	Br	Br	CN	CN	CN	Me	Me	Me
R^2	H	Me	COOEt	Me	Et	COOEt	H	Me	COOEt

Figure 6.10: Synthesis of indolizine derivatives (**40a–r**).

Park et al. [33] obtained a new family of indolizine-based chalcone hybrids, *via* the pathway presented in Figure 6.11, that included a base mediated aldol condensation of indolizine (**43**) bearing a 7-acetyl group with various aldehydes with heterocyclic skeleton.

From the series, derivative (**45**) with R = Ph as substituent, showed the best inhibitory activity against human lymphoma U932 cells (IC_{50} = 1.12 μM), the testing being realized using as positive references doxorubicin (IC_{50} = 0.28 μM) and cisplatin (IC_{50} > 30 μM). Moreover, compound (**45**) showed superior apoptotic activity in lymphoma cells to that shown by cisplatin, but inferior to the one shown by doxorubicin, which had similar cytotoxicity.

New indolizine derivatives (**48**) were synthesized under solvent- and metal-free conditions by Liu et al. (Figure 6.12) [34]. All compounds were tested for their cytotoxicity against HepG2 (p53-wild), A549, and HeLa cell lines. Compound (**48c**) exhibited the most significant inhibition of proliferation in HepG2 cells. The mechanistic investigations revealed that indolizine (**48c**) induced apoptosis via the mitochondrial p53 pathway in HepG2 cells, being a potential candidate for the growth inhibition of liver cancer cell [34].

A novel series of 14 hybrids with 3-chlorophenyl (1-(5-phenyl-1,3,4-oxadiazol-2-yl) indolizin-3-yl) methanone structure (**54**) were synthesized using the pathway presented in Figure 6.13 [35].

The antiproliferative activity of the synthesized compounds was evaluated against breast carcinoma MCF-7 cell line using as positive control the standard drug doxorubicin. The most active compounds (**54a**) and (**54b**) exhibited cytotoxic activity with IC_{50} values of 20.64 and 23.48 μM, respectively.

6.2.2 1*H*-pyrrolo[2,3-*b*]pyridines-*b* (7-azaindoles)

Due to the special structure providing a scaffold that contains a hydrogen-bond donor and acceptor in a rigid three-atom system [36–39], 7-azaindole derivatives show the

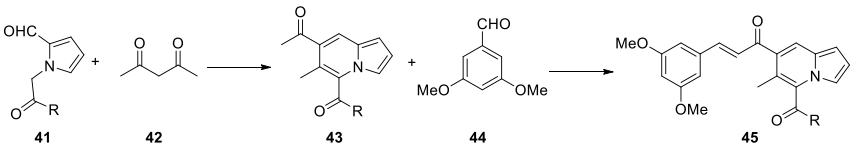


Figure 6.11: Synthesis of indolizine derivative (**45**).

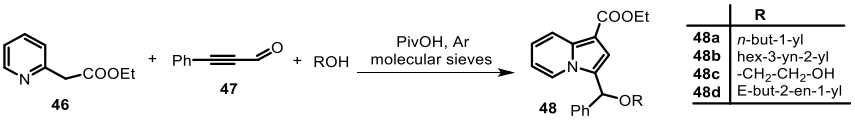


Figure 6.12: Synthesis of indolizine derivatives (**48**).

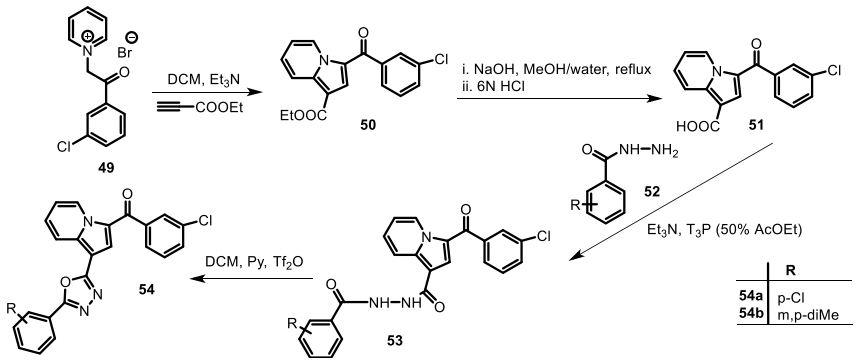


Figure 6.13: Synthesis of indolizine derivatives (**54**).

most interesting pharmacological and biological properties of all azaindoles, including anticancer. There are already three anticancer FDA-approved drugs: Vemurafenib (Figure 6.14) used for the treatment of BRAF V600E mutation metastases produced by melanoma [40], Pexidartinib (Figure 6.14) used for the treatment of rare tenosynovial giant cell tumor [41] and Pemigatinib (Figure 6.14) used for the treatment of cholangiocarcinoma [36], and also several natural compounds with antiproliferative potency (Variolin B and deoxyvariolin B (Figure 6.14)), that contain 7-azaindole ring as core.

Thus, the design of 7-azaindole derivatives is receiving increasing attention and there are nowadays plenty of synthetic methods implemented to build the 7-azaindole ring (e.g., Hemetsberger reaction, Chichibabin-like cyclization, intramolecular Suzuki-Miyaura palladium-catalyzed reaction, Sonogashira coupling reactions, tandem palladium-catalyzed amination reaction, Reissert-type reaction, Stille cross-coupling reaction, intramolecular palladium-catalyzed Heck reaction, multistep reaction from

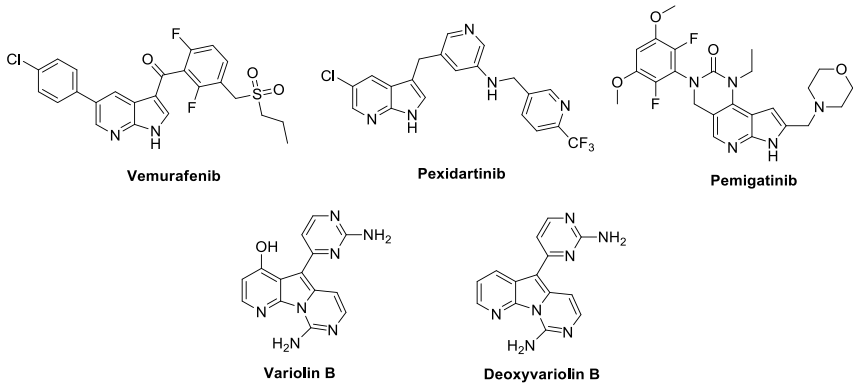


Figure 6.14: Structures of FDA approved azaindolic anticancer drugs (Vemurafenib, Pexidartinib, Pemigatinib) and azaindolic natural compounds Variolin B and Deoxyvariolin B.

pyrrole or from 2-aminopicoline, three-component Knoevenagel reaction followed by Michael addition, etc.) [37–39].

Diana et al. [42] synthesized a new series of phenylthiazolyl-pyrrolo[2,3-*b*]pyridines-*b* by a Hantzsch reaction between aryl thioamides (**55a–e**) and 3-bromoacetyl-pyrrolo [2,3-*b*]pyridines (**56a–b**) (Figure 6.15), and studied the anticancer properties of the new compounds against NCI'panel of 60 tumor cell lines of leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, and breast cancer. The anticancer testing of all synthesized derivatives (**57a–e**) and (**58a–e**) was initially performed at a single concentration (10^{-5} M). From all compounds, derivatives (**57a**), (**57e**), (**58a**), (**58b**), (**58c, d**) were selected, for full evaluation at five different concentrations (10^{-4}–10^{-8} M). *N*-methyl derivative (**58c**) had a good selectivity against the melanoma cell line MDA-MB-435 (pGI_{50} = 6.50) and ovarian cell line NCI/ADR-RES (pGI_{50} = 5.93). Also, this compound show a slight selectivity for the leukemia (pGI_{50} = 4.90–6.59), colon (pGI_{50} = 5.17–6.18), and renal cancer (pGI_{50} = 5.16–7.50) cell lines.

Aly et al. [43] synthesized several pyrazole compounds, one of the derivative (compound (**62**)) having incorporated a 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* core. Derivative (**62**) was synthesized and tested for the anticancer properties against liver (HEPG2) and colon (HCT) human tumor cell lines. The synthesis started with the reaction of 4-aminoantipyrine (**59**) with bromoacetophenone (**60**), that leaded to the acylated intermediate (**61**). The skeleton of pyrrolo[2,3-*b*]pyridine was generated by the reaction of compound (**61**) with two molecules of malononitrile in sodium ethoxide (Figure 6.16) [43].

The *in vitro* anticancer evaluation revealed that derivative (**62**) exhibited potency against HCT colon cell line (IC_{50} = 7.4 μg/mL).

The synthesis and testing of the antitumor activity of hybrid derivative (**65**) containing a 1*H*-pyrrolo[2,3-*b*]pyridinone*H*-pyrrolo[2,3-*b* unit was performed by Zhang et al. [44]. Thus, the reaction of 1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one (**63**) with the pyrrole derivative (**64**) generated the desired compound (**65**) (Figure 6.17).

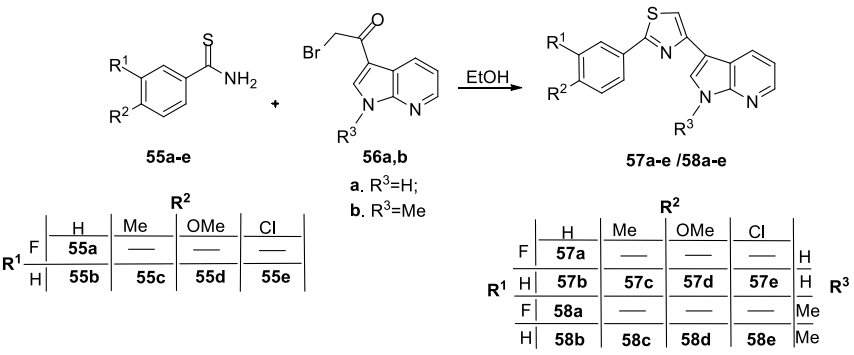


Figure 6.15: Synthesis of new phenylthiazolyl-pyrrolo[2,3-*b*]pyridine derivatives (**57**/**58**).

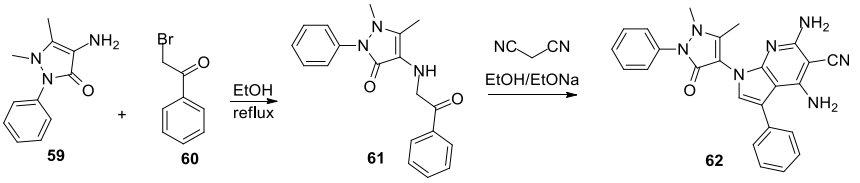


Figure 6.16: Synthesis of pyrrolo[2,3-*b*]pyridine-5-carbonitrile derivative (**62**).

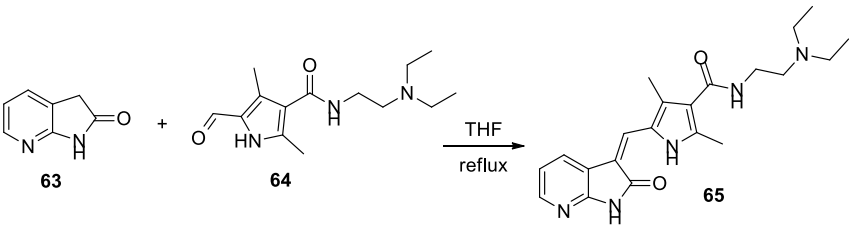


Figure 6.17: Synthesis of 1*H*-pyrrolo[2,3-*b*]pyridinone*H*-pyrrolo[2,3-*b* derivative (**65**).

The antiproliferative activity of the 1*H*-pyrrolo[2,3-*b*]pyridinone*H*-pyrrolo[2,3-*b* derivative (**65**) was investigated *in vitro* against A375 melanoma cells, SMMC liver cancer cells and MCF-7 breast cancer cells. This compound showed the best antitumor activity against A375 cells with the IC_{50} of 25.38 μg/mL. The mechanistic investigations realized by the same group, revealed that the pathway of compound (**65**) to inhibit the proliferation of A375 cells could include the activation of caspase-3 and caspase-9.

Lee et al. [45] have designed and synthesized a novel series of azaindolylsulfonamides with anticancer properties. The desired derivatives were obtained through a sequence of reactions as presented in Figure 6.18. Thus, the first step consisted in the reaction between 7-azaindole (**66**) or 2,3-dihydro-7-azaindole (**66′**), and *para*- and *meta*-bromobenzenesulfonyl chloride (**67a, b**), respectively, in the presence of KOH and tetrabutylammonium hydrogen sulfate (TBAHS). The obtained products (**68a, b**) and (**68′a, b**) were subjected to a Heck reaction leading the *tert*-butyl cinnamates intermediates (**69a, b** and **69′a, b**) that were hydrolyzed to generate the corresponding cinnamic acids (**70a, b** and **70′a, b**). Next step consisted in the amidation of compounds (**70a, b)** and (**70′a, b)**, respectively, with *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (NH_2OTHP), and subsequent hydrolysis to afford the desired azaindolylsulfonamides (**71a, b**) and (**71′a, b**) (Figure 6.18) [45].

The anticancer properties of the new azaindolylsulfonamide derivatives (**71a, b**) and (**71′a, b**) have been studied against four different human cancer cell lines: KB oral carcinoma, H460 lung carcinoma, PC3 prostate carcinoma and HCT116 colorectal carcinoma. The most active compound of the series against all human tumor cell lines was 7-azaindole derivative (**71b**). This compound displayed values of IC_{50} in the range

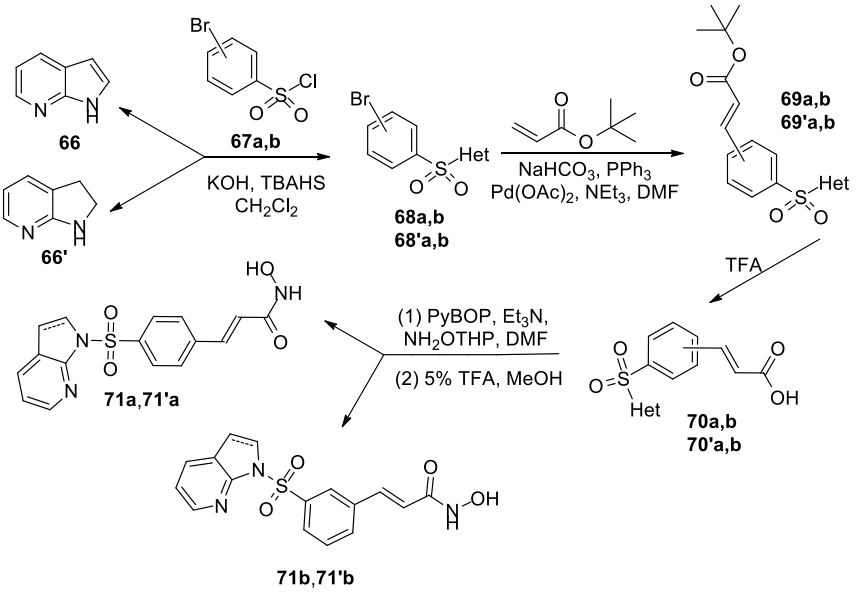


Figure 6.18: Synthesis pathway for azaindolylsulfonamides (**71**).

of 0.39–2.51 μM (against 11 diverse cancer cell lines). Also, compound (**71a**) showed anticancer potency with IC_{50} = 0.87 μM against KB oral carcinoma cell line, and compound (**71'b**) with IC_{50} = 4.11 μM against PC3 prostate carcinoma cell line. Further pharmacological studies indicated that compound (**71b**) is a potent HDAC inhibitor (IC_{50} = 0.1 μM), it is highly selective for histone deacetylase 6 (HDAC6), and showed a good pharmacokinetic profile (with 33% of oral bioavailability in mice). Also, after oral treatment with 25 and 100 mg/kg of compound (**71b**), TGI values of 55.8 and 66.9% were obtained in human colorectal HCT116 xenograft models. All reported data show that azaindolylsulfonylcinnamic hydroxamate (**71b**), has a great potential as promising anticancer agent.

A new series of PARP-1 inhibitors with 7-azaindole-1-carboxamide scaffold was designed and synthesized by Cincinelli et al. [46]. First, 7-azaindole *N*-oxide (**72**) was obtained by the oxidation reaction of 7-azaindole (**66**) with *meta*-chloroperoxybenzoic acid (MCPAB), then by the treatment with benzoyl bromide (PhCOBr) in the presence of hexamethyldisilazane (HMDS), *N*-acyl-7-azaindole derivative (**73**) was generated. Basic elimination of the acyl group leaded 6-bromo-7-azaindole derivative (**74**). Using Suzuki coupling of derivative (**74**) with appropriate boronic acids, 7-azaindole derivatives substituted in position 6 with different aromatic and heteroaromatic moieties (**75b–l**) have been obtained. The reaction of derivatives (**75g, h, j, k**) with 4-nitrophenylchloroformate, in the presence of tetrabutylammonium bromide generated *p*-nitrosubstituted derivatives (**76g, h, j, k**). In the final step,

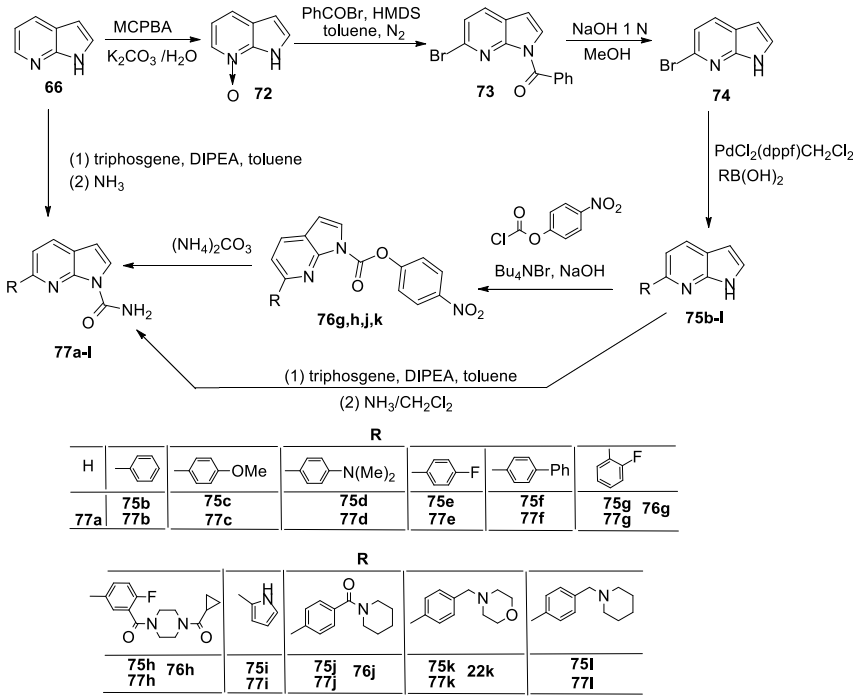


Figure 6.19: Synthetic route for the synthesis of 7-azaindole derivatives (**77**).

7-azaindole-1-carboxamides (**77a–l**) were synthesized by the reaction of compounds (**76g, h, j, k**) with ammonium carbonate. Compounds (**77**) were also obtained by treatment the derivatives (**66**) or (**75b–l**) with triphosgene, followed by the treatment with NH_3 (Figure 6.19) [46].

The study involving the evaluation of 7-azaindole-1-carboxamides (**77a–l**) for their inhibitory activity against recombinant human PARP-1 expressed as GST fusion protein, showed compound (**77l**) to exhibit significant potency against the enzyme with IC_{50} = 0.07 μM [46]. Another compound with a good activity was derivative (**77b**) which with the IC_{50} value of 0.27 μM. The *in vivo* antitumor activity of the 7-azaindole-1-carboxamide (**77l**) against the MX1 breast carcinoma growth in nude mice was also investigated [46]. A significant antitumor activity (TVI-tumor volume inhibition, around 35%), was observed at the treatment with a dose 100 mg/kg of compound (**77l**), the effect being similar with the one produced by the standard Olaparib (tested at 167 mg/kg).

Cheng et al. [47] have developed new aza- and diaza-*bis*indoles in order to test their antitumor activity. The synthesis of aza-*bis*indole (**80**) consisted in the condensation of 1*H*-indol-3-yl acetate (**78a**) or 1*H*-pyrrolo[2,3-*b*]pyridin-3-yl acetate (**78b**) with isatin (**79**) in acid or basic conditions. Also, aza- and diaza-*bis*indoles (**82**) and (**83**),

respectively, were obtained using condensation of (**78a, b**) with indoline-2,3-dione (**81**) in acid condition (Figure 6.20) [47]. The antiproliferative activity of the new aza- and diaza-*bis*indoles (**80**), (**82**) and (**83**) was evaluated on human cancer cell lines LXFL529L (large cell lung tumor xenograft cells), MCF7 (breast adenocarcinoma cells) and HT29 (colon adenocarcinoma cells), using the sulforhodamine B colorimetric (SRB) assay [47]. All tested compounds were more active against LXFL529L cell line (IC_{50} = 4.4 μM for compound (**80**); IC_{50} = 2.9 μM for compound (**82**); IC_{50} = 0.06 μM for compound (**83**)), compared with the standard Indirubin (IC_{50} = 9.9 μM).

Platinum (II) oxalato complexes (**86**), (**87**) and (**88**) including 7-azaindole derivatives as coligands were synthesized by Štarha et al. [48] in order to study their antitumor properties. The synthesis of the desired new 7-azaindole complexes (**86–88**) was achieved by treatment of various 7-azaindole-3,4-substituted derivatives (**84a–c**) with $K_2[Pt(ox)_2]\cdot 2H_2O$ (**85**) (Figure 6.21) [48].

The antiproliferative activity of new complexes was determined against the various human cancer cell lines: HOS osteosarcoma, MCF7 breast adenocarcinoma, G361 melanoma, HeLa cervix carcinoma, A2780 ovarian carcinoma, A2780R cisplatin-

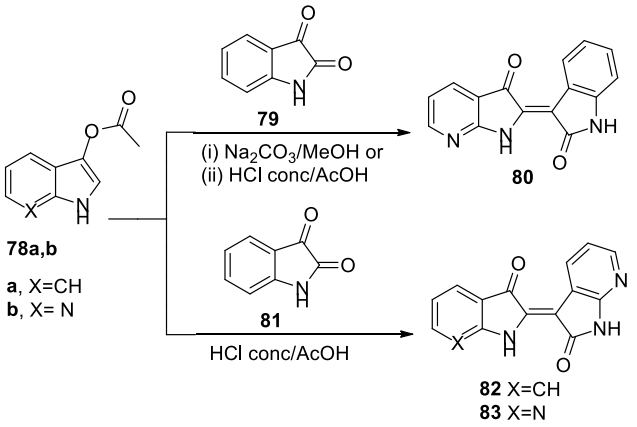


Figure 6.20: Synthesis of aza- and diaza-*bis*indoles (**80**), (**82**) and (**83**) using condensation reactions.

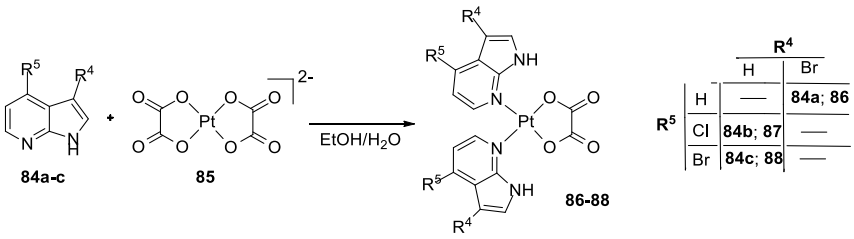


Figure 6.21: Synthesis of 7-azaindole complexes (**86–88**).

resistant ovarian carcinoma, A549 lung carcinoma and LNCaP prostate carcinoma. The testing revealed that only complex (**86**) exhibited moderate *in vitro* antitumor activity against HOS, MCF7, G361, HeLa and A2780 with IC_{50} in the range of 17–32 μM.

The same group synthesized novel cis-dichloridoplatinum (II) complexes (**91–95**) using 7-azaindole ligands and studied their cytotoxic activity [49]. Thus, by the reaction of different 3,4,5-substituted 7-azaindoles (**89a–e**) with potassium tetrachloroplatinate (II), complexes (**91–95**) have been obtained (Figure 6.22) [49].

They studied *in vitro* cytotoxicity of the complexes (**91–95**) and also of the cisplatin (as positive control) against eight human cancer cell lines. Complexes (**91–93**) containing monosubstituted 7-azaindole ligands showed superior *in vitro* cytotoxic effect against A2780, A2780R, HOS, MCF7 and HeLa cell lines (IC_{50} values ranging from 2.7 to 17.1 μM) as compared with cisplatin. Complex (**93**) with 4-bromo-1*H*-pyrrolo[2,3-*b*] pyridine*H*-pyrrolo[2,3-*b* scaffold exhibited the best cytotoxic activity, against all cell lines tested, with IC_{50} values in the range of 2.7–11.1 μM. Halogeno substitution of the second position on the 7-azaindole moiety was detrimental, leading to either decreased solubility (**94**) or decreased biological effect (**95**) of such complexes.

In the continuation of their research in the field of complexes with 1*H*-pyrrolo[2,3-*b*] pyridine*H*-pyrrolo[2,3-*b*, Štarha et al. [50], synthesized complexes with gold (I) triphenylphosphane using substituted 7-azaindoles as ligands. Thus, by the reaction between various 2,3,4,5-substituted 1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**96a–h**) and chloro(-triphenylphosphine)gold(I) (**97**), in acetone, they obtained complexes [Au(*n*aza)(PPh_3)] (**98a–h**) (Figure 6.23) [50].

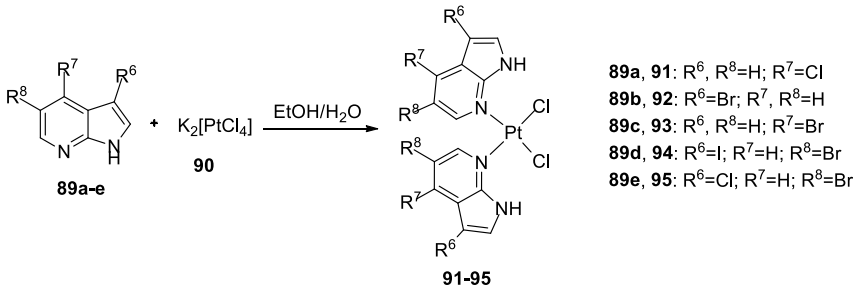


Figure 6.22: Synthesis of complexes cis-[$PtCl_2$(*n*aza)$_2$] (**91–95**).

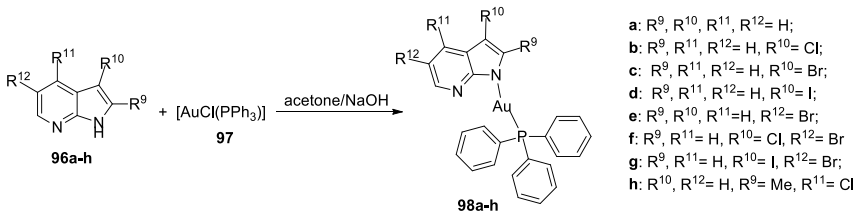
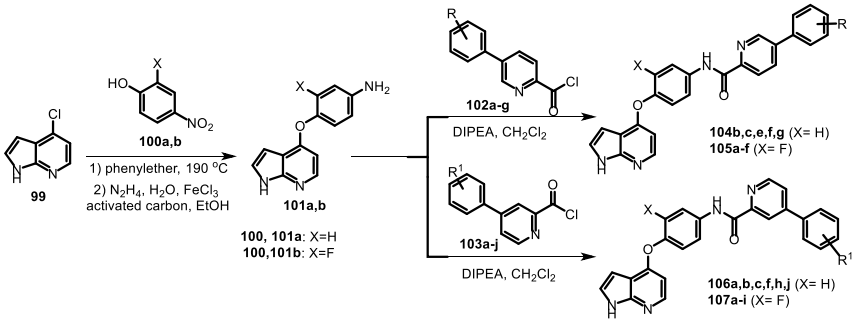


Figure 6.23: Synthetic route for obtained of complexes [Au(*n*aza)(PPh_3)] (**98**).

The *in vitro* cytotoxicity of the synthesized complexes was evaluated using cisplatin for comparative purposes. The activity of gold (I) complexes [Au(*n*a-za)(PPh_3)] (**98a–h**) and cisplatin was carried out against A2780 ovarian cancer, A2780R cisplatin-resistant ovarian cancer and MRC-5 normal fibroblast cell lines. The data furnished by this study revealed that the complexes with unsubstituted or 3-iodo, 5-bromo-, 2-methyl-4-chloro-substituted 1*H*-pyrrolo[2,3-*b*]pyridines-*b* (**98a, d, e, h**) were more potent against the A2780 cells (IC_{50} = 3.8 μM for (**98a**), IC_{50} = 3.5 μM for (**98d**), IC_{50} = 3.1 μM for (**98e**), IC_{50} = 2.8 μM for (**98h**)) than conventional cisplatin (IC_{50} = 20.3 μM). Complexes with 3-Cl-, 3-Br-, 3-Cl-5-Br- and 3-I-5-Br- as substituents (**98b, c, f, g**) showed antitumor activity comparable with cisplatin against the same cell lines. The treatment of the A2780 cells with complexes (**98a**) and (**98e**) (at IC_{50} concentrations) led to cell cycle modifications (G2-arrest) different from those caused by the treatment with cisplatin.

Zhu et al. [51] synthesized novel series of 1*H*-pyrrolo[2,3-*b*]pyridine *H*-pyrrolo[2,3-*b* derivatives substituted with phenylpicolinamide group, designed as *c*-Met inhibitors*c*-. To obtain the desired derivatives, they started from 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**99**) in reactions with 2-unsubstituted or 2-fluoro-substituted 4-nitrophenols (**100a, b**), followed by reduction of the nitro group, when 1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**101a, b**) were obtained. In the next step, the acylation of compounds (**101a, b**) with acyl chloride (**102a–g**) and (**103a–j**), respectively, were carried out to give 1*H*-pyrrolo [2,3-*b*]pyridine derivatives (**104b, c, e, f, h**), (**105a–f**), (**106a, b, c, f, h, j**) and (**107a–i**) (Figure 6.24) [51].

The four series of derivatives (**104–107**) were evaluated for their cytotoxicity against A549 lung cancer, PC-3 prostate cancer and MCF-7 breast cancer cell lines, but also for the IC_{50} values against *c*-Met kinase *c- in vitro*, in both cases Foretinib being used as reference compound [51]. Eighteen of the tested compounds showed



	R¹									
	H	3-Me	4-Me	2,4-diMe	4-Et	4-OMe	4-F	4-CF_3	2,4-diF	4-Cl
103	a	b	c	d	e	f	g	h	i	j
106	a	b	c	-	-	f	-	h	-	j
107	a	b	c	d	e	f	g	h	i	-

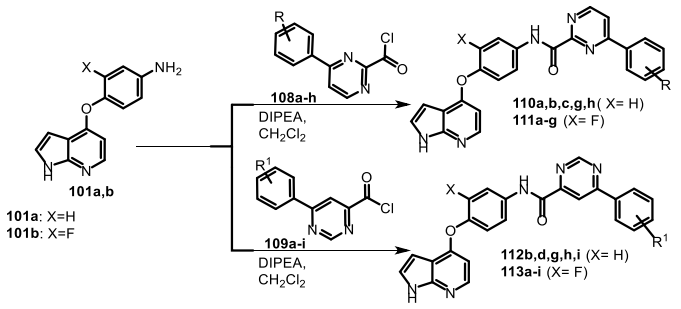
	R						
	H	4-Me	4-OMe	4-F	4-Cl	2,4-diF	4-Et
102	a	b	c	d	e	f	g
104	-	b	c	-	e	f	g
105	a	b	c	d	e	f	-

Figure 6.24: Synthesis of novel pyrrolo[2,3-*b*]pyridine derivatives (**104–107**).

high cytotoxicity, seven of them with equal or superior values than the ones shown by the positive control Foretinib, against one or more cell lines. The series (**107a–i**) exhibited the best activity, while the most active compound was (**107f**) bearing a 3-fluorophenyl-5-(4-methoxyphenyl)picolinamide moiety (IC_{50} = 1.04 μM against A549 cell line, compared with IC_{50} = 0.64 μM of Foretinib; IC_{50} = 0.02 μM against PC-3 cell line, compared with IC_{50} = 0.39 μM of Foretinib; IC_{50} = 6.22 μM against MCF-7 cell line, compared with IC_{50} = 9.47 μM of Foretinib; IC_{50} = 0.69 μM against *c*-Met kinase, compared with IC_{50} = 0.014 μM of Foretinib).

The same authors synthesized new series of 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo [2,3-*b* derivatives substituted with phenylpyrimidine-carboxamide, designed as *c*-Met inhibitors.*c*- [52] Using the same synthetic method as described above [51], they obtained four new series of 6-(phenyl)pyrimidine-4-carboxamide substituted 1*H*-pyrrolo [2,3-*b*]pyridine derivatives (**110–113**) (Figure 6.25) [52].

All derivatives (**110–113**) were evaluated for the cytotoxicity against A549 lung cancer, PC-3 prostate cancer and MCF-7 breast cancer cell lines, 11 of them being equal or more potent than Foretinib against one or more cell lines. Compound with the highest cytotoxic activity against all test cell lines was the 1*H*-pyrrolo[2,3-*b*]pyridine *H*-pyrrolo[2,3-*b* derivative (**111e**) with IC_{50} values of: 0.14 μM against A549 cell line (compared with IC_{50} = 0.64 μM of Foretinib), 0.24 μM against PC-3 cell line (compared with IC_{50} = 0.39 μM of Foretinib), 0.02 μM against MCF-7 cell line (compared with IC_{50} = 9.47 μM of Foretinib). Compounds (**111e**), (**112g**), (**112h**), (**113a**) were evaluated *in vitro* for the IC_{50} values against *c*-Met kinase*c*-, the most potent compound being (**111e**) with IC_{50} = 0.12 μM, compared with IC_{50} = 0.014 μM of Foretinib. Anyway, in case of both reports, further studies should be carried out in order to reveal mechanistic insights of the active compounds [51, 52].



	R¹								
	H	4-OMe	4-F	4-Cl	4-CF_3	4-Br	4-Me	3-Cl	4-NO_2
109	a	b	c	d	e	f	g	h	i
112	-	b	-	d	-	-	g	h	i
113	a	b	c	d	e	f	g	-	-

	R							
	4-OMe	4-F	4-Cl	4-CF_3	H	4-Br	4-Me	3-Cl
108	a	b	c	d	e	f	g	h
110	a	b	c	-	-	-	g	h
111	a	b	c	d	e	f	g	-

Figure 6.25: Synthesis of new series of pyrrolo[2,3-*b*]pyridine derivatives (**110–113**).

Spanò et al. [53, 54] designed and synthesized several series of nortopsentin (a natural compound isolated from Topsentia genitrix sponge) analogues. One of the series (compounds (**116**)) contains 3-thiazol-1*H*-pyrrolo[2,3-*b*]pyridine *H*-pyrrolo[2,3-*b* core in the structure. The synthetic route consisted in the reaction of various 3,5-substituted-1*H*-pyrrolo[2,3-*b*]pyridines-*b* (**114a–d**) with bromoacetyl bromide leading to 3-acyl-1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**115a–d**). To provide desired 3-thiazol-1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**116a–d**), compounds (**115**) were coupled with naphthalene-2-carbothioamide in EtOH (Figure 6.26) [53].

3-Thiazol-1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivatives (**116a–d**), were tested for cytotoxicity at a single dose (10^{-5} M) against HCT 116 colorectal carcinoma, MDA-MB-435 melanoma and MCF-7 breast cancer [53]. The results showed that only derivative (**116d**) showed moderate cytotoxic activity against all tested cell lines.

Tang et al. [55] continued the study in the field of *c*-Met kinase*c*- inhibitors, with the design and synthesis of new 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivatives substituted with dihydropyridazine moiety. Thus, intermediates (**119, 120**) were generated in two steps: etherification of 2-unsubstituted or 2-fluoro-substituted 4-nitrophenols (**100a, b**) with 4-chloro derivatives (**117, 118**) and reduction of nitro group using hydrazine. Further acylation with various dihydropyridazine-3-carbonyl chlorides (**121a–l**) gave the desired *c*-Met kinase inhibitors with 7-azaindole skeleton (**122–125**) (Figure 6.27) [55].

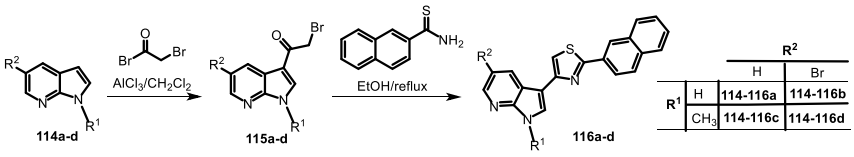


Figure 6.26: Synthetic route for synthesis of 3-thiazol-pyrrolo[2,3-*b*]pyridine derivatives (**116**).

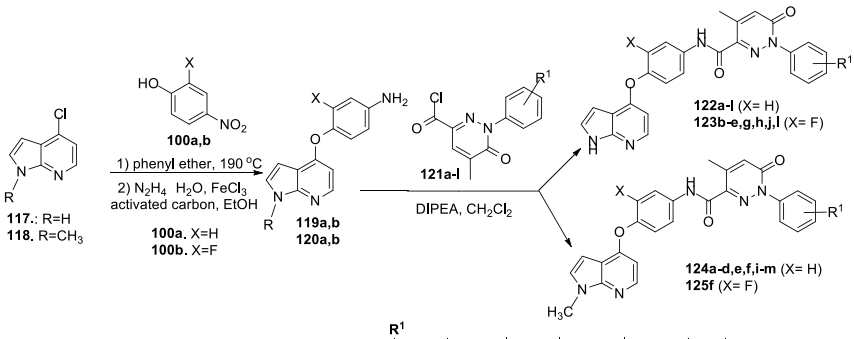


Figure 6.27: Synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**122–125**).

The evaluation of synthesized compounds against *c*-Met kinase*c*- and HT29, A549, H460, and U87MG cancer cell lines, was realized using Foretinib as positive control [55]. Twelve derivatives were found to be more potent against one or more cell lines than Foretinib. Derivative (**123d**) was the most promising compound with the IC_{50} values (0.039 μM against the HT29; 0.046 μM against A549; 0.091 μM against H460 and 0.76 μM against U87MG cell lines) superior to Foretinib. Compound (**123d**) also, proved to be the most potent compound in the series with the IC_{50} value 1.06 nM. Analysis of SARs indicated that electron withdrawing groups (such as F) in the place of substituent X on aminophenyl ring increased the anticancer potency. Also, the number, position, and electrical properties of the substituents R^1 on the phenyl ring prove to have an important influence on the potency, while substituent R of azaindole ring appears to be less important for the anticancer properties.

Yuan et al. [56] designed and synthesized a series of derivatives hydroxamic acid derivatives, for the evaluation as DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors. One of the derivatives synthesized is compound (**129**) that contains a 7-azaindole core. The first step of its synthesis consisted in the amide coupling of amine (**126**) and acid (**127**), when 3-carboxamido substituted 1*H*-pyrrolo [2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivative (**128**) was obtained. The target derivative (**129**) was generated by transformation of the ester residue of the compound (**128**) into hydroxamic acid using hydroxylamine and sodium methoxide (Figure 6.28).

The antiproliferative activity of derivative (**129**) was evaluated against two different human cancer cells line: myeloid leukemia K562 and histiocytic lymphoma U937. An inhibitory activity of tumor cells almost 10 times lower compared to SAHA (vorinostat) was registered for the tested compound (IC_{50} = 17.95 μM for compound (**129**) compared with IC_{50} = 1.91 μM for SAHA, against K562 cell line; IC_{50} = 17.85 μM for compound (**129**) compared with IC_{50} = 0.54 μM for SAHA, against U937 cell line).

Another series of nortopsentin analogues having in their structure units of 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* was synthesized by Parrino et al. [57]. The synthesis of target 7-azaindole derivatives (**133–135**) is based on the Hantzsch reaction between thioamides (**132**) and acylated derivatives of *N*-substituted 1*H*-pyrrolo[2,3-*b*] pyridine (**131**) synthesized by acylation of *N*-substituted 7-azaindoles (**130**) (Figure 6.29) [57].

The novel synthesized derivatives (**133–135**) were submitted to the NCI for the study of the cytotoxic activity against a panel of 60 tumor cell line. Compounds (**133e, q**) and

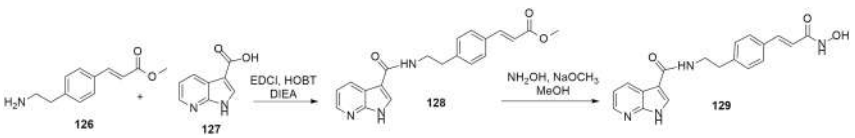
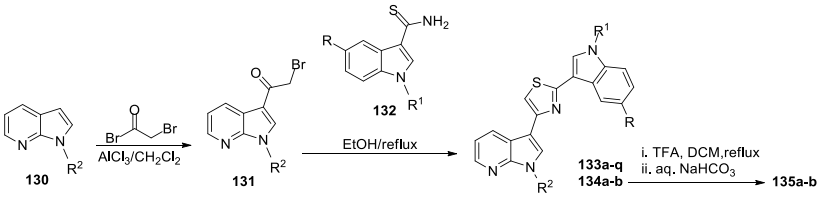


Figure 6.28: Synthesis pathway of pyrrolo[2,3-*b*]pyridine derivative (**129**).



133a: R= H; R^1= CH_2CH_2OMe; R^2= H;
133b: R= H; R^1= CH_2CH_2OMe; R^2= Me;
133c: R= H; R^1, R^2= CH_2CH_2OMe;
133d: R= H; R^1= Me; R^2= CH_2CH_2OMe;
133e: R=OMe; R^1= CH_2CH_2OMe; R^2= H;
133f: R=OMe; R^1= CH_2CH_2OMe; R^2= Me;
133g: R=OMe; R^1, R^2= CH_2CH_2OMe;
133h: R=OMe; R^1= Me; R^2= CH_2CH_2OMe;
133i: R= Br; R^1= CH_2CH_2OMe;R^2= H;
133j: R= Br; R^1= CH_2CH_2OMe;R^2= Me;
133k: R= Br; R^1, R^2= CH_2CH_2OMe;
133l: R= Br; R^1= Me; R^2= CH_2CH_2OMe;
133m: R= F; R^1= CH_2CH_2OMe; R^2= H;
133n: R= F; R^1= CH_2CH_2OMe; R^2= Me;
133o: R= F; R^1, R^2= CH_2CH_2OMe;
133p: R= F; R^1= Me; R^2= CH_2CH_2OMe;
133q: R= F; R^1= H; R^2= CH_2CH_2OMe;
134a: R= H; R^1= Boc; R^2= CH_2CH_2OMe;
134b: R= Br; R^1= Boc; R^2= CH_2CH_2OMe;
135a: R= H; R^1= H; R^2= CH_2CH_2OMe;
135b: R= Br; R^1= H; R^2= CH_2CH_2OMe;

Figure 6.29: Synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**133–135**).

(**135a, b**) showed sufficient inhibitory properties against almost all the 60 human tumor cell lines, to be selected for further screenings at five concentrations (10^{-4}–10^{-8} M) on the full panel [57]. The most active compound (**133e**) expressed GI_{50} values ranging from low micromolar to nanomolar level (12.6–0.03 μM), but also high selectivity against tumor cells and excellent potency in causing mitotic failure. Moreover, compound (**133e**) showed inhibitory properties against CDK1 activity with IC_{50} value similar to roscovitine and purvalanol A (used as positive control). The mechanistic investigation revealed that antiproliferative action on MCF-7 cells (breast cancer) can be associated with externalization of plasma membrane phosphatidylserine and DNA fragmentation, accompanied to the cell cycle progression disruption. The study concluded that a possible growth inhibitory mechanism induced on MCF-7 cells by (**133e**), was CDK1/cyclin B inhibition-induced G2/M phase arrest. SAR analysis indicated that substitution of the nitrogen atom of the indole and/or 7-azaindole moiety with 2-methoxyethyl group led to improved biological activity [57].

A series of nucleoside analogs obtained by coupling of 3′-*C*-ethynyl-*β*-D-ribofuranose sugar scaffold, with variously modified purine nucleobases were developed by Hulpia et al. [58], in order to test their antitumor activity. For the synthesis of 1,7-dideazapurine (7-azaindole or pyrrolo[2,3-*b*]pyridine) nucleoside series, the first step consisted in a substitution reaction of 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivatives (**137**)/(**137′a, b**) with acetoxytetrahydrofuran derivative (**136**) using *bis*(trimethylsilyl)acetamide (BSA) and trimethylsilyl trifluoromethanesulfonate (TMSOTf), when intermediates (**138**)/(**140a, b**) were obtained. These intermediates were converted in the target nucleoside (**139**)/(**141a, b**) in two steps depicted in Figure 6.30.

The antitumor activity of novel pyrrolo[2,3-*b*]pyridine derivatives (**139**) and (**141a, b**) was studied against three cancer cell lines: L1210 (murine leukemia), CEM (human lymphocytic) and HeLa (human cervix carcinoma). The results showed that the most

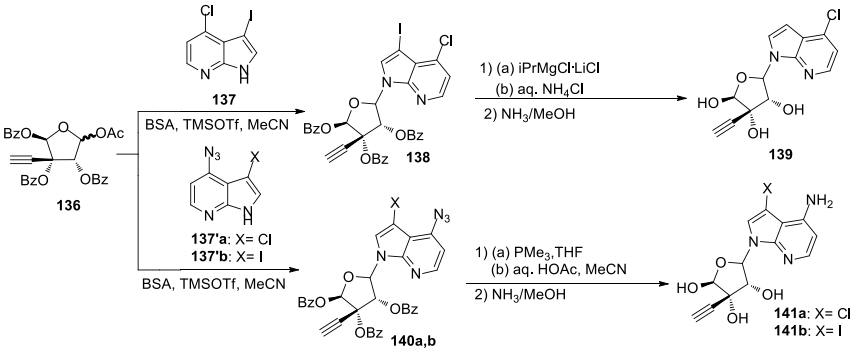
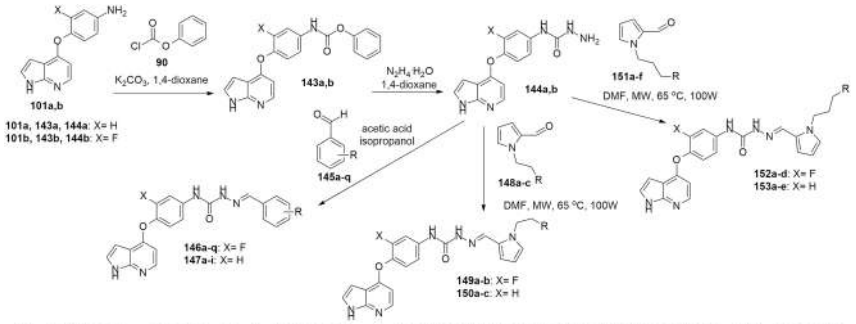


Figure 6.30: Pathway of synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**139**) and (**141**).

active compound was derivative (**141b**) with IC_{50} = 0.044 μM against L1210 cell line, IC_{50} = 0.18 μM against HeLa cell line and IC_{50} = 0.31 μM against CEM cell line.

As continuation of their work in the field of *c*-Met kinase*c*- inhibitors, Wang's group reported the synthesis of 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivatives bearing aromatic hydrazone moiety [59]. The synthesis consisted of several steps, starting from 1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**101a, b**) obtained as described in Figure 6.25 [52, 53]. The acylation of derivatives (**101a, b**) was achieved by using phenyl carbonochloridate (**142**), when the acyl derivatives (**143a, b**) were generated. Their reaction with hydrazine hydrate lead to compounds (**144a, b**) that were subjected to condensation with various substituted aldehydes to yield the desired 1*H*-pyrrolo[2,3-*b*]pyridine derivatives (**146/147, 149/150, 152/153**) bearing an aromatic hydrazone moiety (Figure 6.31).



146 a. R= H; b. R= 3-Br; c. R= 4-Br; d. R= 4-F; e. R= 4-CN; f. R= 3-NO_2; g. R= 4-NO_2; h. R= 2,3-di Cl; i. R= 3,4-di Cl; j. R= 2-Cl-4-F; k. R= 4-OH; l. R= 4-OMe; m.R= 2,4-di OH; n. R= 2,3,4-tri OMe; o. R= 3,4,5-tri OMe; p. R= 4-OH-3,5-di $C(CH_3)_3$; q. R= 3-Br-4-OH; r. R= 3,5-diBr-4-OH
147 a. R= H; b. R= 4-Br; c. R= 4-F; d. R= 4-NO_2; e. R= 2,3-di Cl; f. R= 4-OH; g. R= 4-OMe; h. R= 2,3,4-tri OMe; i. R= 2-Br-4-OH;
149 a. R= $N(Me)_2$; b. R= morpholin-*N*-yl;
150 a. R= $N(Me)_2$; b. R= $N(Et)_2$; c. R= morpholin-*N*-yl;
152 a. R= $N(Et)_2$; b. R= piperidin-*N*-yl; c. R= *N*-methylpiperazin-N-yl; d. R= pyrrollidin-*N*-yl;
153 a. R= $N(Me)_2$; b. R= morpholin-*N*-yl; c. R= piperidin-*N*-yl; d. R= *N*-methylpiperazin-*N*-yl; e. R= pyrrollidin-*N*-yl

Figure 6.31: Synthesis of 1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* derivatives (**146/147, 149/150** and **152/153**).

All compounds were investigated for the IC_{50} values against four cancer cell lines (A549, HepG2, MCF-7 and PC-3), most of the compounds showing moderate to excellent cytotoxicity activity. Compounds (**146c**) and (**153e**) were further evaluated for the activity against several kinases (*c*-Met, Flt-3, VEGFR-2 and EGFR). The results obtained in case of both tested compounds, showed a good selectivity for *c*-Met kinase *c*-. Compound (**146c**), the most promising in terms of antiproliferative activity (with IC_{50} values of: 0.82 μM against A549, 1.00 μM against HepG2, 0.93 μM against MCF-7, 0.92 μM against PC-3 cell lines and 0.506 μM against *c*-Met kinase) was also investigated in terms of dose-dependent, time-dependent and cell apoptosis. The obtained data showed that derivative (**146c**) induces apoptosis of A549 cells by arresting the cell cycle progression in G2/M phase. SAR study (supported also by docking calculations) indicated phenyl hydrazone derivatives (**146** and **147**) being superior to the heterocyclic hydrazone derivatives (**149–150** and **152–153**). Also, while the introduction of fluorine atoms on the aminophenoxy groups had an impact on the biological profile of the compounds of the phenylhydrazone series, no significant effect was observed in the pyrrole hydrazone series of compounds.

The same group, using a similar strategy, reported the synthesis and the *in vitro* antitumor activity of four new series of pyrrolo[2,3-*b*]pyridine derivatives bearing pyridazinone moiety (**156–159**) [60]. Thus, acyl chloride derivatives (**155)** (obtained in four steps from the corresponding substituted anilines) were coupled with key 7-azaindole intermediates (**154**) generated as described in ref [55] (Figure 6.32).

The antitumor properties were evaluated for all synthesized compounds against four cancer cell lines (A549, HepG2, MCF-7 and PC-3), while the activity against *c*-Met, Flt-3, VEGFR-2, c-Kit and EGFR kinase was evaluated only for the most promising compound (**156g**). Compound (**156g**) showed superior activity than positive control Foretinib against A549 (IC_{50} = 2.19 μM), HepG2 (IC_{50} = 1.32 μM), MCF-7 (IC_{50} = 6.27 μM) and PC-3 (IC_{50} = 4.63 μM) cell lines. The same compound proved to be selective against *c*-Met kinase*c*- and able to induce apoptosis of HepG2 cells. SARs study revealed that target compounds containing R = H and R^1 = H were favorable to the activity, while EWGs on aryl group (R^2) also increased the activity of compounds.

A series of 24 new pyrrolo[2,3-*b*]pyridine derivatives bearing 4-oxoquinoline moiety were designed, synthesized and investigated for the *in vitro* antiproliferative properties against A549, HepG2 and MCF-7 cancer cell lines, by Liu et al. [61]. Last step

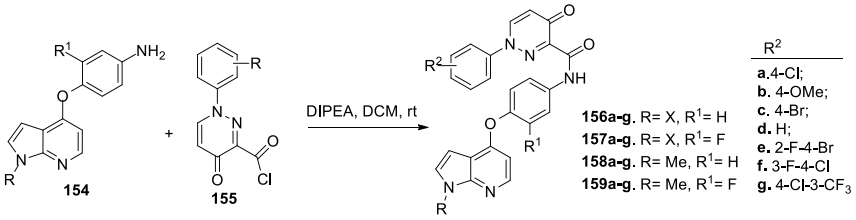


Figure 6.32: Synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**156–159**).

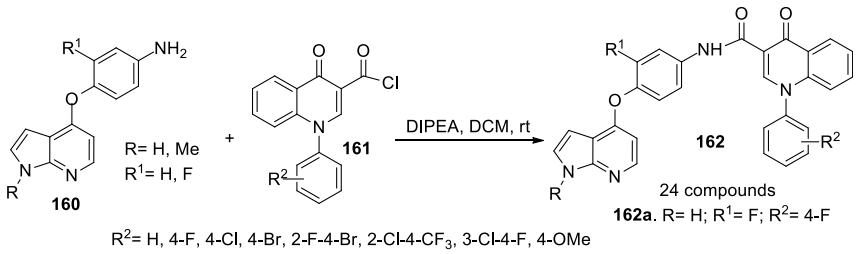


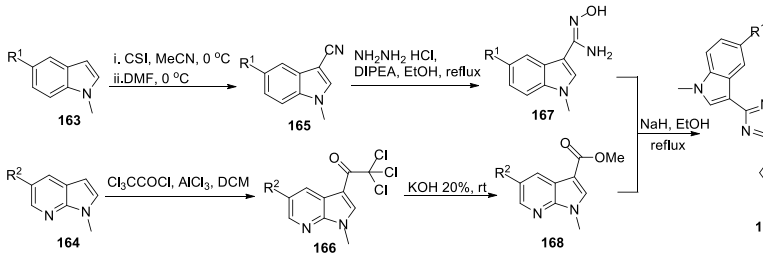
Figure 6.33: Synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**162**).

of the synthesis consisted in the reaction of anilines (**160**) (synthesized in two steps from 4-chloroazaindole or 1-methyl-4-chloroazaindole) with acyl chlorides (**161**) (generated in a multistep synthesis by the same group) (Figure 6.33) [61].

Most of the compounds showed moderate to high potency, eight of them being more effective than Forentinib against at least one cancer cell lines. The most promising compound is (**162a**) (Figure 6.33) that also proved good selectivity on *c*-Met kinase (IC_{50} = 17 nM) over other six tyrosine kinases (Flt-3, c-Kit, VEGFR-2, ALK, PDGFR-β and RON). SARs study suggested that the anticancer activity is superior when R = H and R^2 = single EWGs (such as R^2 = F).

New analogs of nortopsentin that contain a 7-azaindole moiety (**169a–o**), were efficiently synthesized by Cascioferro et al. [62], by reaction between two different key intermediates, *N*′-hydroxy-1-methyl-1*H*-indole-3-carboximidamides (**167**) and methyl-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b*-3-carboxylates (**168**), both compounds being synthesized as presented in Figure 6.34.

Among all prescreened derivatives (against the HCT-116 colon cancer cell line), the most active compounds (**169k**) and (**169n**) were further evaluated for the antiproliferative profile against MCF-7, HeLa and CaCo2 human tumor cells. Both compounds exhibited IC_{50} values in the micromolar and submicromolar range, with



169

a. R^1= H; R^2=H	**d.** R^1= Br; R^2=H	**g.** R^1= F; R^2=H	**j.** R^1= Cl; R^2=H	**m.**R^1= OMe; R^2=H
b. R^1= H; R^2=Br	**e.** R^1= Br; R^2=Br	**h.** R^1= F; R^2=Br	**k.** R^1= Cl; R^2=Br	**n.**R^1= OMe; R^2=Br
c. R^1= H; R^2=F	**f.** d. R^1= Br; R^2=F	**i.** R^1= F; R^2=F	**l.** R^1= Cl; R^2=F	**o.** R^1= OMe; R^2=F

Figure 6.34: Synthesis of pyrrolo[2,3-*b*]pyridine derivatives (**169a–o**).

superior values for compound (**169k**) derivative. Both derivatives caused cell cycle arrest of MCF-7 in the G0–G1 phase. This suggests an apoptotic mechanism of the antiproliferative effect that can be associated with externalization of plasma membrane phosphatidylserine, chromatin condensation and membrane blebbing. Also compounds (**169k**) and (**169n**) proved to exhibit preferential toxicity toward cancer cells.

Dongare et al. [63] designed and synthesized novel 7-azaindole derivatives bearing a hydrazone unit, and studied their anticancer properties. The syntheses consisted in the condensation of hydrazide derivative (**170**) (obtained from 2-methyl-1*H*-pyrrolo[2,3-*b*]pyridine*H*-pyrrolo[2,3-*b* (**171**) in seven steps by the same authors) and various aldehydes (**172**) and (**173**), when new pyrrolo[2,3-*b*]pyridine derivatives bearing the hydrazone unit (**174a–c**) and (**175a–d**) were generated (Figure 6.35) [63].

The *in vitro* antiproliferative activity of all the synthesized compounds was investigated against MCF-7 breast cancer cell line. The results showed that compounds (**175b–d**) were the most potent with GI_{50} values of 22.3 μM for **175b**, 24.9 μM for **175c** and 29.6 μM for **175d**.

Diao et al. [64] synthesized a series of new 3-amino-1*H*-7-azaindoles using a one-pot sequence. Thus, the cyclization reaction between ethyl(3-cyanopyridin-2-yl) carbamate (**176**) and different α-bromoketones (**177a–e**), afforded the desired 3-amino-1*H*-7-azaindoles (**178a–e**) (Figure 6.36).

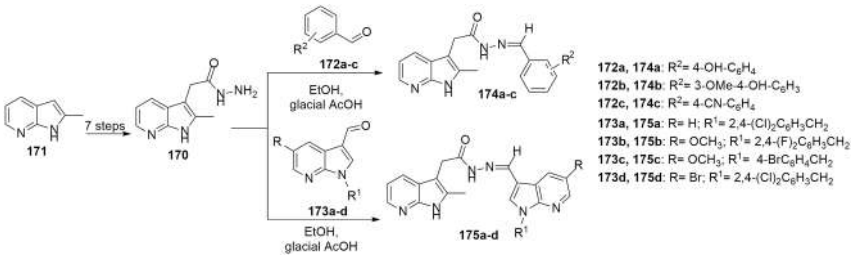


Figure 6.35: Synthesis of 7-azaindole derivatives bearing the hydrazone unit (**174**) and (**175**).

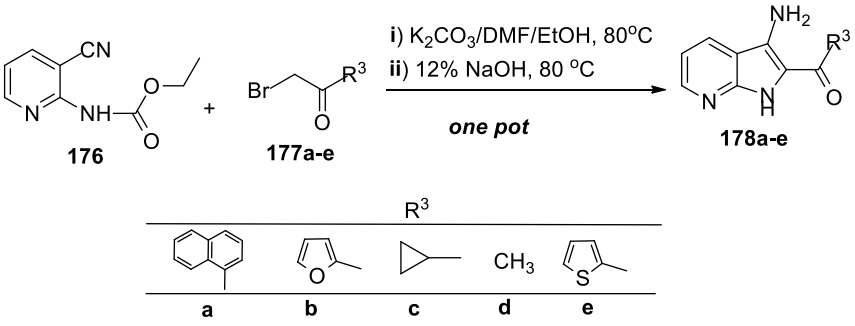


Figure 6.36: Synthesis of new 3-amino-1*H*-7-azaindoles (**178**).

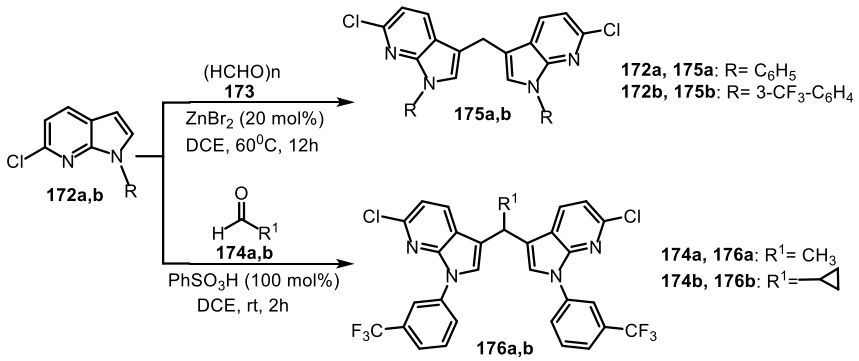


Figure 6.37: Synthesis of new *bis*-7-azaindoles (**175**) and (**176**).

The cytotoxic activity of the derivatives (**178a–e**) was determined *in vitro* against HeLa, HepG2, MDA-MB-231 and MCF-7 cancer cell lines. The most potent compound proved to be derivative (**178a**) that showed IC_{50} values of 3.7 μM (against HeLa), 8.0 μM (against HepG2), and 19.9 μM (against HepG2), these values being 13-, 5-, and 1.4-times higher activity compared to fluorouracil [64].

Lee et al. [65] synthesized new *bis*-7-azaindoles and studied their anticancer properties against human breast adenocarcinoma cells (MCF-7) and human ovarian carcinoma cells (SKOV-3). The synthesis of desired *bis*-7-azaindoles (**175a, b**) and (**176a, b**) were done using Lewis acid-mediated cross-coupling reactions between *N*-substituted 7-azaindoles (**172a, b**) and several aldehydes (**173**)/(**174a, b**), using $ZnBr_2$ or $PhSO_3H$ as Lewis acid (Figure 6.37) [65].

The cytotoxicity study revealed that *bis*-7-azaindoles (**175b**) and (**176b**) were the most active compounds against both studied cell lines with IC_{50} values of 8.3 (**176b**) and 9.3 μM (**175b**) against MCF-7 breast adenocarcinoma cells, and 12.5 (**176b**) and 13.6 μM (**175b**) against SKOV-3 ovarian carcinoma cells, these values being competitive with doxorubicin used as positive control.

6.2.3 1*H*-pyrrolo[2,3-*c*]pyridines (6-azaindoles)

6-Azaindoles are probably less known in the series of azaindoles, but however, there are several 6-azaindole derivatives reported to show biological activity, including anticancer. For synthesis of 6-azaindoles, there are also reported strategies that include the building of pyrrole ring (starting from pyridine derivatives) or the construction of pyridine ring (starting from pyrrole derivatives) [38, 39].

In 2012, Ganser et al. [66] synthesized 3-azaindolyl-4-arylmaleimide derivative (**181**) in few steps. First, treatment of 2-chloro-3-nitropyridine (**177**) with vinylmagnesium bromide and subsequent catalytic hydrogenation of 7-chloro-1*H*-pyrrolo

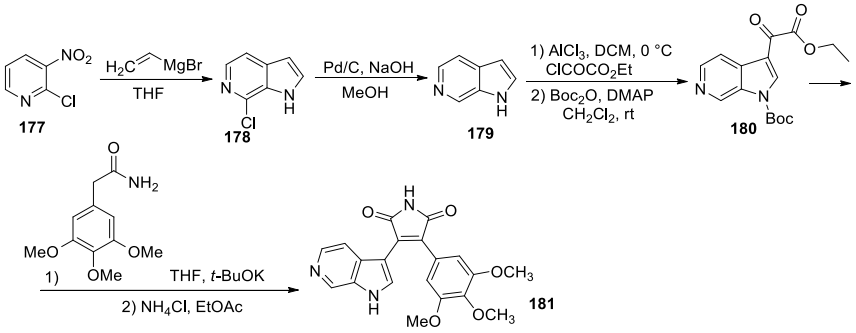


Figure 6.38: Synthesis of 3-azaindolyl-4-arylmaleimide (**181**).

[2,3-*c*]pyridine*H*-pyrrolo[2,3-*c* (**178**) produced 6-azaindole (**179**). The acylation with ethyl chlorooxoacetate in the presence of aluminum chloride ($AlCl_3$) in excess, gave the corresponding azaindole ketoester, which was converted into the protected form (**180**), using Boc anhydride. The target maleimide (**181**) was finally obtained in a *one pot* reaction of azaindole glyoxyl ester (**180**), 4-(3,4,5-trimethoxyphenyl)acetamide and potassium *tert*-butoxide (*t*-BuOK) (Figure 6.38) [66].

The antiangiogenic properties and kinase inhibition potency of azaindolyl-maleimide (**181**) as well as the kinase selectivity was evaluated, using *in vivo* chick embryo assay and kinase assays, respectively. Important inhibition of VEGFR and GSK-3β protein kinase was found, with IC_{50} values of 48 nM for VEGRF2 and 9 nM for GSK-3β. Inhibition of FLT-3 kinase (known to have a decisive function in cell proliferation and differentiation), was also registered for compound (**181**), with IC_{50} value of 18 nM. Also, compound (**181**) exhibited antiproliferative properties against leukemic and endothelial cells, as well as against HT-29 cells in monotherapy [66].

Starting from 2-bromo-3-nitropyridine (**182**), Lee et al. [67] reported the synthesis of a new class of 6-azaindole-1-benzenesulfonamides (**185a–k**) by using the conditions of the Bartoli indole synthesis [68]. Treatment of 7-bromo-6-azaindole (**183**) with 4-methoxybenzenesulfonyl chloride yielded the corresponding N^1-substituted derivative (**184**) that was finally engaged in a Suzuki coupling reaction with various substituted phenylboronic acids to obtain the 6-azaindole-1-benznesulfonamides (**185a–k**) (Figure 6.39) [67].

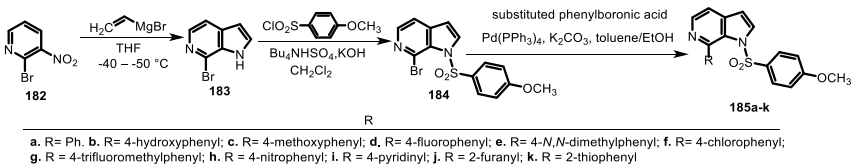


Figure 6.39: Synthesis of 6-azaindole-1-benznesulfonamides (**185a–k**).

The antiproliferative activity of compounds (**185a–k**) was evaluated against cervical carcinoma (KB cells), colorectal carcinoma (HT29 cells), stomach carcinoma (MKN45 cells) and lung carcinoma (H460 cells) cell lines: [67]. Compound (**185j**) showed the most potent anticancer activity against all cancer cell lines, with IC_{50} value of 21.1 nM (against KB), 32.0 nM (against HT29), 27.5 nM (against MKN45) and 40.0 nM (against H460). Also, compound (**185j**) inhibited tubulin polymerization by binding to the colchicine binding site of tubulin and induced cell cycle arrest in the G2/M phase [67]. The *in vivo* evaluation of compound (**185j**) using the HT-29 xenograft nude mice model revealed that it acts as a vascular disrupting agent and exhibits substantial dose-dependent inhibitory activity.

Liu et al. [69] synthesized 6-azaindole derivatives (**189a–b**) as potential inhibitors of tyrosine kinases receptors (FGFRs), which are attractive targets for cancer treatment. The reaction of 3-iodo-4-methylbenzoic acid (**186**) with oxalyl chloride gave the benzoyl chloride (**187**), which was reacted with 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline to produce the amide (**188**). Final coupling with the appropriate TMS-substituted alkyne generated the target compounds (**189a–b**) (Figure 6.40) [69]. The FGFR1/KDR enzymatic and FGFR1 translocated KG1 cellular activities for compounds (**189a–b**) revealed a good selectivity for FGFR1 over KDR. Compound (**189b**) exhibited potent inhibitory activity against FGFR1 (IC_{50} = 1.1 nM), weak KDR inhibitory activity (IC_{50} = 63.5 nM) and a potent KG1 cellular activity (IC_{50} = 10.0 nM) [69].

Based on the PROTACs (Proteolysis Targeting Chimeras) approaches, Zhang et al. [70] have synthesized pyrrolopyridone derivatives as potential BRD4 (Bromodomain-containing protein 4) degraders, BRD4 being known to play a decisive role in the epigenetic regulation of gene transcription. The pyrrolopyridone derivatives were obtained using a multi-step procedure, starting with nucleophilic substitution reaction of 2-bromo-1-fluoro-4-nitrobenzene (**190**) with 2,4-difluorophenol (**191**) to afford the intermediary compound (**192**). Further reactions, which include reductions, sulfonylation, condensation, *N*-protection/deprotection, demethylation, *N*-methylation, Suzuki coupling and a final amide condensation with propargylamine (12 steps), afforded pyrrolo[2,3-*c*]pyridine-2-carboxamide intermediate (**193**) (Figure 6.41) [70].

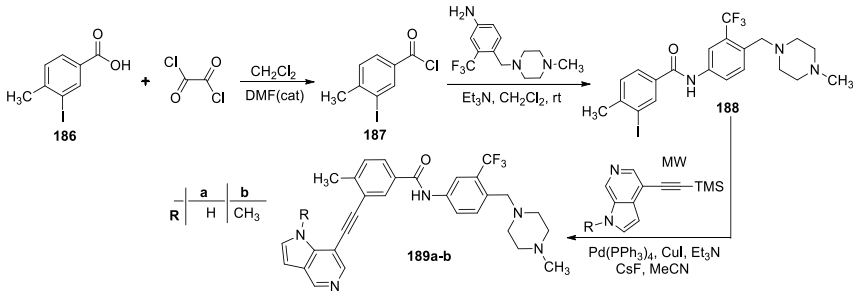


Figure 6.40: Synthesis of 6-azaindole derivatives (**189**).

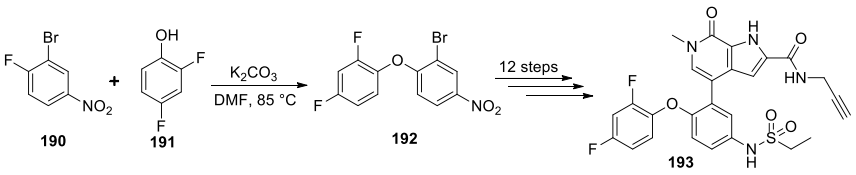


Figure 6.41: Synthesis of pyrrolo[2,3-*c*]pyridine-2-carboxamide intermediate (**193**).

Tosylation of polyethylene glycols (**194**) using 4-tosyl chloride delivered compounds (**195**) that next, by nucleophilic substitution reaction with sodium azide, produced the intermediates (**196**). Subsequent nucleophilic substitution of *tert*-butyl bromoacetate using azides (**196**) yielded the intermediates (**197**) that were transformed into the carboxylic acids (**198**) by deprotection with trifluoroacetic acid. Compounds (**198**) were reacted with thionyl chloride to provide the corresponding acyl chlorides, which were converted to pyrrolo[2,3-*c*]pyridine-2-carboxamides (**199**) by condensation with lenalidomide. Finally, compounds (**199**) were engaged in a "click reaction" with intermediate (**193**) (Figure 6.41) to produce target pyrrolopyridone derivatives (**200a–d**) (Figure 6.42) [70].

The antiproliferative activity of compounds (**200a–d**) against BxPC3 cell line (pancreatic cancer) as well as their potency as BRD4 BD1 protein degraders was evaluated. All the compounds showed potent binding affinities against BRD4 BD1, with the most potent compound (**200a**) showing IC_{50} value of 2.7 nM and significantly

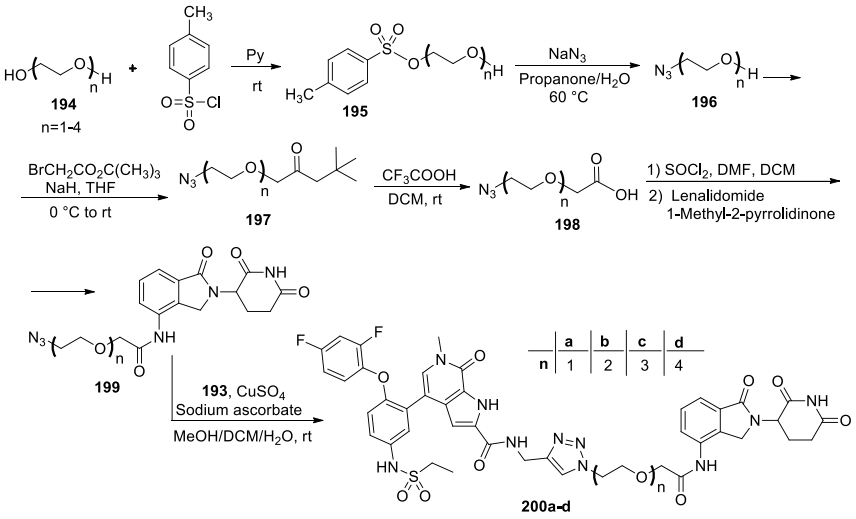


Figure 6.42: Synthesis of pyrrolopyridone derivatives (**200**).

inducing the degradation of BRD4 in a time-dependent manner, which resulted in the continuous decrease of c-Myc expression. Compound (**200a**) also exhibited the highest antiproliferative activity on BxPC3 cell line (IC_{50} = 0.165 μM/0.245 μM), revealing a good therapeutic potential for human pancreatic cancer. Also, the study of intracellular antitumor mechanism indicated that degrader (**200a**) could effectively induce the cell cycle arrest and apoptosis [70].

6.2.4 1*H*-pyrrolo[3,2-*b*]pyridines (4-azaindoles)

4-Azaindole in an important moiety in the structure of several biologically potent compounds, including antitumor derivatives [38, 39]. Similar strategies to those used for building other azaindoles, can be also used for their synthesis (e.g. Fischer reaction, Michael addition, Doebner-Knoevenagel reaction, 1,3-dipolar cycloaddition Suzuki-Miyaura or Sonogashira coupling reactions, domino synthesis) [36, 38, 39].

Kim et al. [71] reported the synthesis of pyrrolo[3,2-*b*]pyridine-*b* derivatives (**204**) containing both 5-benzylamide and 4′-amide moieties in their structure, in order to evaluate their antiproliferative activity against melanoma. Pyrrolo[3,2-*b*]pyridine (**202**) was obtained starting from 5-aminopicolinic acid (**201**) in six steps. Treatment with benzylamine generated the corresponding benzylamide (**203**) that was subjected to a condensation with substituted benzoic acid derivatives to yield the target pyrrolo[3,2-*b*] pyridines (**204a–d**) (Figure 6.43) [71].

Compounds (**204a–d**) were tested *in vitro* for their anticancer activity against A375 melanoma cell line and HS 27 fibroblast cell line. Both, compounds (**204a**) and (**204c**) showed excellent activity against A375 cell line, with low IC_{50} values of 0.7 and 0.9 μM, respectively. Also, the activity on HS 27 human fibroblast cell line was satisfactory, with IC_{50} values of 1.5 μM (compound **204a**) and 2.6 μM (compound **204c**) [71].

Novel 4-azaindole derivatives as potential TGFβ receptor kinase inhibitors were reported by Zhang et al. [72], since it is accepted that the multifunctional cytokine TGFβ have a crucial role in the regulation of anti-tumor immunity. The 4-azaindole derivatives (**209a–d**) were obtained starting with a typical Sonogashira coupling reaction

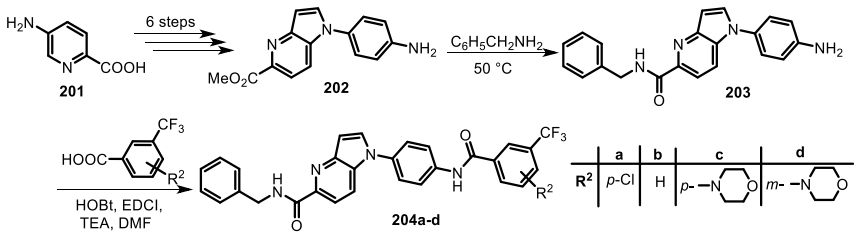


Figure 6.43: Synthesis of pyrrolo[3,2-*b*]pyridine-*b* derivatives (**204**).

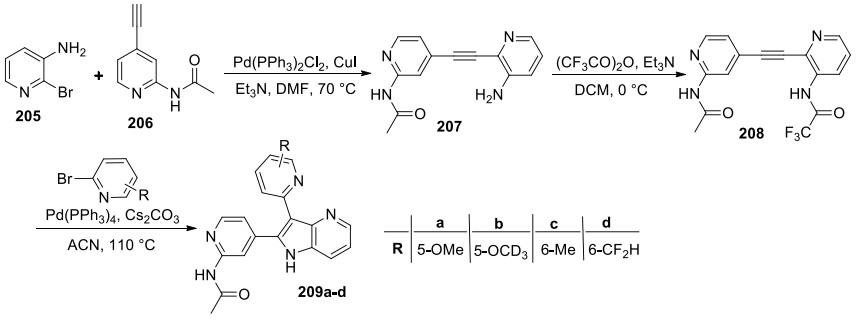


Figure 6.44: Synthesis of pyrrolo[3,2-*b*]pyridinyl-acetamides (**209a–d**).

of bromopiridine (**205**) with pyridinylacetamide (**206**) that generated the alkyne intermediate (**207**). Compound (**207**) was subsequently acylated using trifluoroacetic anhydride to afford derivative (**208**). Final treatment with 2-bromopyridine derivatives under a modified Cacchi palladium mediated domino alkylation-cyclization reaction yielded target pyrrolo[3,2-*b*]pyrridinyl-acetamides (**209a–d**) (Figure 6.44) [72].

Compounds (**209a–d**) were evaluated for their antitumor potential, by binding to cell-surface serine and threonine kinase receptors TGFβ receptor type I (TGFβRI) and type II (TGFβRII). Compound (**209b**) was identified as a good inhibitor of TGFβ receptor kinase, with important selectivity for TGFβ receptor 1 kinase (IC_{50} = 0.002 μM). Also, the synergic oral administration of compound (**209b**) with anti-PD-1 antibody led to persisting anticancer activity in MC38 tumor model, and no observed toxicity [72].

Based on the above-mentioned high *in vitro* cytotoxicity of platinum (II) complexes containing 7-azaindoles [48–50], Štarha et al. [73, 74] reported the synthesis of four new diiodido platinum (II) complexes that possess a 4-azaindole moiety. First alkylation of 4-azaindole (**210**) using sodium hydride and 2-bromopropane yielded the corresponding N^1-isopropyl-4-azaindole (**210a**). Complexes (**212a–b**) were obtained by initial treatment of potassium tetrachloroplatinate (II) $K_2[PtCl_4]$ (**211**) with potassium iodide, through a tetraiodoplatinate (II) intermediate $K_2[PtI_4]$, followed by subsequent treatment with 4-azaindoles (**210**) and (**210a**). Starting also from $K_2[PtCl_4]$ (**211**) and following a four steps procedure, the $K[PtI_3(NH_3)]$ intermediate (**213**) was obtained. Final engagement in a reaction with 4-azaindoles (**210**) and (**210a**) afforded complexes (**214a–b**) (Figure 6.45) [74].

Complexes (**212**) and (**214**) were evaluated for their *in vitro* cytotoxicity against three cancer cell lines: ovarian carcinoma A2780 cells, ovarian carcinoma A2780R cells (with acquired resistance to cisplatin) and colon carcinoma HT-29 cells (intrinsically resistant to cisplatin). Complexes (**212b**) and (**214b**) showed significant cytotoxic activity on A2780 and A2780R cell lines, with IC_{50} values in the range of 2.9–32.4 μM, suggesting a positive effect of N^1-alkylation on biological profile of the compounds. The higher cytotoxicity of complex (**212b**) at both the A2780 and A2780R cells resulted in

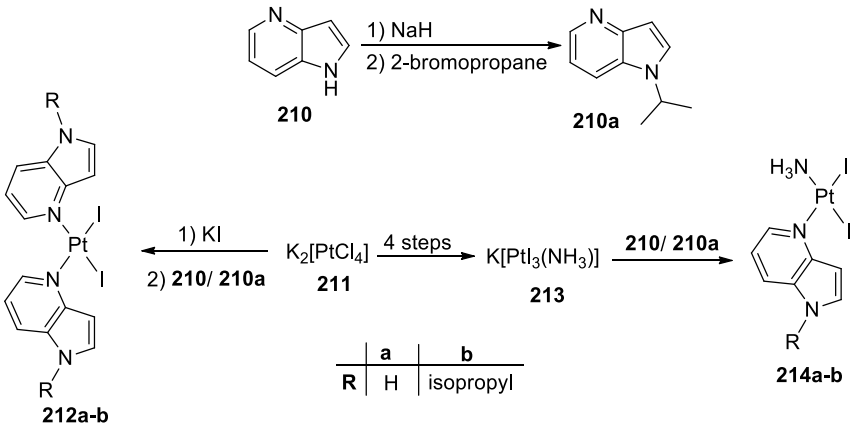


Figure 6.45: Synthesis of diiodido platinum (II) complexes (**212**) and (**214**).

the low resistance factor (RF ~ 0.8), indicating its ability to defeat the acquired resistance of these cancer cells against cisplatin. Also, the *in vitro* antiproliferative activity of all four complexes (**212a–b**) and (**214a–b**) against the HT-29 cells was evaluated. IC_{50} values of 4.2–8.0 μM were obtained, superior to the ones obtained with the reference cisplatin (IC_{50} > 50 μM) [74].

Starting from 4-azaindole (**210**), Swamy et al. [75] synthesized a series of amide derivatives of azaindole-oxazoles (**221a–h**) using a multi-step procedure. Firstly, treatment of 4-azaindole with hexamethylenetetramine (HMTA) generated aldehyde (**215**), that by condensation with hydroxylamine hydrochloride afforded oxime (**216**). Further reaction with acetic anhydride gave the nitrile (**217**) that was subjected to partial hydrolysis with aqueous NaOH in ethanol to yield amide (**218**). Amide (**218**) was then reacted with 3-bromo-2-oxopropanoate to afford the oxazole-ester (**219**) that undergoes basic hydrolysis with aqueous NaOH to generate the corresponding oxazole-acid intermediate (**220**). Finally, condensation of acid (**220**) with various aromatic amines afforded the target amides (**221a–h**) (Figure 6.46) [75].

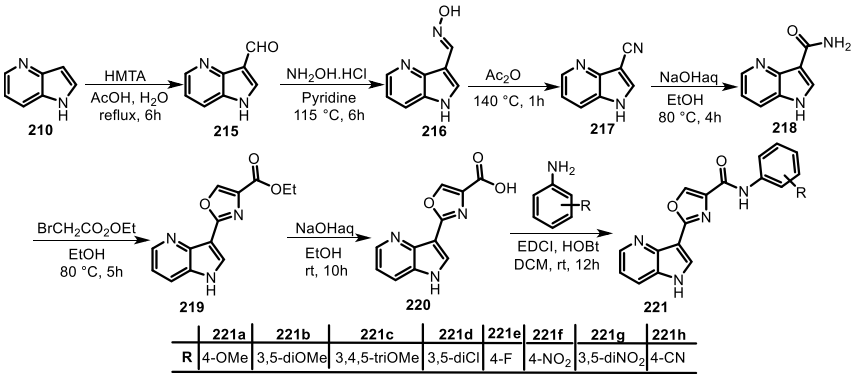


Figure 6.46: Synthesis of amide derivatives of azaindole-oxazoles (**221**).

The amide derivatives (**221a–h**) were tested for their anticancer activity against MCF7 breast cancer, A549 lung cancer and A375 melanoma cell lines. All tested compounds showed moderate to excellent anticancer activity with the highest activities for compounds (**221d**) and (**221g**) that showed IC_{50} values of 0.036 and 0.034 μM, respectively, against MCF7cell (breast cancer). Also, compound (**221f**) exhibited potent anticancer activity on both MCF7 cells (breast) and A549 cells (lung), with IC_{50} values of 0.22, and 0.14 μM, respectively. Furthermore, molecular docking study on EGFR revealed that both potent compounds (**221f**) and (**221g**) strongly interact with protein EGFR receptor [75].

6.2.5 1*H*-pyrrolo[3,2-*c*]pyridines (5-azaindoles)

5-Azaindole core was also found in the structure of synthetic derivatives that provided interesting pharmacological properties [38, 39], but there are only few showing anticancer properties. Their synthetic development is based on reactions as: isocyanide rearrangement, [3 + 2] cycloaddition, Hemetsberger reaction, intramolecular Suzuki-Miyaura reaction, Sonogashira coupling reactions, copper-catalyzed three-component coupling reaction, or multistep reaction from pyrrole [36, 38, 39].

El-Gamal et al. [76] reported the synthesis of a new *bis*-amide with pyrrolo[3,2-*c*]pyridine-*c* moiety. Synthesis of the target compound (**229**) involves the first oxidation of pyrrolo[2,3-*b*]pyridine (**66**) using 3-chloroperoxybenzoic acid (**222**) to obtain the corresponding 7-hydroxy-1*H*-pyrrolo[2,3-*b*]pyridinium 3-chloroperoxybenzoate (**223**) which was converted into the *N*-oxide compound (**224**) when treated with potassium carbonate. Subsequent heating with methanesulfonyl chloride, yielded 4-chloro-pyrrolo[2,3-*b*]pyridine (**225**). Heating of derivative (**225**) with *m*-nitroaniline led to nucleophilic substitution of the 4-chloro group, and subsequent rearrangement of the resulting secondary amine generated hydrochloride salt of pyrrolo[3,2-*c*]pyridine (**226**). Further treatment with benzoyl chloride generated amide derivative (**227**). Reduction of nitro group of compound (**227**) using $Pd–C/H_2$ led to the corresponding amino derivative (**228**), which was subjected to condensation with 3-(trifluoromethyl)-4-morpholinobenzoic acid to produce the *bis*-amide derivative with pyrrolo[3,2-*c*]pyridine (5-azaindole) scaffold (**229**) (Figure 6.47) [76].

Evaluation of kinase inhibitory activity of compound (**229**) over 47 different kinases revealed that it showed selectivity for FMS kinase (IC_{50} = 96 nM), a type III receptor tyrosine kinase that by binding to the macrophage or monocyte colony-stimulating factor (M-CSF or CSF-1), influences the cancer biology [76]. In a subsequent study realized by the same group, compound (**229**) inhibited the proliferation of IGF-1-induced cell and tumor transformation of JB6 Cl41 cells by binding to Raf-1 [77].

Following the same protocol starting from 7-azaindole (**66**) [76], El-Gamal et al. [78, 79] reported the synthesis of a new series of diarylureas and diarylamides derivatives that contain a pyrrolo[3,2-*c*]pyridine-*c* scaffold in their structure. Diarylurea derivative (**231a**)

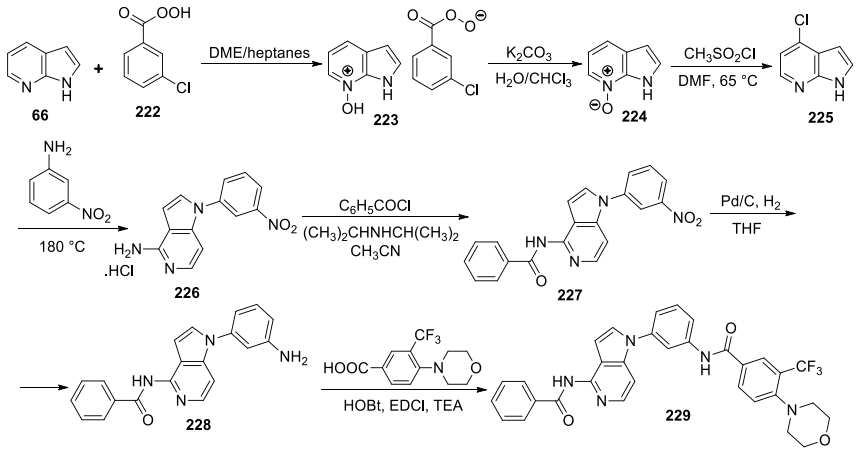


Figure 6.47: Synthesis of *bis*-amide derivative (**229**).

was obtained from the key intermediate (**230a**, *para*-amino substituted), while the diarylureas (**231b–f**) were obtained from compound (**230b**, *meta*-amino substituted), when treated with the appropriate aryl isocyanates. The *bis*-amide derivatives (**232a–h)** were obtained by condensation of amino group of compounds (**230a–b**) with the corresponding aromatic carboxylic acids in the presence of HOBt/EDCI/triethylamine. Thus, compounds (**232a–e**) were obtained from amines (**230a**), while compounds (**232f–h**) were synthesized from amines (**231b**) (Figure 6.48) [78, 79].

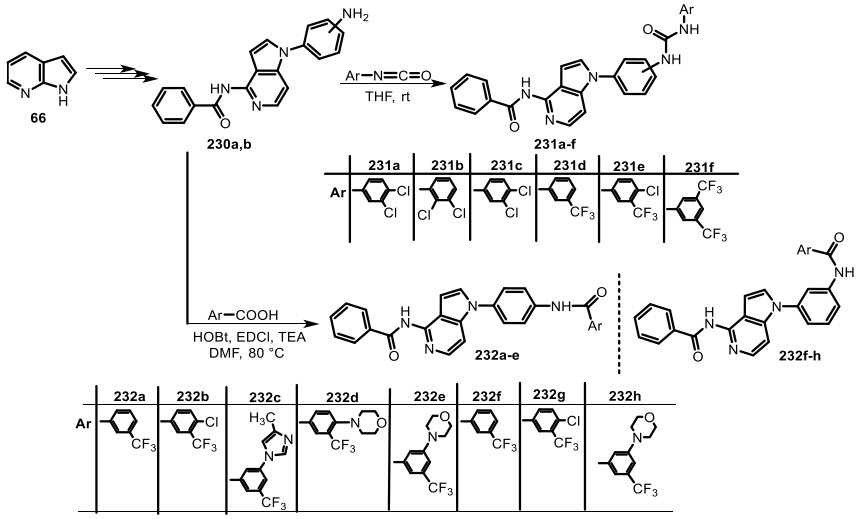


Figure 6.48: Synthesis of pyrrolo[3,2-*c*]pyridines (**231**) and (**232**).

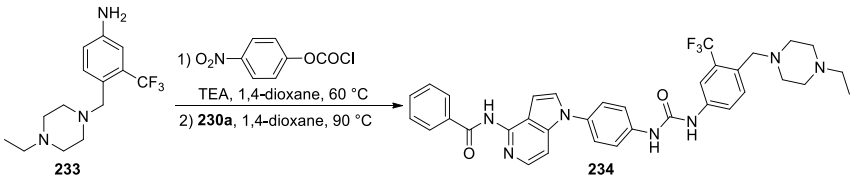


Figure 6.49: Synthesis of *N*-ethylpiperazinyl diarylurea derivative (**234**).

Also, *N*-ethylpiperazinyl diarylurea derivative (**234**) was obtained by a two step procedure that involves first, reaction of the amine (**233**) with 4-nitrophenyl chloroformate followed by the subsequent reaction with amine (**230a**) (Figure 6.49) [78].

Both, pyrrolo[3,2-*c*]pyridines based diarylureas and diarylamides were *in vitro* evaluated for their anticancer properties against A375P melanoma cell line. Among all the tested derivatives, the diarylureas (**231c**), (**231e**) and (**234**) showed the highest activity against A375P human melanoma cell line with IC_{50} values less than 0.1 μM. Also, compounds (**231a)**, (**232a**) and (**232c–e**), were efficient against A375P cell proliferation, with IC_{50} ranging from 0.1 to 0.8 μM [78, 79]. The most potent diarylamide (**231e**) and diarylurea (**234**) showed high efficacy against nine melanoma cell lines, with nanomolar IC_{50} values over three and 7 cell lines, respectively [78]. In the *bis*-amide series (**232a–h**), compounds (**232f–h**) showed the highest antiproliferative activity on A375P cell line, with IC_{50} values less than 0.1 μM [79]. The diarylureas (**231b**) and (**231d–e**) also exhibited a good antitumor profile on A375P human melanoma cell line, with IC_{50} values in the range of 0.1–0.7 μM. It is worth to highlight the antiproliferative activity of *bis*-amide derivative (**232f**) when tested over the NCI panel of nine melanoma cell lines (IC_{50} = 17–100 nM) [79].

The same group reported a new series of 18 pyrrolo[3,2-*c*]pyridines based diarylureas and diarylamides [80] with general structure (**235**) (Figure 6.50) synthesized following similar strategy as presented above [76–79].

All synthesized derivatives were evaluated for inhibitory properties against FMS kinase. Compound (**235a**) (Figure 6.50) showed the best poteny among all tested analogues (IC_{50} = 30 nM). Therefore, the leading compound was tested over a panel of 40

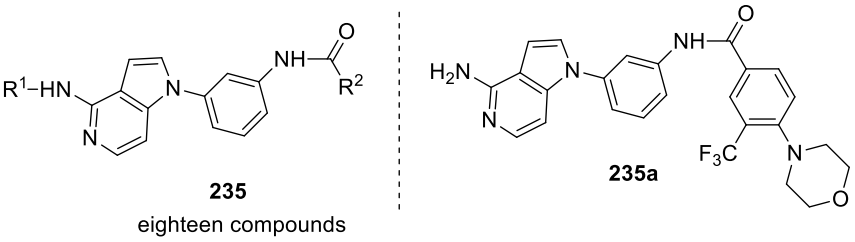


Figure 6.50: Structure of 5-azaindole derivatives (**235**).

kinases, and exhibited good selectivity for FMS kinase. Compound (**235a**) was also tested against bone marrow-derived macrophages (BMDM) and provided an IC_{50} value of 84 nM. The antiproliferative activity was evaluated against a panel of six ovarian, two prostate, and five breast cancer cell lines, compound (**235a**) providing good selectivity toward cancer cells (IC_{50} = 0.15–1.78 μM) than normal fibroblasts [80].

Starting from *N*-substituted 4-chloro-pyrrolo[3,2-*c*]pyridines (**236a, b**) and adapting a previously described procedure [81], Prudent et al. [82] achieved the synthesis of new 5-azaindole derivatives, as a part of their developed strategy to find new cell-permeable microtubule-targeting agents. The lithiation of pyrrolo[3,2-*c*]pyridines (**236a, b**) followed by treatment with corresponding acetophenones/benzophenone produced derivatives (**237a, b**), which on treatment with acetic anhydride and further dehydration yielded alkenes (**238a, b**). Final hydrogenation of alkenes (**238a, b**) afforded target derivatives (**239a–d**) (Figure 6.51) [82].

The anticancer potential of derivatives (**237–239**) was evaluated against 11 cancer cell lines, including cervix, kidney, lung and breast cancer. Compounds (**238a**) and (**239a**) were identified as good reversible microtubule-depolymerizing agents that exhibit important cytostatic efficacy on the tested human cancers, including MDR cells. As far the mechanism of action, azaindoles (**238a**) and (**239a**) are potent cytostatic compounds by arresting the cell cycle at the G2/M phase. *In vitro* assays revealed that both azaindole derivatives also inhibited tubulin polymerization. They were also found to efficiently inhibit angiogenesis and tumor growth in chorioallantoic breast cancer xenografts [82].

In 2016, Liu et al. [83] reported the synthesis of a series of new 5-azaindole derivatives which combine into their structure the imidazo[1,2-*a*]pyridine nucleus. The target compounds were designed as potential inhibitors of *c*-Met*c*-, a tyrosine kinase receptor known to be aberrantly activated in human cancers via mutation, amplification or protein overexpression [84]. The synthesis involved in the first step the treatment of imidazo[1,2-*a*]pyridine-sulfonic acid (**240**) with phosphorus oxychloride to produce the corresponding sulfonyl chloride (**241**). Subsequent *N*-alkylation of 5-azaindole generated compound (**242**) which was subjected to a Suzuki coupling reaction using various phenyl boronic acids, to provide the final compounds (**243a–c**) (Figure 6.52) [83].

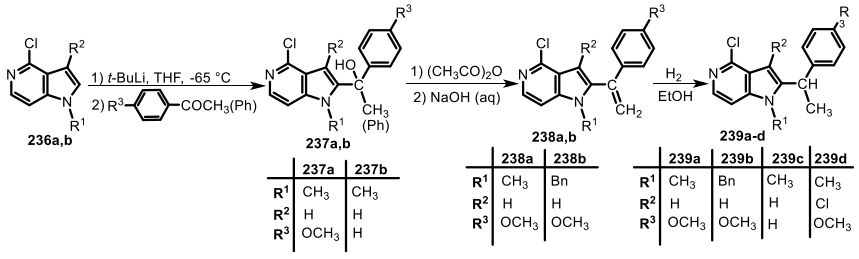


Figure 6.51: Synthesis of 5-azaindole derivatives (**237**–**239**).

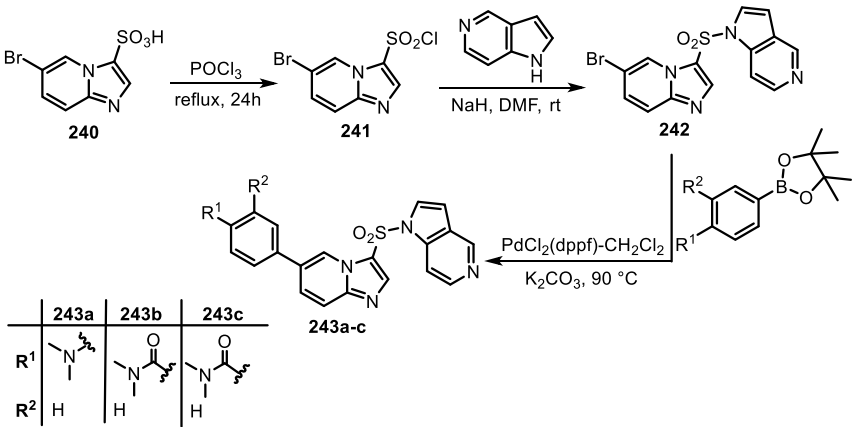


Figure 6.52: Synthesis of 5-azaindole derivatives (**243**).

The docking optimizations combined with the results of enzymatic and cellular assays revealed that compound (**243c**) inhibits the *c*-Met kinase*c*- activity (IC_{50} = 12.8 nmol/L). Also, compound (**243c**) inhibited in a dose-dependent manner the phosphorylation of *c*-Met and its key downstream Akt and ERK signaling cascades in *c*-Met aberrant human EBC-1 cancer cells. Moreover, compound (**243c**) dose-dependently reduced *c*-Met-mediated cell scattering of MDCK cells [83].

Dong et al. [85] designed and synthesized a novel 1*H*-pyrrolo[3,2-*c*]pyridine-*c*-2-carboxamide derivative as NAMPT and HDAC dual inhibitor. The synthesis started with the reaction of 2-(1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)acetic acid (**244**) with methyl 4-(aminomethyl)benzoate (**245**) using *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP), when was generated the carboxamido group from desired compound. The next two steps consisted in hydrolysis reaction of ester group, and coupling reaction with *o*-phenylenediamine, when the target compound (**247**) was obtained (Figure 6.53).

The *in vitro* cytotoxicity of novel synthesized compound (**247**) was studied against HCT116 colorectal carcinoma, MDA-MB-231 breast cancer and HepG2 liver cancer cell lines. The results showed that compound (**247**) was more active against HepG2 cells (IC_{50} = 1.7 μM) compared with the activity against HCT116 cells (IC_{50} = 4.0 μM) and MDA-MB-231 cells (IC_{50} = 48 μM) [85].

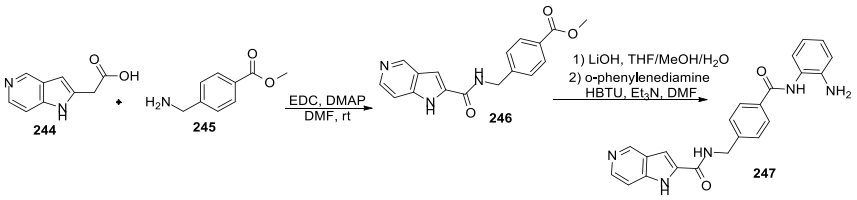


Figure 6.53: Synthetic route of synthesis of 1*H*-pyrrolo[3,2-*c*]pyridine-*c* derivative (**247**).

6.2.6 2*H*-pyrrolo[3,4-*c*]pyridines

Even if the most important biological properties of pyrrolo[3,4-*c*]pyridine-*c* derivatives are the analgesic and sedative effects, there are also several pyrrolo[3,4-*c*]pyridines reported to possess other pharmacological profile, including anticancer [86].

Kalai et al. [87] designed and synthesized novel dihydropyrrolo[3,4-*c*]pyridine *N*-oxide derivative and studied its cytotoxicity against two different human cancer cell lines (A2780 and MCF-7), but also against healthy cardiac cells (H9c2). Target derivative (**250**) was synthesized by *N*-alkylation of 3,5-*bis*(4-fluorobenzylidene)piperidin-4-one (**248**) with halide (**249**) (Figure 6.54) [87].

The results of anticancer activity showed that compound (**250**) had moderate cytotoxicity against A2780 (5.26% viability) and limited toxicity toward MCF-7 (42.71% viability) and H9c2 (46.57% viability) [87].

Novel potent inhibitors of human nicotinamide phosphoribosyltransferase (NAMPT), with 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-*c* unit, were developed by Dragovich et al. [88]. NAMPT inhibition is considered one of the anticancer therapy strategies, thus blocking NAMPT activity may damage the cell growth. The synthesis of desired compounds consisted in the reaction of various starting benzylic amines (**251a–e**) with 4-nitrophenyl carbonochloridate (**252**) to give the corresponding 4-nitro-phenyl carbamates (**253a–e**). The carbamates derivative (**253a–e**) condensed in next step with 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (**254**) to generated the desired potential inhibitors of NAMPT (**255a–e**) (Figure 6.55) [88].

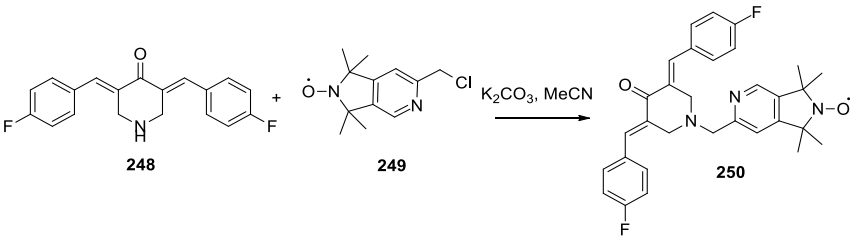


Figure 6.54: Synthesis of novel dihydropyrrolo[3,4-*c*]pyridine *N*-oxide derivative (**250**).

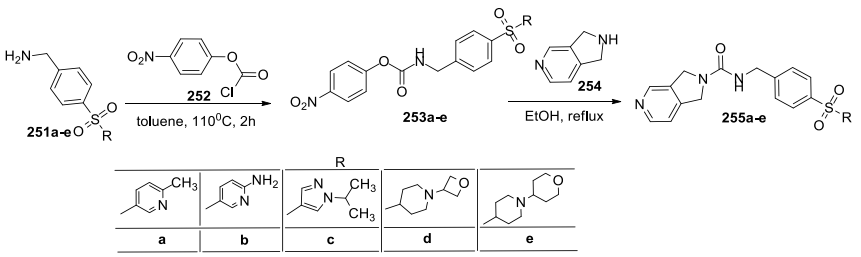


Figure 6.55: Synthetic route of pyrrolo[3,4-*c*]pyridine-*c* derivatives (**255**).

The activity of the synthesized derivatives (**255a–e**) against NAMPT was strong (IC_{50} in the range of 0.011 μM (for compound (**255a**)) and 0.020 μM (for compound (**255b**)). Tested compounds showed also *in vitro* good stability against human liver microsomes. Antiproliferative evaluation revealed effects of compound (**255a**) against various human tumor cell lines: HT1080 fibrosarcoma (IC_{50} = 25 nM), PC3 prostate carcinoma (IC_{50} = 36 nM), MiaPaCa2 pancreatic cancer (IC_{50} = 70 nM) and HCT116 colorectal carcinoma (IC_{50} = 20 nM) [88].

Lam et al. [89] reported synthesis of 1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one *H*-pyrrolo[3,4-*c* derivatives (**262**) for being tested as inhibitors of Spleen Tyrosine Kinase (SYK). The synthesis of the desired derivatives was performed in a multistep sequence that started with transformation in three steps of 2,6-dichloro-5-fluoronicotinic acid (**256**) to lead 4,6-dichloro-2-(2,4-dimethoxybenzyl)-7-fluoro-1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one (**257**) that by treatment with *tert*-butyl (1*S*,2*R*)-2-amino-cyclohexylcarbamate (**258**), using *N*,*N*-diisopropylethylamine (DIEA), was converted into derivative (**259**). Next step consisted in Suzuki coupling with two different boronic acids (**260a, b**), when derivatives (**261a, b**) were obtained. The final step, Boc deprotection, gave the desired 1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one derivatives (**262a, b**) (Figure 6.56) [89].

Biological evaluation revealed that compound (**262b**) inhibits cellular proliferation in SYK-dependent DLBCL and FLT3-dependent AML cell lines, and shows notable tumor growth inhibition in SYK- and FLT3-dependent xenograft models [89]. For this compound the clinical trials are ongoing, consisting in treatment of advanced solid tumors and lymphoma malignancies.

Wojcicka et al. [90] synthesized new *N*-substituted 1*H*-pyrrolo[3,4-*c*]pyridine-*c*-1,3(2*H*)-diones (**265**) and (**267**) and studied their anticancer properties against human leukemia cells MV-4-11. The target compounds were obtained by reaction of 4-methyl-

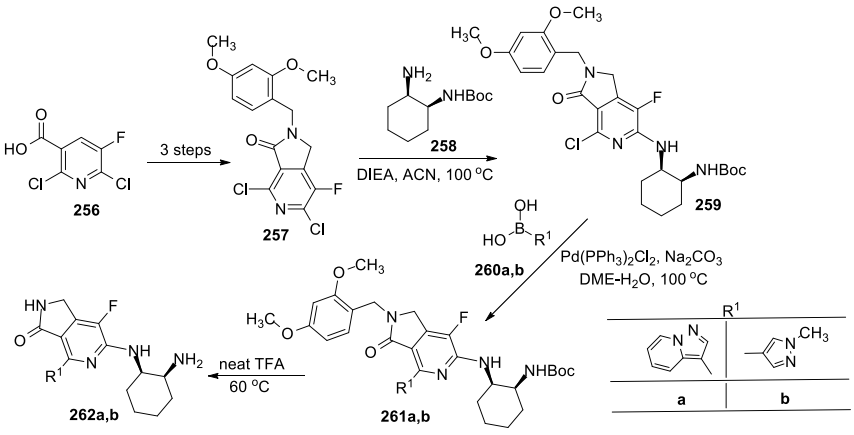


Figure 6.56: Synthesis of 1*H*-pyrrolo[3,4-*c*]pyridin-3(2*H*)-one *H*-pyrrolo[3,4-*c*derivatives (**252**).

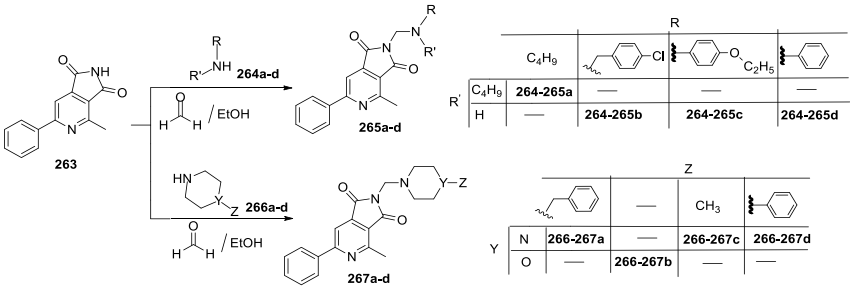


Figure 6.57: Synthesis of 1*H*-pyrrolo[3,4-*c*]pyridine-*c*-1,3(2*H*)-diones (**265**) and (**257**).

6-phenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione (**263**) with amines (**264a–d**) or substituted piperazine and morpholine derivatives (**266a–d**), using formaldehyde in ethanol (Figure 6.57).

The anticancer studies showed that all the tested compounds have good activity, the most active compound from the series being (**265d**) with IC_{50} value of 21.8 μg/mL [90].

6.3 Fused pyrrolo-(iso)quinolines

Quinoline and isoquinoline are considered privileged scaffolds in anticancer medicinal chemistry having shown their controlling effects on cancer treatment *via* various pathways. Both moieties are versatile bicyclic heterocycles with huge therapeutic potential, being very accessible for designing new drugs [91, 92].

In the search for new medicines with improved pharmacological properties and less side-effects, besides multiple strategies of derivatization, the fusion of (iso)quinoline with a pyrrole ring (another important pharmacophore) leaded to new fused pyrrolo-(iso)quinoline cores, part of them being the scaffold of compounds providing anticancer potential (Figure 6.58).

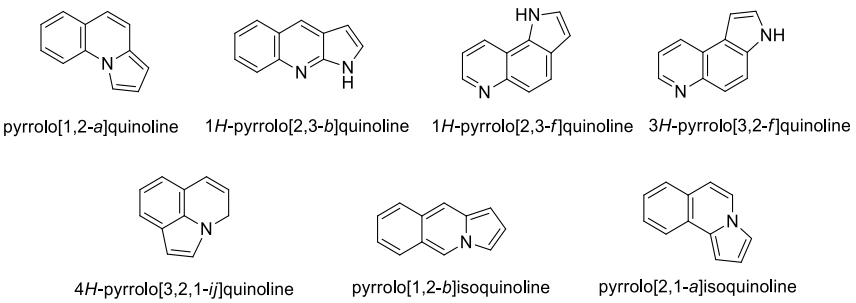


Figure 6.58: Structures of pyrrolo-(iso)quinoline systems present in compounds with anticancer properties.

In this subchapter, the focus is on the recent reports of potential exploitation of the above scaffolds (Figure 6.58) in design and synthesis of new anticancer derivatives. Each next section is focused on the synthesis and the anticancer properties of derivatives containing each of these heterocyclic ring systems.

6.3.1 Pyrrolo[1,2-*a*]quinoline

There are several natural alkaloids that contain reduced forms of pyrrolo[1,2-*a*]quinoline-*a*, the most known being gephyrotoxin, a muscarin antagonist [93]. Other pyrrolo [1,2-*a*]quinoline derivatives have been shown to exert diverse biological activities [94]. Thus, synthetic methodologies to build that system are continuously improving starting from quinolines, pyrroles, and *N*-acyclic compounds which undergo one or double cyclization reactions [94].

Kemnitzer et al. [95] reported the synthesis of a family of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines as novel apoptosis inducers through caspases activation. Their study had as model compound, the commercially available 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinoline derivative, known for its selective activity against several breast cancer cell lines. The goal of this research was to study the influence of the substituents of position three of pyrrolo[1,2-*a*]quinoline-*a*, and of position four of the benzoyl group on the biological properties. The target compounds (**270a–g**) were obtained in two steps: quaternization of quinoline with 2-bromoacetylphenones (**268**), followed by 1,3-dipolar cycloaddition of quinolinium salts (**269a–g**) to acrylonitrile in basic medium, in the presence of TPCD or MnO_2 as oxidant. Compounds (**271a)** and (**271b)** were obtained by nucleophilic substitution from (**269c**), using imidazole and pyrazole, respectively (Figure 6.59) [95].

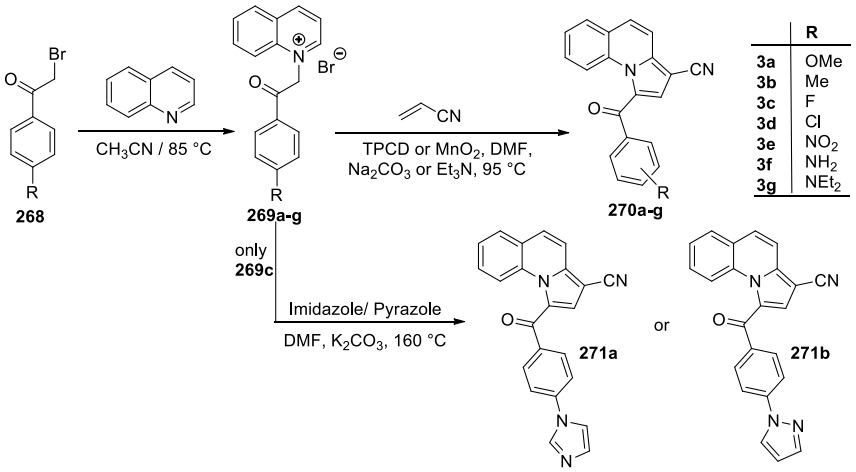


Figure 6.59: Synthesis of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinoline derivatives (**270–271**).

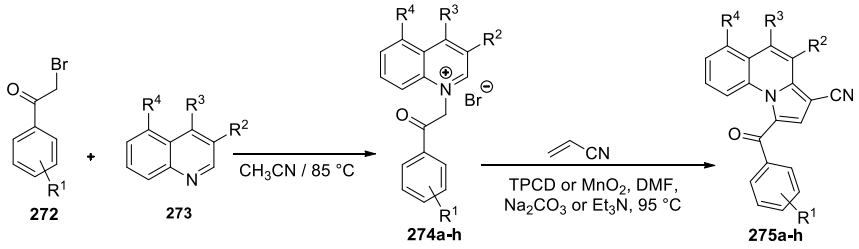
Compounds (**270a–g**) showed different selectivities across the three cancer cell lines (breast cancer cells T47D, colon cancer cells HCT116 and hepatocellular carcinoma cancer cells SNU398) they were tested on. The most active compounds have an imidazole (**271a**), and pyrazole (**271b**) substituent, respectively, at the 4-position of the benzoyl group. Interestingly, these compounds show a different caspase activation profile. Compounds (**271a–b**) also induced apoptosis by inhibiting tubulin polymerization. It was found that 3-cyano group was found to be crucial for activity, while other EWGs at the *para*-position of the benzoyl group like F and Cl are preferred to preserve efficacy and selectivity in the caspase activation assay [95].

The same group, in order to find new candidates as potential anticancer agents, have exploited the 4-, 5-, 6-, 7- and 8-positions of pyrrolo[1,2-*a*]quinolines by synthesizing derivatives (**275**) [96] in a similar manner, as presented in Figure 6.60. The dipolarophile used for 1,3-dipolar cycloaddition of the quinolinium salts (**274**), was acrylonitrile, to obtain compounds (**275a–h**).

SAR analysis showed that substitution at the 6-position by a small group such as Cl, led to compounds with high potency and selectivity against breast cancer cell lines. Thus, compounds (**275c**) and (**275f–h**) showed low nanomolar potency against breast cancer cells T47D in the caspase activation assay. Substitutions at the positions 5- and 8- reduced 3–7 folds the activity, while substitutions of the positions 4- and 7- resulted in inactive compounds [96].

Al-Matarneh et al. [97] reported the synthesis of new pyrrolo[1,2-*a*]quinoline-*a* derivatives by [3 + 2] cycloaddition reaction of the *in situ* generated quinolinium ylides to fumaronitrile (Figure 6.61).

The cycloaddition reaction to fumaronitrile occurred stereoselectively in case of salts (**277a–b**), leading tetrahydropyrrolo[1,2-*a*]quinolines (**278a–b**). In case of salt (**277**c), the



275	R1	R2	R3	R4
a	H	Me	H	H
b	H	H	Me	H
c	H	H	H	Cl
d	H	H	H	NO_2
e	H	H	H	OH
f	*p*-F	H	H	Cl
g	*p*-NO_2	H	H	Cl
h	*m*-OMe	H	H	Cl

Figure 6.60: Synthesis of pyrrolo[1,2-*a*]quinoline-*a* derivatives (**275**).

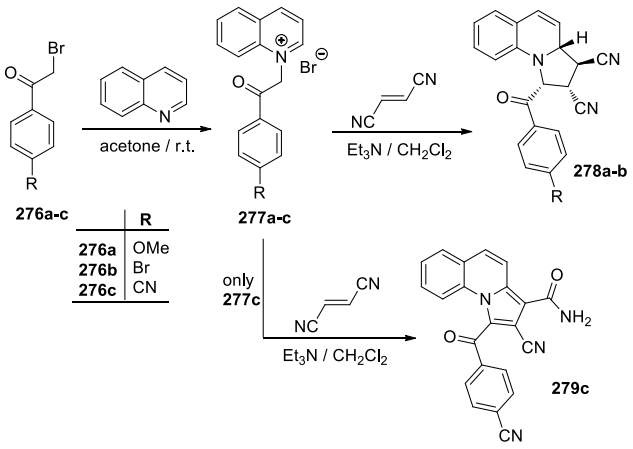


Figure 6.61: Synthesis of (tetrahydro)pyrrolo[1,2-*a*]quinoline-*a* derivatives (**278**).

aromatized 2-cyano-1-(4-cyanobenzoyl)pyrrolo[1,2-*a*]quinoline-*a*-3-carboxamide (**279c**) was obtained, due to the complete aromatization and hydrolysis of one of the cyano groups of pyrrole ring. Compound (**278a**) showed potent anticancer activity with GI_{50} values in the range of 0.197–3.49 μM against the NCI panel of 40 cancer cell lines. *In vitro* assays and molecular docking showed that compound (**278a**) also posses tubulin interaction properties by binding the colchicine site of tubulin [97].

6.3.2 Pyrrolo[2,3-*b*]quinoline

The pyrrolo[2,3-*b*]quinoline-*b* is a privileged scaffold present in active biologically molecules such as blebbistatin (a myosin II inhibitor) [98] and PGP-4008 (a selective P-glycoprotein (P-gp) mediated drug release (MDR) modulator) [99]. Thus, biological profile and interesting molecular architecture arouse interest to develop facile synthetic approach to build pyrrolo[2,3-*b*]quinoline structure motif [100, 101].

Searching for multiple drug resistance (MDR) modulators, Lee et al. [99] identified a novel series of substituted dihydopyrroloquinolines that selectively inhibit the function of P-glycoprotein (Pgp) without modulating multidrug resistance-related protein 1 (MRP1). Condensation of 2-aminobenzonitrile (**280**) with pyrrolidinone (**281**) afforded the corresponding 2-(1-benzylpyrrolidin-2-ylidenemethyl)benzonitrile (**282**). LDA reduction of cyano group furnished amine (**283**) that was subjected to alkylation and acetylation, respectively, to yield compounds (**284a–d**)/(**285a–d**) (Figure 6.62).

Biological evaluation demonstrated the capacity of all tested compounds modulate Pgp in a highly selective manner with maximal efficacy exhibited by compound (**285a**). Further *in vivo* testing demonstrated its potency to reverse Pgp mediated MDR in

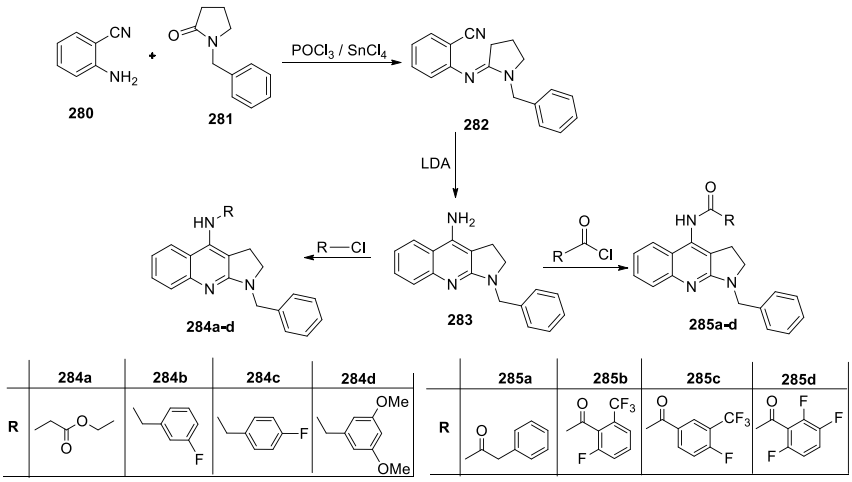


Figure 6.62: Synthesis pathway of dihydopyrroloquinolines (**284**) and (**285**).

a solid tumor model, and also an excellent pharmacokinetic profile that make compound (**285a**) a good candidate for clinical utility [99].

6.3.3 Pyrrolo[2,3-*f*]quinoline

The most known compound with pyrrolo[2,3-*f*]quinoline-*f* skeleton is undoubtedly pyrroloquinoline quinone (PQQ), that serves as a redox cofactor of a number of prokaryotic dehydrogenases, such as alcohol and sugar dehydrogenases, and is exploited for the potential health benefits and pharmacological applications [102]. There are also several reports of pyrrolo[2,3-*f*]quinoline derivatives with anticancer properties presented below.

Vlachou et al. [103] reported an efficient two-step method for the synthesis of pyrrolo [2,3-*f*]quinolines. The treatment of 5-nitroquinoline (**286**) with 4-chlorophenoxyacetonitrile in the presence of *t*BuOK, led to the corresponding nucleophilic substitution product (**287**). Further, catalytic hydrogenation gave the desired 1*H*-pyrrolo[2,3-*f*]quinoline-*f* (**288**). NaH mediated alkylation using 2-chloro-*N*,*N*-dialkylethylamine hydrochloride yielded the corresponding *N*-alkylated derivatives (**289a–b**) (Figure 6.63).

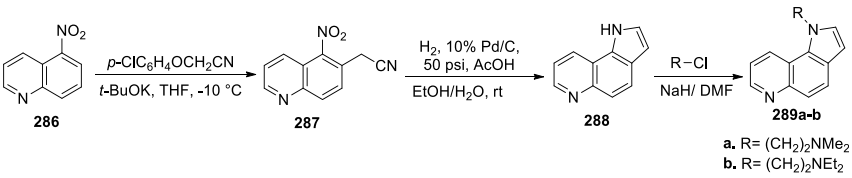


Figure 6.63: Synthesis of *N*-alkyl-1*H*-pyrrolo[2,3-*f*]quinolines (**289a–b**).

Pyrroloquinolines (**289a–b**) exhibited significant cytotoxic activity on five ovarian carcinoma cell lines, being more active against cisplatin resistant CH1cisR cell line (IC_{50} = 17.0 for (**289a**) and 16.5 μM for (**289b**)) compared to the corresponding parent CH1 line (both IC_{50} > 25 μM) [103].

Tsotinis et al. [104] also synthesized a series of new C2-substituted pyrrolo[2,3-*f*] quinolines (**296a–d**), by a strategy that developed a new four-step procedure for synthesis of the key intermediate (**295**). In the first step, oxidation of 6-methylquinoline (**290**) with SeO_2 leaded 6-quinolinecarboxaldehyde (**291**) that was subjected to nucleophilic attack of the carbanion generated *in situ* from the reaction of ester derivative (**292**) and sodium methoxide, to form the azido-6-quinolinopropenoic acid methyl ester (**293**). Key intermediate (**293**) was then cyclized to the ester (**294**) and by subsequent hydrolysis yielded the desired 1*H*-pyrrolo[2,3-*f*]quinolino-2-carboxylic acid (**295**). The target derivatives (**296a–d**) were prepared by treatment of carboxylic acid (**295**) with the appropriate amines in the presence of 1,1′-carbonyldiimidazole (CDI) (Figure 6.64).

From compounds (**296a–d**) *in vitro* evaluated for their cytotoxic properties against a four tumor cell lines panel, only compound (**296d**) showed antiproliferative activity against the human non-small lung cancer cell line NSCLC-N16-L16 (IC_{50} = 10.2 μM) [104].

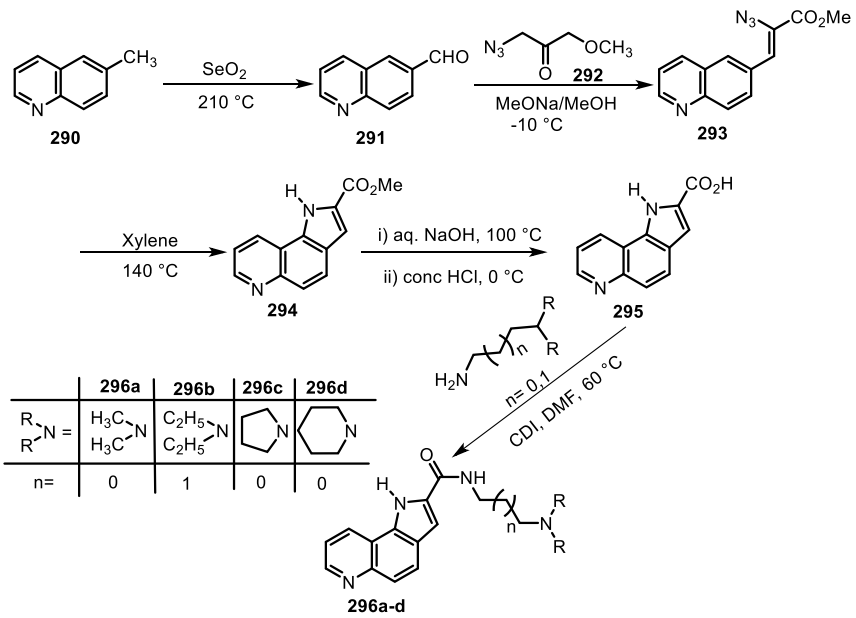


Figure 6.64: Synthesis of pyrrolo[2,3-*f*]quinoline-*f*-2-carboxamides (**296a–d**).

6.3.4 Pyrrolo[3,2-*f*]quinoline

Pyrrolo[3,2-*f*]quinoline is a less studied scaffold with great biological potential. However, we found several reports regarding synthesis and anticancer properties of such derivatives, discussed below.

Ferlin et al. [105] have intensively studied the synthesis and antiproliferative activity of various pyrrolo[3,2-*f*]quinoline-*f* derivatives, by introduction in different positions, the methanesulfon-anisidide residue characteristic to the known anticancer drug amsacrine (*m*-AMSA), as well the analogues lacking the *m*-methoxy substituent. Firstly, they reported the synthesis of 7-(un)substituted pyrrolo[3,2-*f*]quinolines, starting with catalytic reduction of 5-nitroindole (**297**). The acid-catalyzed condensation of the obtained 5-aminoindole (**298**) with ethyl acetoacetate yielded the enamine-derivative crotonate, which then underwent thermal cyclization to generate 7-methyl-9-hydroxy-pyrrolo[3,2-*f*]quinoline (**299**). When intermediate (**298**) was treated with diethyl ethoxymethylene malonate, it produced the corresponding enamine acrylate which was subjected to regioselective cyclization, to yield 9-hydroxy-pyrrolo[3,2-*f*] quinoline ethyl ester (**300**). Hydrolysis in alkaline medium was followed by thermal decarboxylation to afford derivative (**301**). Phosphorus oxychloride treatment of intermediates (**299**) and (**301**) allowed nucleophilic substitution of the hydroxyl group at C-9 with chlorine, to yield compounds (**302a–b**). Subsequent substitution of the chlorine with *p*-methanesulfonamido-aniline produced target pyrrolo[3,2-*f*]quinoline derivatives (**303a–b**) as hydrochlorides (Figure 6.65) [105].

Compounds (**303a–b**) were tested for their antineoplastic potential against the NCI's panels of cancer cell lines, compound (**303a**) showing potency against leukemia

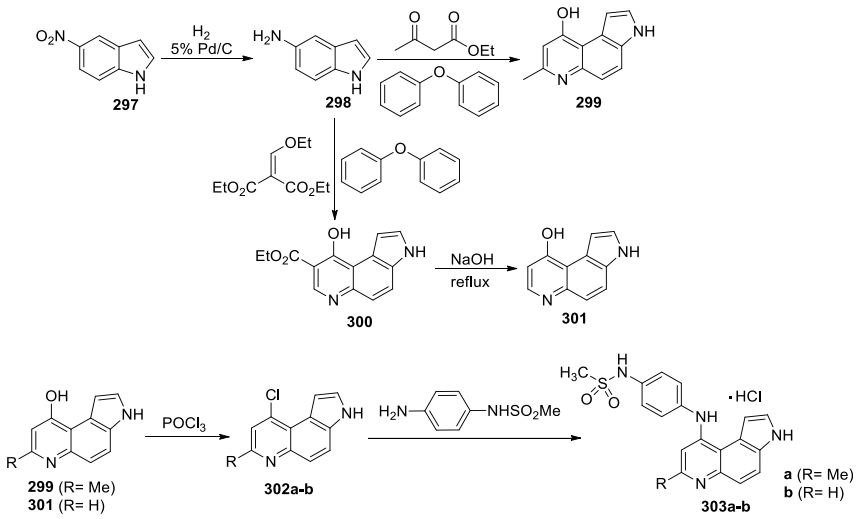


Figure 6.65: Synthesis pathway of pyrrolo[3,2-*f*]quinoline-*f* derivatives (**303**).

subpanel of cell lines, but also, against other cells obtained from solid tumors like CNS-, melanoma- and prostate-derived cells [105].

In order to further investigate the anticancer properties of the pyrrolo[3,2-*f*]quinoline-*f* family, the same group [106] also synthesized different substituted pyrrolo[3,2-*f*]quinolines. Thus, pyrrolo[3,2-*f*]quinolines (**305**) were first obtained by catalytic hydrogenation of the chlorinated analogue (**304**), then a classical Vilsmeier–Haach reaction was performed to obtain the 1-formyl derivatives (**306**). Condensation with *p*-methanesulfonamido-anilines and selective reduction using $NaBH_3CN$ gave the target compounds (**308**) in good yields, which were easily transformed into the water-soluble dihydrochlorides (**309a–d**) (Figure 6.66).

After *in vitro* primary NCI antitumor screening of compounds (**309**), derivative (**309b**) having the *m*-methoxy showed remarkable potency against leukemia cells, the best activity being achieved against CCRF-CEM and HL-60 cells (for both, GI_{50} = 34 nM) [106]. Comparison with previously investigated regioisomers [105], shows that modulation of activity is controlled by the position and conformational freedom of side-chain groups.

Ferlin's research group also directed on the synthesis of new pyrrolo[3,2-*f*]quinolines to act as DNA-direct alkylating agents [107]. Thus, 9-chloropyrroloquinoline derivative (**310**) was first alkylated using MeI in DMF, then the nucleophilic substitution of the chlorine atom with aniline derivatives (**312a–b**) yielded the target compounds (**313a–d**), with compound (**313a**) as hydrochloride (Figure 6.67).

Compounds (**313a–d**) were tested on human tumor cell lines HeLa and HL-60, with a good antiproliferative activity for compounds (**313b**) and (**313d**), providing IC_{50} values in the range of 0.45–0.66 μM, and no cytotoxic effects. Supplementary denaturation-renaturation experiments evidenced their ability to form cross-links with the double helix of DNA [107].

Ferlin et al. also reported the synthesis of new water soluble 3*H*-pyrrolo[3,2-*f*]quinolin-9-ones-*f* possessing one or two (2-diethylamino-ethyl) side chains in their

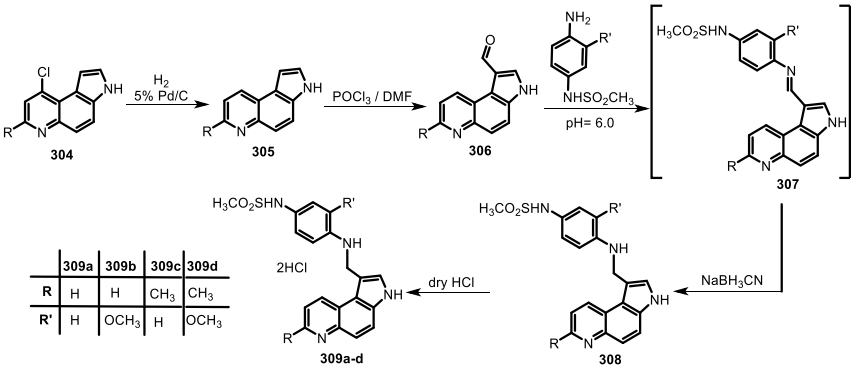


Figure 6.66: Synthesis of the substituted pyrrolo[3,2-*f*]quinolines (**309**).

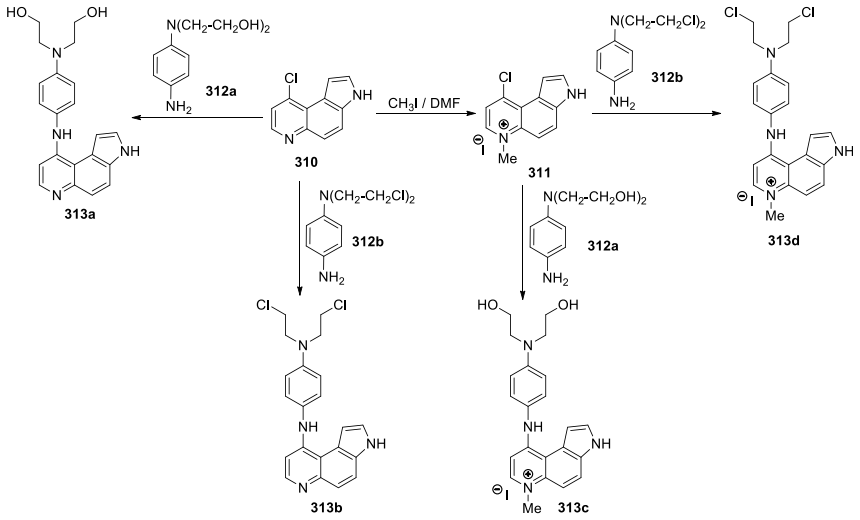


Figure 6.67: Synthesis of the amino-substituted pyrrolo[3,2-*f*]quinolines (**313**).

structures [108]. Catalytic reduction of the nitro-indole derivative (**314**) produced the corresponding amino-derivative (**315**), which by condensation with ethylacetoacetate furnished the enamine compound (**316**). By thermal cyclization, the quinolinone (**317**) is obtained. Finally, the treatment of compound (**317**) with (2-chloroethyl)-diethylamine gave a separable mixture of 3*N*-mono- and 3*N*,9-*bis*-functionalized substituted pyrroloquinolines (**318**) and (**319**), with a good water solubility profile (Figure 6.68) [108].

Pyrroloquinolines (**318–319**) were evaluated for their antiproliferative properties against 2008 ovarian cancer, HL60 leukemia, A431 cervix cancer, A549 lung cancer, MCF-7 breast cancer and A375 melanoma cell lines. Water soluble compound (**318**)

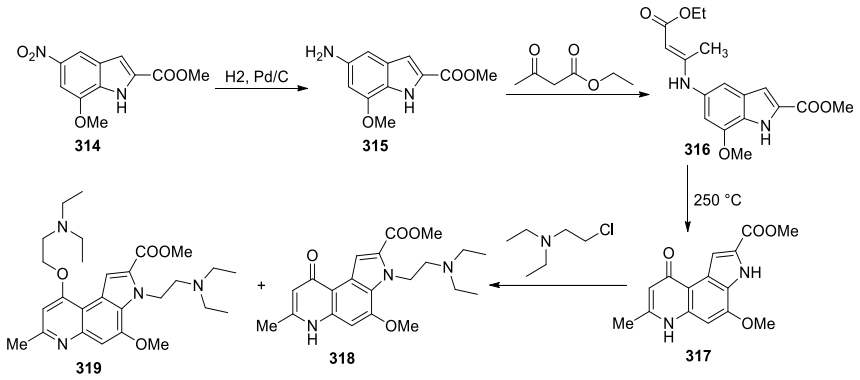


Figure 6.68: Synthesis pathway of substituted pyrrolo[3,2-*f*]quinolin-9-ones (**318**) and (**319**).

showed the highest cytotoxicity against the melanoma cell line (IC_{50} = 6.23 μM) and HL60 cells (IC_{50} = 4.78 μM) [108].

The same group continued their research in the field [108], studying the influence of the small substituents, as well as the insertion of suitable substituents in order to increase the solubility in water of biological active 3*H*-pyrrolo[3,2-*f*]-quinolin-9-ones derivatives. The target compounds were obtained in few steps, starting with the *N*-alkylation of 5-nitroindole (**320**) with ethyl acrylate or trimethoxybenzyl bromide, in the presence of NaH, followed by catalytic reduction to achieve amines (**322**). Condensation with ethyl benzoylacetate and subsequent thermal cyclization yielded target pyrrolo[3,2-*f*]quinolin-9-ones (**324a–b**) (Figure 6.69) [109].

Compounds (**324a–b**) were screened for their *in vitro* antiproliferative activity against a panel of 11 human solid and leukemic tumor cell lines, and showed good activity in all cell lines, with the GI_{50} values against the leukemia cells in the range of 0.5–10 nm, and against the solid tumor cells in the range of 0.4–1.4 μm. Moreover, compound (**324a**) exhibited potent inhibition of tubulin polymerization (IC_{50} = 0.76 μM) [109].

Dalla Via et al. [110] prepared 9-chloro-pyrrolo[3,2-*f*]quinoline-*f* (**325**) by the multi-step pathway previously reported by Ferlin et al. [105] starting from 5-aminoindole and diethyl ethoxymethylene malonate. Further treatment with *p*-amino-acetophenone, yielded 9-(4-acetylanilino)-3*H*-pyrrolo[3,2-*f*]quinoline (**326**), as mono-hydrochloride (Figure 6.70).

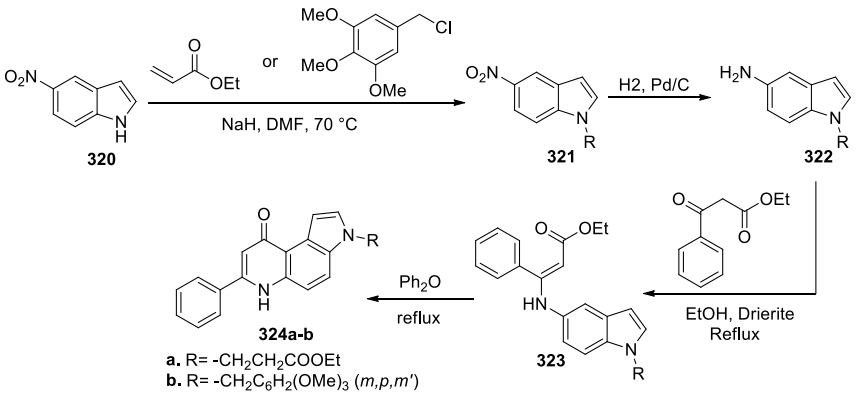


Figure 6.69: Synthesis of pyrrolo[3,2-*f*]quinolin-9-ones (**324**).

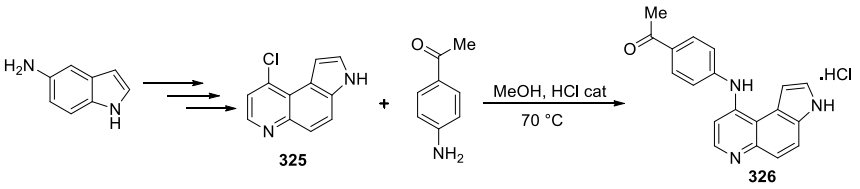


Figure 6.70: Synthesis of pyrrolo[3,2-*f*]quinoline-*f* (**326**).

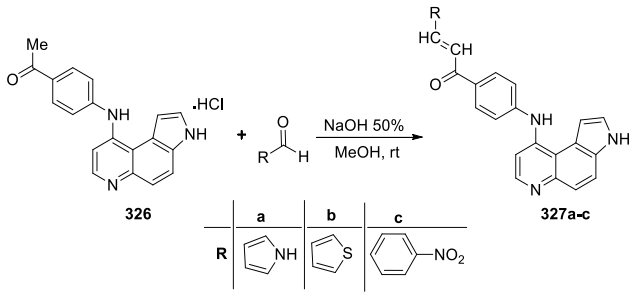


Figure 6.71: Synthesis of pyrroloquinoline-linked chalcones (**327a–c**).

The screening of pyrroloquinoline (**326**) carried out against HL-60, HeLa, JR8 and OVCAR-3 human tumor cell lines, showed high antiproliferative activity by a mechanism that includes formation of an intercalative complex with DNA, inhibition of DNA topoisomerase II and blockage of the cell cycle in G2/M phase [110].

The same authors [111] synthesized pyrroloquinoline-linked chalcones (**327a–c**) *via* a Claisen-Schmidt condensation of anilino-3*H*-pyrrolo[3,2-*f*]quinoline-*f* (**326**) with the appropriate aromatic aldehydes (Figure 6.71).

The growth inhibition ability of compounds (**327a–c**) was evaluated against HL-60, HeLa and JR8 human tumor cell lines, with particularly good cytotoxic activity against melanoma cell line JR8 (IC_{50} = 1.2–3.3 μM) and an efficient intercalative complexation with DNA [111].

6.3.5 Pyrrolo[3,2,1-*ij*]quinoline

Pyrrolo[3,2,1-*ij*]quinoline system exists in many compounds that have been reported to possess a broad spectrum of biological activities, including antiproliferative activity [112].

Inspired by antileukemic compound (**328**) (Figure 6.72) reported by Matesic et al. [112], Pao's group synthesized using ultrasound, several 6-substituted-2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-*ij* derivatives (**331a–f**) *via* a Bischler-type reaction of ethanone (**330**) obtained from 1,2,3,4-tetrahydroquinoline (**329**) (Figure 6.72) [113].

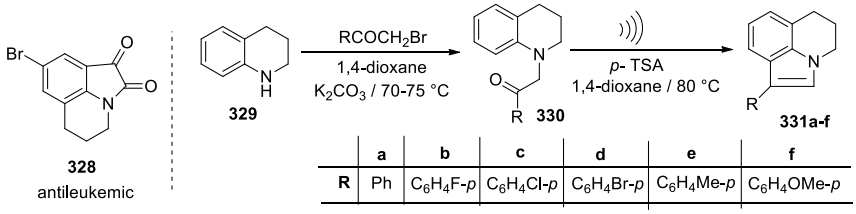


Figure 6.72: Synthesis of 2,3-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-*ij* derivatives (**331**).

Compounds (**331a–f**) were *in vitro* evaluated for their antiproliferative properties against leukemia, derivatives (**331b**), (**331e**) and (**331f**) showing significant and selective cytotoxic effect against K562 Leukemia cells [113].

Starting from commercially available 2-substituted-dichloropirimidine/triazine (**332**), Zhang et al. [114] prepared a series of 5,6-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinoline-*ij* derivatives in order to overcome the drug resistance derived from T790M/L858R double mutations. First, coupling compounds (**332**) with 5,6-dihydro-4*H*-pyrrolo [3,2,1-*ij*]quinoline (**333**) in the presence of $AlCl_3$ produced the intermediates (**334**), which on further treatment with 4-fluoro-2-methoxy-5-nitroaniline yielded the corresponding substitution compound (**335**). Fluoro substitution with *N*,*N*,*N*-trimethylethane-1,2-diamine and subsequent reduction of the nitro group yielded amines (**337**). Acylation with acryloyl chloride followed by the elimination of HCl using sodium hydroxide afforded the desired final molecules (**338**) (Figure 6.73).

Compound (**338a**) proved good pharmacokinetic properties and significantly inhibited the enzymatic function of EGFR T790M/L858R (IC_{50} = 2.6 nM) and NSCLC H1975 (IC_{50} = 102.6 nM) cell lines. For the same compound, a strong antiproliferative activity against the H1975 non-small cell lung cancer cells bearing T790M/L858R was registered. Promising results have also been obtained at *in vivo* anticancer screening in a human NSCLC (H1975) xenograft mouse model [114].

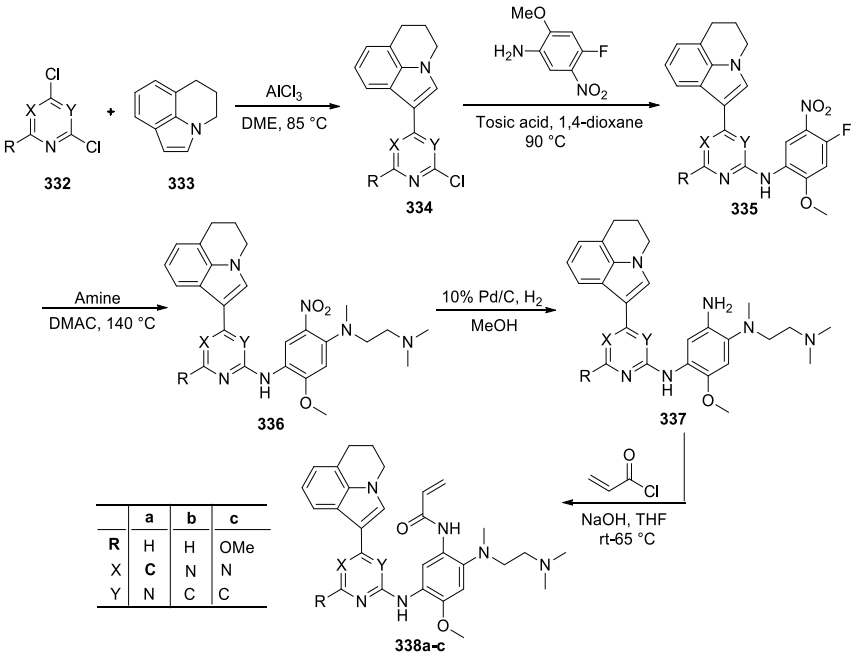


Figure 6.73: Synthesis pathway of pyrrolo[3,2,1-*ij*]quinoline-*ij* derivatives (**338**).

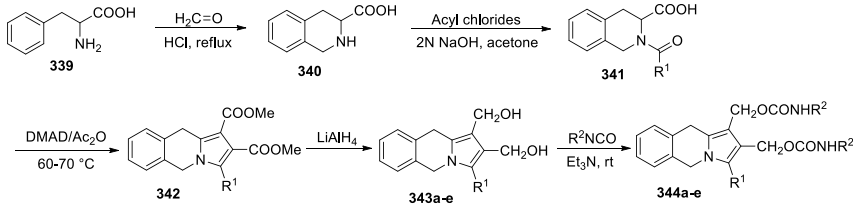
6.3.6 Pyrrolo[1,2-*b*]isoquinoline

There are only several reports regarding the biological potential of pyrrolo[1,2-*b*]isoquinoline-*b* derivatives, including anticancer properties. However, pyrrolo[1,2-*b*]isoquinolone scaffold was found in the structure of lycorine group of Amaryllidaceae alkaloids and natural alkaloids with phenanthroindolizidine skeleton [115].

Chaniyara et al. [116] prepared *bis*(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**343a–e**) and their *bis*(alkylcarbamate) derivatives (**344a–e**) by first treating of the commercially available D,L-phenylalanine (**339**) with formaldehyde and concentrated HCl. Further reaction with various acyl chlorides gave the *N*-acyl-3-carboxy-1,2,3,4-tetrahydroisoquinolines (**341**). Cycloaddition with dimethyl acetylenedicarboxylate (DMAD) yielded derivative (**342**) that was subjected to the reduction with $LiAlH_4$ to afford *bis*(hydroxymethyl) derivatives (**343a–e**). Further treatment with methyl-, ethyl- or *iso*-propylisocyanate in the presence of Et_3N, furnished the desired *bis*(alkylcarbamate)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-*b*-1-yl derivatives (**344a–e**) (Figure 6.74).

Both *bis*(hydroxymethyl) derivatives (**343a–e**) and their *bis*-alkylated derivatives (**344a–e**) were tested *in vitro* against various human solid tumor cell lines. A comparison of their cytotoxicities revealed that *bis*(hydroxymethyl) derivatives are more potent than their corresponding *bis*(alkylcarbamates) in the series of C3-alkyl derivatives, while the *bis*(alkylcarbamates) are generally more efficient than their counterparts in the series of 3-phenyl-substituted derivatives. It is noteworthy that 1,2-*bis*(hydroxymethyl)-3-methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-*b* (**343a**) has shown a wide-range of anticancer activities and as a consequence, it was evaluated for antitumor studies in animal models. The results showed it can cause total tumor remission or important suppression in nude mice bearing human breast (MX-1) and ovarian (SK-OV-3) xenografts, respectively, and is also able to cross-link with DNA [116].

In 2020, Patel et al. [117] reported the synthesis of 12,13-*bis*hydroxymethyl-9,14-dihydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline-*b* derivatives (**354a–e**) through a



	a	b	c	d	e
R^1	Me	Et	Ph	C_6H_4F-*p*	C_6H_4Cl-*p*
R^2	Me	Et	*i*-Pr	Me	Et

Figure 6.74: Synthesis of 5,10-dihydropyrrolo[1,2-*b*]isoquinoline-*b* derivatives (**344**).

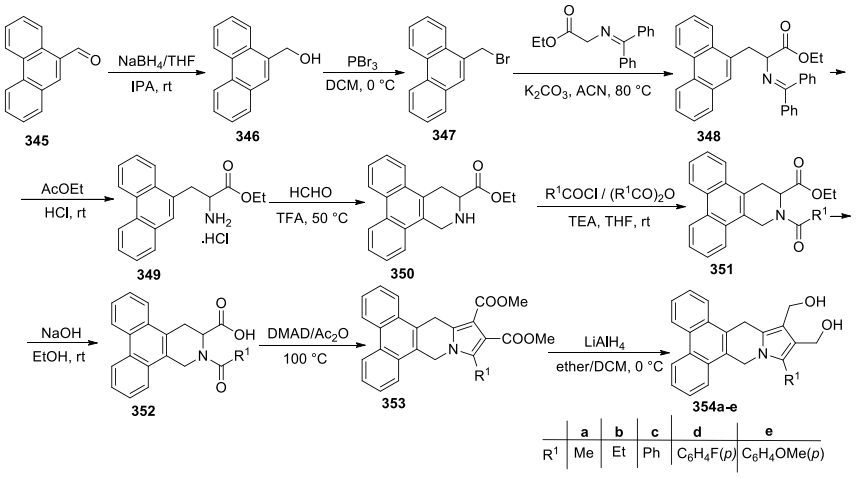


Figure 6.75: Synthesis of benzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolines (**354**).

multi-step protocol, starting with the reduction of 9-phenanthrene carboxaldehyde (**345**) and treatment of the corresponding alcohol (**346**) with PBr_3 to afford 9-(bromomethyl)phenanthrene (**347**). The reaction of (**347**) with diphenylmethyleneglycine ethyl ester afforded compound (**348**) that after HCl acid treatment yielded amine (**349**). The Pictet-Spengler cyclization of (**349**) generated dibenzo[*f*,*h*]isoquinoline (**350**). Reaction with various acyl chlorides or anhydrides produced the *N*-acetyl derivatives (**351**) that were subjected to hydrolysis to give the corresponding carboxylic acid (**352**). Cyclization with DMAD, followed of the reduction with $LiAlH_4$ finally yielded the target *bis*(hydroxymethyl) derivatives (**354a–e**) (Figure 6.75).

Compounds (**354a–e**) were investigated *in vitro* for their antiproliferative activity against human CCRF-CEM lymphoblastic leukemia and several other solid tumor cells. The most cytotoxic compound of the series (**354a**) showed IC_{50} values of 0.36 μM against CCRF/CEM cells, and 0.18 μM against H460 cells. Also, compounds (**354a–b**) were able to induce DNA interstrand cross-links [117].

6.3.7 Pyrrolo[2,1-*a*]isoquinoline

Pyrrolo[2,1-*a*]isoquinoline, in particular 5,6-dihydropyrrolo[2,1-*a*]isoquinoline is a common scaffold of multiple alkaloids (crispines, trolline, lamellarins) endowed with diverse biological action, including antitumor, antibacterial, antifungal and antiviral activities [118–122]. Since it has been demonstrated that pyrrolo[2,1-*a*]isoquinoline-*a* derivatives possess anticancer potency [123, 124], especially antileukemic activity, many research groups have focused on the synthesis of various pyrrolo[2,1-*a*]isoquinoline derivatives as potential antitumor agents [94, 118]. There are two important

strategies to approach this tricyclic system: starting from suitable substituted pyrroles (transition metal catalyzed: Heck reaction, Mizoroki–Heck reaction; intramolecular Friedel–Crafts-type cyclization) and construction of the annulated pyrrole ring on *N*-functionalized isoquinoline derivatives (based on 1,3-dipolar cycloaddition reaction, Baylis–Hillman reaction, several condensation-type reactions, intramolecular cyclization reaction, etc.) [94, 118].

Kakhki et al. [125] reported the synthesis of dihydroisoquinoline derivatives (**359a–d**) using a three-step protocol, starting from *β*-arylethylamine (**355**) and 3,4-dimethoxyphenyl acetic acid (**356**) under free solvent condition. The Bischler–Napieralski cyclization of amide (**357**) in the presence of $POCl_3$, resulted in generation of 3,4-dihydroisoquinoline (**358**) which by subsequent treatment with various phenacyl bromides yielded the desired 2-aryl-5,6-dihydropyrrolo[2,1-*a*]isoquinolines-*a* (**359a–d**) (Figure 6.76).

Cytotoxic activity of compounds (**359a–d**) was assessed against MCF7 (human breast cancer), Hep-G2 (human liver carcinoma), A549 (human lung cancer), HeLa (human cervix cancer) and T47D (human breast cancer) cell lines All tested compounds exhibited satisfactory activity against HepG2 cells, while compound (**359b**) showed the best potency against all tested cell lines with the highest efficacy toward HepG2 (IC_{50} = 4.2 μM) [125].

Following a similar five step procedure, the same group synthesized a new series of 1,2-diaryl-5,6-dihydropyrrolo[2,1-*a*]isoquinolines-*a* (**367a–d**), the synthetic pathway being represented in Figure 6.77 [126].

Compounds (**367a–d**) were screened for their anticancer activity against MCF-7, MDA-MB231, T47D, A549 and HeLa human cancer cell lines. All compounds demonstrated significant potency against HeLa cell line, while compounds (**367a**) and (**367c**) were the most potent against all types of breast cancer lines, activity mediated through estrogen receptors [126].

Chávez-Santos et al. [127] synthesized 5,6-dihydropyrrolo[2,1-*a*]isoquinolines-*a* (**371a–e**), starting from various *α*,*β*-unsaturated esters (**368**), available *via* different

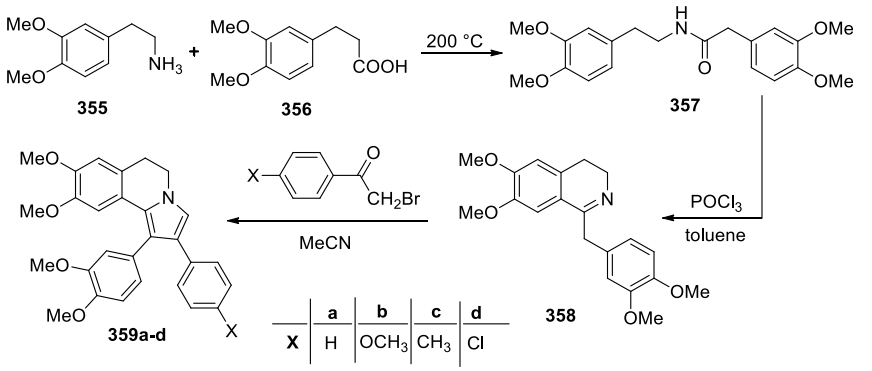


Figure 6.76: Synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinolines (**359a–d**)-*a*.

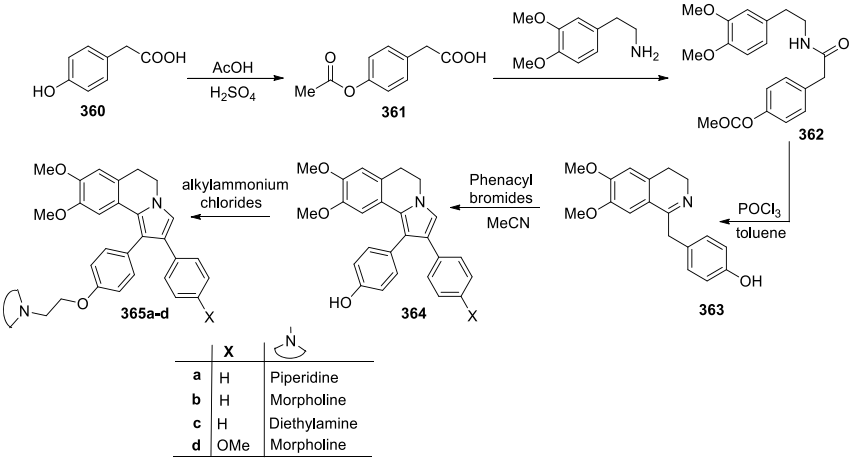


Figure 6.77: Synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinoline derivatives (**367**).

protocols [128, 129]. Treatment with monomethylated TosMIC, prepared from the commercially available TosMIC according to van Leusen's protocol, afforded the 2,3,4-polysubstituted pyrroles (**369**). Further *N*-alkylation using 2-bromo-4,5-dimethoxyphenethyl 4-methylbenzenesulfonate generated derivatives (**370**). Cyclization of the *N*-alkyl-pyrroles (**370**) was achieved using radical oxidative conditions in the presence of tributyltin hydride (n-Bu_3SnH) and dilauroyl peroxide (DLP) to afford the final tetrasubstituted-5,6-dihydropyrroloisoquinolines (**371a, 371c, 371e**). The piperazinyl compound (**371b**) was prepared from compound (**371a**) by deformylation with hydrazine hydrate, while pyrroloisoquinoline (**371d**) was prepared after removal of the benzyl group from the ether (**371c**) (Figure 6.78).

The cytotoxic activity of compounds (**371a–e**) was evaluated on six human cancer cells lines, with spectacular activity for compound (**371d**) compared to irinotecan used

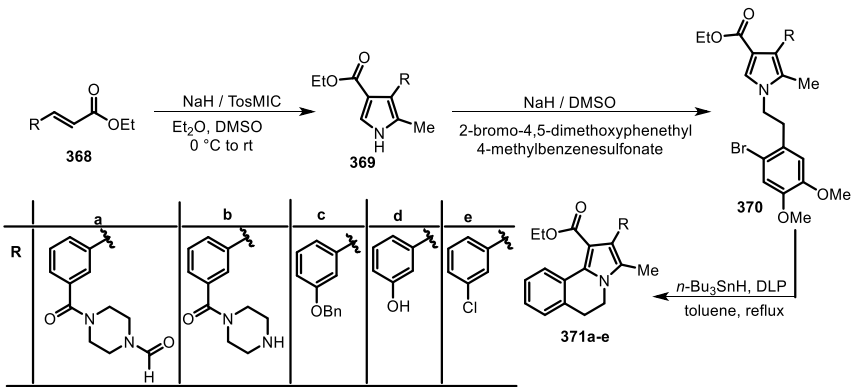


Figure 6.78: Synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinoline derivatives (**371a–e**).

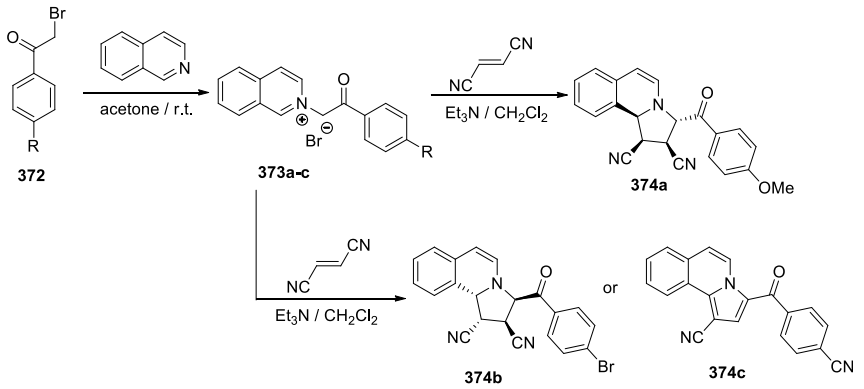


Figure 6.79: Synthesis of pyrrolo[2,1-*a*]isoquinoline-*a* derivatives (**374**).

as reference: almost two thousand times more active against colon HCT-15 cell line (IC_{50} = 0.01 μM) and about six hundred 40 times more efficient against lung SKLU-1 cell line (IC_{50} = 0.05 μM). Also, compound (**371e**) showed more activity in the U-251(CNS) cell line (IC_{50} = 0.37 μM) and leukemia K-562 cell line (IC_{50} = 0.33 μM) compared to the reference etoposide, as well as higher activity in the breast cancer cell line MCF-7 [128].

Synthesis of pyrrolo[2,1-*a*]isoquinolines (**374a–c**) was reported by Al-Matarneh et al. [97] by a sequence including the direct *N*-alkylation of isoquinoline using *para*-substituted 2-bromoacetophenones (**372**), and the Huisgen [3 + 2] cycloaddition of the *in situ* generated ylides (from the salts (**373**) in basic medium). Two types of compounds were thus obtained: dicyano-substituted fused pyrroles (**374a–b**) and the monocyano-substituted fused pyrrole (**374c**), formed due to the elimination of one cyano group under triethylamine excess and oxidative aromatization (Figure 6.79) [97].

The antiproliferative activity of compounds (**374a–c**) was evaluated against NCI's panel of 60 cell lines. Compound (**374a**) showed selective inhibition of cell growth for ovarian cancer OVCAR-8 cells and colon cancer SW-620 cells, while compound (**374c**), showed moderate inhibition of several cancer cell lines [97].

6.4 Conclusions

This literature survey showed that derivatives containing fused pyrrolopyridine and pyrrolo-(iso)quinoline as scaffold, exhibit antiproliferative potency on a large spectrum of cancer cell lines by targeting various enzymatic and signaling pathways. The synthetic versatile, unique molecular architecture specific for each discussed fused system confer excellent biological properties that were exploited in medicinal chemistry in the last years. From all described anticancer derivatives (containing an indolizine, 7-azaindole, 6-azaindole, 4-azaindole, 5-azaindole, 2*H*-pyrrolo[3,4-*c*]pyridine-*c*,

pyrrolo[1,2-*a*]quinoline-*a*, pyrrolo[2,3-*b*]quinoline-*b*, pyrrolo[2,3-*f*]quinoline-*f*, pyrrolo [3,2-*f*]quinoline-*f*, pyrrolo[3,2,1-*ij*]quinoline-*ij*, pyrrolo[1,2-*b*]isoquinoline-*b* or pyrrolo [2,1-*a*]isoquinoline-*a* as core), we remarked a huge potential of some indolizine, 7-azaindole, pyrrolo[1,2-*a*]quinoline and pyrrolo[2,1-*a*]isoquinoline based derivatives to be used as anticancer drugs in the future. However, we expect in the coming years an increasing number of studies in the anticancer field having in the spotlight these fused pyrroloheterocyles.

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7 Updates on the versatile quinoline heterocycles as anticancer agents

Abstract: Quinoline motifs have befallen significant molecules due to their assortment of interest in medicine, chemical synthesis, coordination chemistry, also in the field of applied chemistry. Therefore, various researchers have produced these molecules as objective structures and studied their natal potential. The current chapter endows with concise attention about cancer, anticancer agents, sources (natural) of quinoline, and together with an innovative scope of quinoline-related medicines. Further, the present section gives knowledge concerned with the anticancer activity of synthesized quinolines and their derivatives.

Keywords: anticancer agents; classical methods; heterocyclic compounds; marketed drugs; natural sources; quinolines.

7.1 Overview of cancer and anticancer agents

7.1.1 Definition

The disease that causes cells to uncontrolled growth, resulting in tumor and abridged function in the immune system is referred to as cancer. All cancer diseases are finally lethal if participants do not take the proper medication.

7.1.2 Causes of cancer

a. **Chemical or toxic compound exposures:** Aromatic hydrocarbons like benzene, toluene, glass wool, some metal like nickel, chromium (VI) and cadmium, arsenic, vinyl chloride (chloroethene), benzidine (1,1′-biphenyl-4,4′-diamine), nitrosamines (*N*-nitrosamines), tobacco (nicotine and harmine are the active ingredients), and aflatoxin (produced by fungi), etc.

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b. **Expose to radiation:** Radioactive elements like uranium and radon, ultra-violet rays, radiation from α, β, γ, and X-ray emanating sources.
c. **Pathogens:** Epstein-Barr virus, human papillomavirus, hepatitis B and C viruses, Kaposi's sarcoma-associated herpesvirus, Merkel cell polyomavirus, *Schistosoma spp*., and *Helicobacter pylori*; other bacterial strains are being researched as probable agents.

d. **Genetics:** Numerous definite neoplasm have been associated with human genes, these include ovarian, breast, melanoma, etc.
e. **Diet and exercise:** Over 30–35% of cancer deaths are mainly related to unhealthy diet, physical apathy, and obesity.

7.1.3 Cancer symptoms

The six symptoms were described by the American Cancer Society for cancer that may be present are as follows [1].

1) Changes in habits of bowel or bladder
2) Incurable throat pain
3) Bizarre blood loss or liberation
4) Thickening in the breast, testicles, or elsewhere
5) Digestive disorders
6) Irritating or dry cough

7.1.4 Types of cancer

The precise types of cancers originate in apiece common categories are as follows:

- **Carcinoma:** Carcinoma is the most widespread variety of cancer. That instigates in the epithelial tissue (skin) or tissues that line interior vital body organs, such as kidney or liver. It may reach other body parts, or be confined to the primary location.
- **Sarcoma:** It instigates in the bones or tissues (soft) of the body (cartilage, muscle, fat, blood vessels, fibrous tissue, or other supportive tissue).
- **Leukemia:** The leukemia is a cancer of the blood cells. It starts in blood generation tissues including bone marrow and lymphatic system. Leukemia is mainly of four types: Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia, Acute Lymphocytic Leukemia, and Chronic Lymphocytic Leukemia.
- **Lymphoma and myeloma:** This type of cancer initiates in infection-fighting cells of the immune system, called lymphocytes. These cells are in the lymph glands, spleen, thymus, bone marrow, and different parts of the body. There are two main types: (1) Non-Hodgkin lymphoma and (2) Hodgkin lymphoma.

Myeloma, is also a type of blood cancer, which develops from cells in the bone marrow (spongy tissue found inside the central cavity of many large bones of the body), called plasma cells.

- **Central nervous system (CNS) cancer:** CNS cancer that starts in the normal cells/brain tissues and spinal cord called 'neurons' and 'glia'. The meningioma, primitive neuroectodermal tumors, gliomas, pituitary tumors, pineal tumors, etc. are the primary tumors of the brain.
- **Mixed Types:** The kind machinery may be within one group or from different groups. A few examples are adenosquamous carcinoma, carcinosarcoma, and teratocarcinoma.

7.1.5 Treatment for cancer

The following general methods are used in cancer treatment [2]:

- **Chemotherapy** is introduced by Paul Ehlrich, aims to use one or more medications to destroy tumor cells. This technique affects by controlling the tumor cells from dividing, growing, and multiplying.
- **Hormone therapy** involves medication that sluggish or ends the growth of cancer that utilizes hormones to produce. It is also called endocrine therapy or hormone treatment.
- **Immunotherapy** engages curative that assists the immune system battle against cancer. The immune system helps our body battle infections and other diseases. It is made up of leukocytes and tissues of the lymph organization.
- **Precision medicine (PM)**, the health care providers can recommend and plan specific care for their patients. It involves using genetic testing (examining DNA, chemical database) to decide the most suitable treatments for a person's particular presentation of cancer. It's sometimes called personalized care or medicine.
- **Radiotherapy (RT, XRT)** is a cancer treatment that uses high doses of radiation to destroy or control the malignant cells and shrink neoplasms. This therapy most often uses X-rays but protons or other types of energy also can be used.
- **Bone marrow transplant** or **Stem cell** can be particularly favorable for patients with blood cancers (such as myeloma, leukemia, or lymphoma). This treatment replaces the bone marrow with healthy cells (either from own body or from a donor). It entails eliminating cells, such as RBC's or WBC's, which chemotherapy has destroyed.
- **Surgical oncology (SO)** is habitually a branch of a curative arrangement when an individual has a tumor cell. The orthopedic doctor may stamp out the tumor and nearby tissue during an operation to reduce or avert the disease extend.

– **Targeted therapies** accomplish purposes within cancerous cells to stop them from dividing. This treatment targets specific genes or proteins that control how cancer cells grow, divide, and spread. The most targeted brachytherapies are small-molecule drugs and monoclonal antibodies (mAbs).

7.1.6 Anticancer drugs:

The anticancer or antineoplastic medicine or drug that is efficient in the management and treatment of malignant or cancerous disease represents a diverse class of medications that are used for the management of cancer [3–5]. The major classes of anticancer drugs include:

7.1.6.1 Alkylating antineoplastic agents

That attaches the hydrocarbon chain to the nitrogenous base of the DNA (AGCT), averting the strands of the double helix from linking as they should. This results in the damage of the DNA strands and disturbing the capacity of the tumor cell to develop, finally leads to the death of neoplasm.

– There are five categories of alkylating agents: Nitrogen mustards, Nitrosoureas, Alkyl sulfonates, Triazines, and Ethylenimines.

7.1.6.2 Cytotoxic agents

Cytotoxic refers poisonous to cells. Cytotoxic chemotherapy drugs interrupt the way cancer cells grow and multiply.

– Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin.

7.1.6.3 Antimetabolites

A chemical that inhibits the use of a metabolite is known as an antimetabolite, which is an added chemical that is a component of usual metabolism. These agents are nucleoside analogs that are intrusive with the nucleoside triphosphates in the synthesis of DNA or RNA or both.

– The purine analogs used as antineoplastic agents are B cell, Azathioprine, Rituximab, Cladribine, Mercaptopurine.

– Pyrimidine analogs include, 5-Fluorouracil, 5-Azacytidine, Cytosine arabinoside, Gemcitabine.

7.1.6.4 Monoclonal Antibodies

Various mAbs are used to cure cancer. They are a type of targeted cancer therapy, which means they are intended to intermingle with precise targets.

– Alemtuzumab, Atezolizumab, Avelumab, Bevacizumab, Blinatumomab, Brentuximab, Trastuzumab.

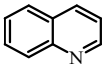
7.1.6.5 *Vinca* alkaloids

These are the set of drugs derived from the Madagascar periwinkle plant (*Catharanthus roseus*). They are well-known clinical cytotoxic agents which inhibit the ability of cancer cells to divide and multiply.

– The major *Vinca* alkaloids include Vincristine, Vinblastine, Vindesine, and Vinorelbine.

7.2 Introduction to quinolines

Quinoline (Figure 7.1) and quinoline derivatives signify a capacious group of compounds, which get conventional interest due to account of an extensive assortment of microbial and pharmacological evaluations. Runge et al. [6] extracted the impure quinoline from coal tar. Gerhardt [7] reported the quinine, strychnine, and cinchonine as a degradation product.



(1)

Figure 7.1: Structure of quinoline.

The quinoline (**1**) is nontoxic, less basic; with acids, it forms salt and the reactions are comparable to those of pyridine and benzene.

Many pharmacologically active and naturally occurring alkaloids such as Cinchona Alkaloids (**2a–d**), Echinopsine (**3**), Vasicine (**4**), Fabrifugine (**5**), Cusparine (**6**), Galipine (**7**), and Cuspareine (**8**) were found to contain quinoline nucleus [8–12]. The structures of some naturally happening significant quinolines are presented in Figure 7.2.

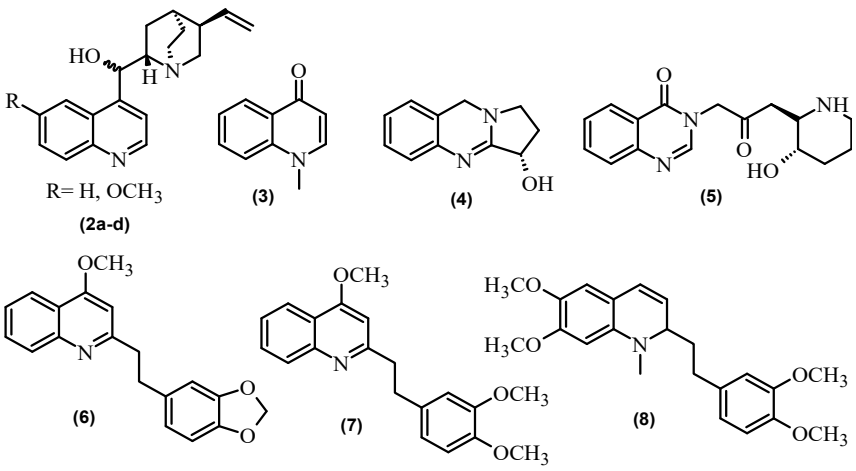


Figure 7.2: Naturally happening significant quinolines alkaloids.

In persistence of our attention in the design, synthesis, and medicinal perspectives of quinoline and its derivatives [13–19], the present chapter signifies the advanced study of quinoline and its derivatives also, planned to present a broad outline of the investigated outcomes in this area. Several methods were adopted to synthesize quinolines and also confer the anticancer activity.

7.3 Synthesis of quinolines by classical methods

The Combes quinoline involves condensation of aniline with β-diketones to form substituted quinolines in presence of acid catalyst through the ring-closing of the Schiff base. In Conrad–Limpach synthesis the reaction of aniline with β-ketoesters to form 4-hydroxyquinolines *via* a Schiff base. The Doebner reaction involves the reaction of aniline with an aldehyde and pyruvic acid to obtain quinoline-4-carboxylic acid. The Doebner–Miller reaction is a chemical reaction to synthesize substituted quinolines from aniline with α, β-unsaturated carbonyl derivatives. The Riehm quinoline synthesis explains the formation of substituted quinolines by prolonged heating of arylamine hydrochlorides with ketones with the use of aluminum chloride or phosphorus pentachloride. The Gould–Jacobs reaction involves the synthesis of 4-hydroxy quinoline from aniline and substituted malonic ester via sequential reactions. In the typical Skraup reaction, acid-catalyzed synthesis quinoline from aniline and glycerol under heating conditions in presence of nitrobenzene. The Povarov synthesis is a multicomponent method that involves the condensation of the aniline with an aldehyde and an activated olefin to produce a tetrahydroquinoline adduct. The camps quinoline synthesis from base-catalyzed intramolecular condensation of

2-acetamido acetophenone. In Friedlaender quinoline synthesis, the o-aminoaryl aldehydes or ketones and ketone possessing α-methylene are used as starting materials. The Knorr quinoline synthesis involves acid-catalyzed conversion of a β-ketoanilide to a 2-hydroxyquinoline. The Niementowski quinoline synthesis involves the conversion of anthranilic acids and ketones/aldehydes to form γ-hydroxyquinoline derivatives. Meth–Cohn and Narine reported an elegant method to synthesize substituted 2-chloro-3-formyl quinolines from substituted acetanilides in the presence of dimethyl formamide and phosphorous oxychloride. The Pfitzinger reaction involves the synthesis of substituted quinoline-4-carboxylic acids from isatin with base and a carbonyl compound [17]. Figure 7.3 enlighten the classical methods for the production of quinolines.

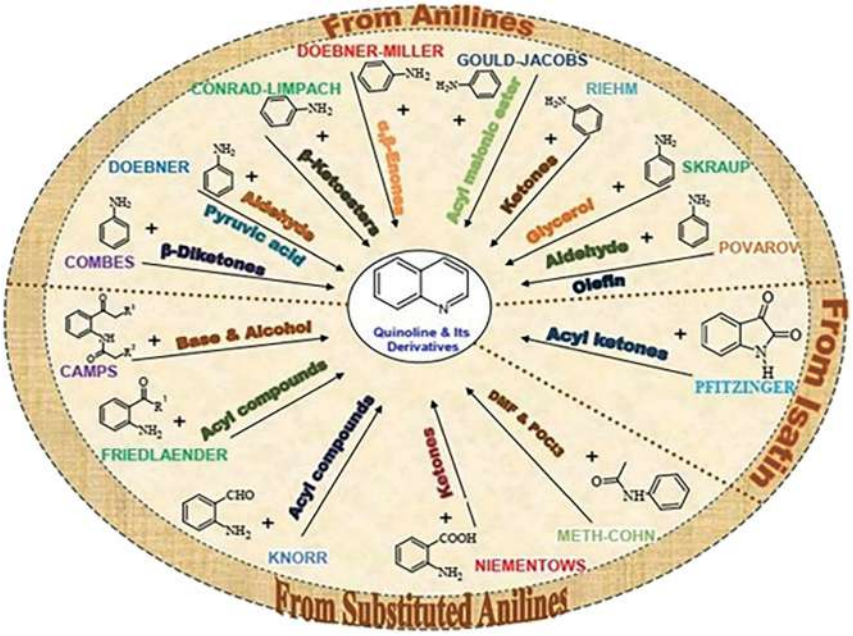


Figure 7.3: The traditional methods for the production of quinolines.

7.4 Quinoline-based anticancer drugs

The quinoline alkaloids derivative from natural sources possesses many biological activities. Various sources of the quinoline derivatives which showed anticancer activity are discussed below.

Camptothecin (**9**) a Chinese tree derivative, revealed by Wall et al. [20] as an anticancer drug and distress topoisomerase I, allowing DNA cleavage, but hindering

ensuing ligation, and resulting in DNA strand breaks. The dictamnine (**10**) known quinoline alkaloid from roots extraction of *Zanthoxylum wutaiense*, was accounted for moderate anticancer property [21]. Haseeb et al. [22] reported more potent anticancer activity of chelidonine (**11**). A noxious polycyclic quinoline salt sanguinarine (**12**) extracted from *Argemone mexicana* [23] and mechanism of toxic effects of animal cells through its action on the Na^+/K^+-ATPase transmembrane protein [24]. The anticancer property of berberine (**13**) from the family of *Berberidaceae* for the cure of colon cancer was investigated by Oritz et al. [25].

The quinoline alkaloid chelerythrine (**14**) from methanolic extract celandine had reported by Haseeb et al. and also disclosed the lung and pancreatic antineoplastic activity [22]. Lavendamycin (**15**) was isolated from *Streptomyces levendulae* and showed antitumor antibiotic activity by Cai et al. [26]. Tan et al. [27] reported quaternary quinoline alkaloid nitidine (**16**) which is isolated from aerial parts of *Zanthoxylum nitidum* for Topoisomerase inhibitor activity and quinoline alkaloid streptonigrin (**17**)

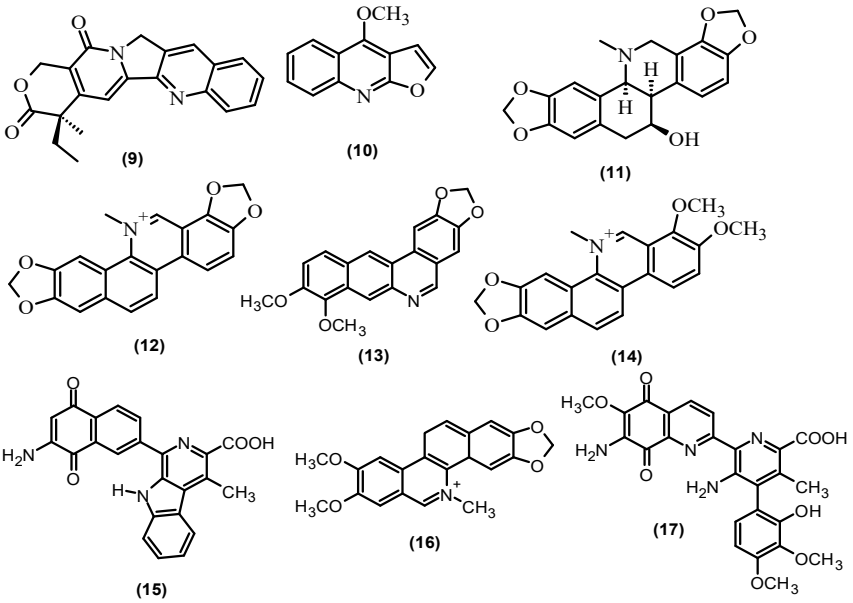


Figure 7.4a: Natural quinolines possess antitumor/anticancer activity.

from *Streptomyces flocculus* was reported by Rao et al. [28] for anticancer agents. The structures of natural quinoline as anticancer drugs (**9–17**) are shown in Figure 7.4a.

In mid of 2012, cabozantinib (**18**) was first permitted as an unfocused tyrosine kinase inhibitor. Firstly it was accepted in the United States in the trade name COMETRIQ™, which is indicated for the cure of metastatic medullary thyroid cancer

[29] and later permitted for the curation of higher renal cell carcinoma. An anticancer enzyme inhibitor irinotecan (**19**) is used in the exploit of colorectal cancer and also restrained the action of topoisomerase-I. It was accepted for the treatment of higher pancreatic cancer in Oct 2015 [30]. A fluoroquinolone antibiotic finafloxacin (**20**) is effective in the curation of sensitive otitis externa caused by the pathogen *Pseudomonas aeruginosa* and *Staphylococcus aureus* [31]. Amsacrine (**21**), used in AML and persisting to be studied in amalgamation with various drugs for the treatment of numerous cancers [32]. Neratinib (**22**) was used as an irretrievable tyrosine-kinase inhibitor of EGFR, HER1, HER2, and HER4. Double blockade by antiangiogenic/HER2 agents (e.g., neratinib) targeting HER and EGFR pathways creates greater embarrassment of human breast cancer cell lines [33].

Talazoparib (**23**) is a tentative antineoplastic agent called a poly ADP ribose polymerase inhibitor, which is creature appraised in breast cancer patients with germline BRCA mutations, as well as other cancer types with deficiencies in DNA damage repair [34]. In association with GlaxoSmithKline, Sitamaquine (**24**) is an 8-aminoquinoline which is being developed by the Walter from Reed Army Institute, as an oral treatment for visceral leishmaniasis. Pre-clinical and subsequent clinical investigations have demonstrated oral efficacy against *Leishmania donovani* [35]. Amgen was developing AMG-208 (**25**), a small molecule inhibitor of c-Met, for the action of cancer. AMG-208 exhibits the effective inhibition of kinase c-Met activity with IC_{50} of 9 nM in a cell-free evaluation. Besides, AMG-208 treatment also leads to the inhibition of HGF-mediated c-Met phosphorylation in PC3 cells with IC_{50} of 46 nM. AMG-208 showed proof of anticancer activity, chiefly in prostate cancer [36].

Foretinib (**26**) is an orally bioavailable small molecule with a potential candidate for the treatment of cancer. It was discovered by Exelixis and is under development by GlaxoSmithKline [37]. A chemotherapy drug Acridine carboxamide (**27**) is being deliberated as an anticancer agent. It belongs to the family of drugs called topoisomerase inhibitors [38]. Pyrazoloacridine (**28**) is an investigational anticancer drug currently under study for the management of solid tumors based on its unique mechanism of action and selectivity for human solid tumor xenograft in mice. Derivatives pyrazoloacridine were originated to exhibit solid tumor selectivity relative to their activity against murine leukemia cells and human lymphoblastoid cells [39]. Figure 7.4b explained the quinoline-based marketed/clinical drugs (**18–28**).

7.5 Anticancer activity of quinolines analogs

Kamal et al. [40] synthesized new curcumin motivated substituted quinoline (**29**) derivatives and studied their activity toward several tumor cell lines. Many new fused quinolines (**30**) were synthesized by Chen and coworkers [41] and were tested *in vitro* against different human cancer cell lines by the MTT method. Novel quinoline (**31**) derivatives were reported by Ghodsi coworkers [42] and were studied for their

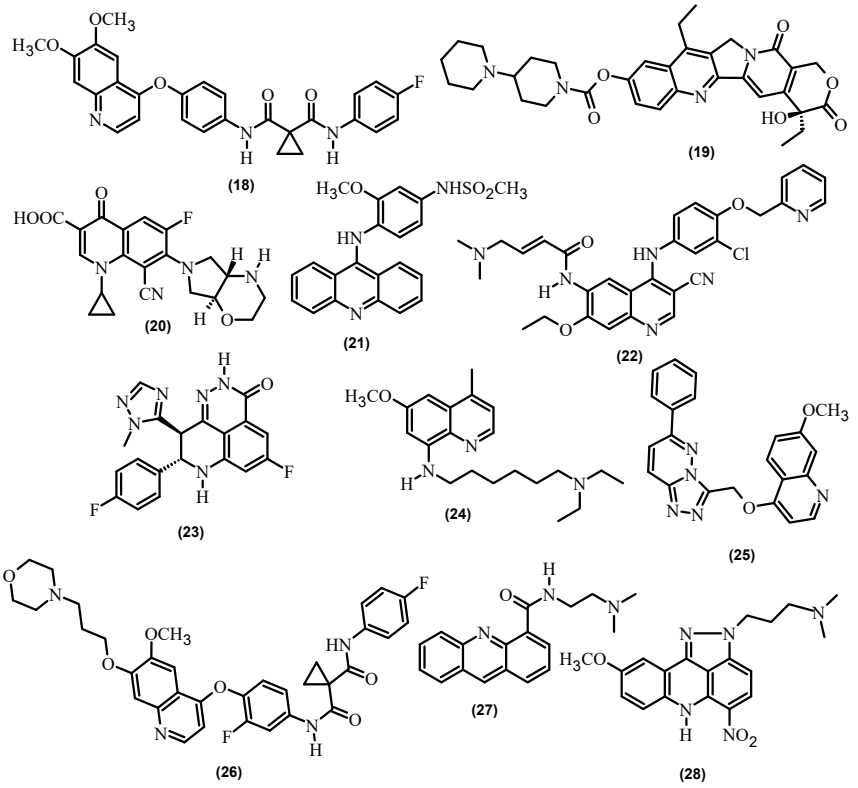


Figure 7.4b: The quinoline-based marketed/clinical drugs.

anticancer activity. Some of the molecules exhibit more potent anticancer activity. The antineoplastic property of novel quinoline (**32**) derivatives was tested against cancer cell lines (human) such as adenocarcinoma cells, prostate cancer cell lines, and adenocarcinomic epithelial cells by Ghodsi and coworkers [43]. Catto et al. [44] disclosed the anticancer activity of pyridazino[4,3-c]quinolines (**33 a–c**) against two human cancer cell lines. The design and development of indole intriguing quinolines (**34**) and were tested for anticancer activity by Sidoryk et al. [45]. The new 4-alkynyl-quinoline compounds (**35**) were prepared and their biological activity such as PI3Kα inhibition and antiproliferative effects against two cancer cell lines PC-3 and HCT-116 was carried out by Lu and co-workers [46].

Preparation and anticancer activity of a novel sequence of 2-substituted quinolines (**36**) were reported as lead molecules by Ghodsi et al. [47]. The anticancer property of the developed molecules was tested against human cancer cell lines. Anticancer activity of novel triazolo[1, 5-a] quinoline (**37**) compound was tested against human cancer cell lines by Bassyouni and co-workers [48]. Tang et al. [49] synthesized new analogs of 8-hydroxy

quinoline (**38**) and tested for antineoplastic activity. The *in vitro* anticancer property of new substituted quinoline (**39**) compounds containing cinnolinamides in opposition to c-Met kinase and some representative cell lines was developed by Gong et al. [50].

Zhou et al. [51] presented the preparation and anticancer activity in opposition to c-Met kinase and few representative cell lines of polysubstituted-quinolines (**40**). Al-Ghamdi et al. [52] reported many pyrano and pyrimido fused quinoline (**41 a & b**) compounds and studied their antineoplastic activity. In Figure 7.5a, the structures of quinolines (**29–41 a & b**) showing anticancer activity are presented.

Some novel quinoline (**42**) derivatives to study their antibreast cancer property against human breast cancer MCF-7 and T47D cell lines were reported by Razieh et al. [53]. Cakmak et al. [54] reported several nitro, phenyl, *N*-oxide, cyano, and methoxy substituted quinolines (**43**) were evaluated for their *in vitro* anticancer activity against different cancer cell lines. Design and synthesis of 4-substituted-quinoline (**44**) compounds and tested for their *in vitro* anticancer activity against cancer cell lines by Gong et al. [55]. Canete et al. [56] took advantage of the development of several fused quinoline analogs (**45**) to assess SAR in human gastric (AGS), lung (SK-MES-1), bladder (J82) cancer cell lines, and human normal lung fibroblasts (MCR-5). Design, synthesis, and potential antiprostate cancer activity of quinoline (**46**) derivatives were reported by Zhao et al. [57]. The novel substituted quinoline (**47**) compounds were reported by Al-Bayati and his research group [58] and evaluated their anticancer activity. Design, synthesis, and potential anticancer activity of novel 2, 4-disubstituted quinoline (**48**) derivatives were reported by Satyanarayan et al. [59]. Wang et al. [60] reported the synthesis and anticancer activity of substituted quinolines (**49**) as c-Met kinase inhibitors. Marganakop et al. [61] synthesized some quinoline (**50**) derivatives from 2-chloro-3-formyl quinoline and tested them for their anticancer activities. Novel quinoline (**51**), derivatives were synthesized by structural modification of Sorafenib and evaluated their selective antitumor activities [62]. Several substituted quinolines (**52**) were developed by Scott et al. [63] and they are tested for their anticancer property. Mai et al. [64] developed several 4-hydroxyquinolines (**53**) and studied their anticancer activity. Some c-Met kinase inhibitory quinolones (**54**) were investigated by Wang et al. [60] with $IC_{50} < 1$ nM. These analogs were found to exhibits the inhibition of c-Met phosphorylation in c-Met-dependent cell lines.

Selim and his team [65] presented the preparation and anticancer study of fused quinolines (**55**), against cancer cell lines. Tiwari et al. [66] reported the design and synthesis of polycyclic quinolines (**56**) framework for anticancer activity. These derivatives were evaluated for antineoplastic treatment against colon, prostate, breast, ovarian, and hepatocellular neoplasms and against noncancerous Madin Darby Canine Kidney, mouse embryonic fibroblast, and human embryonic kidney cells. Figure 7.5b represented the quinoline and its derivatives structures (**42–56**) exhibiting antineoplastic activity.

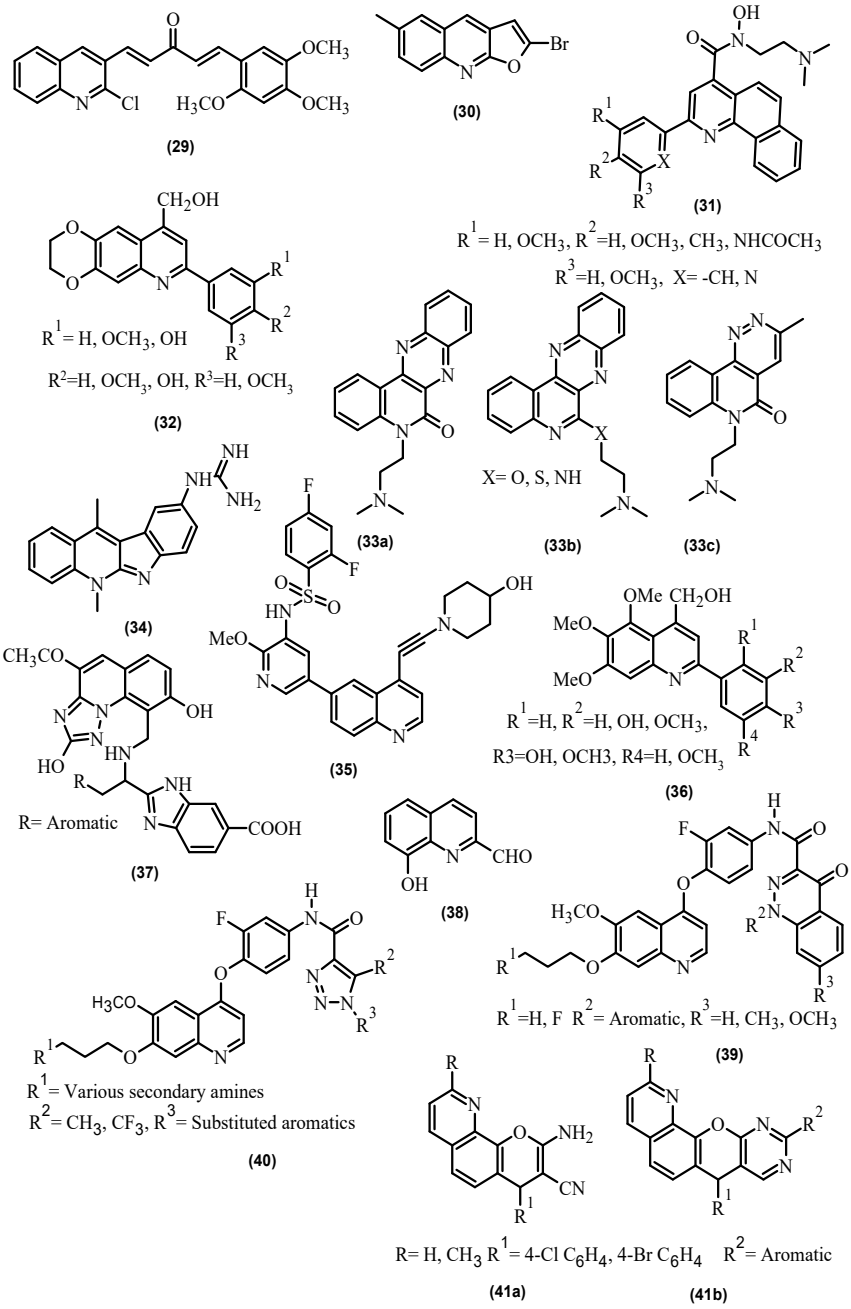


Figure 7.5a: Quinoline and its derivative structures showing antineoplastic activity.

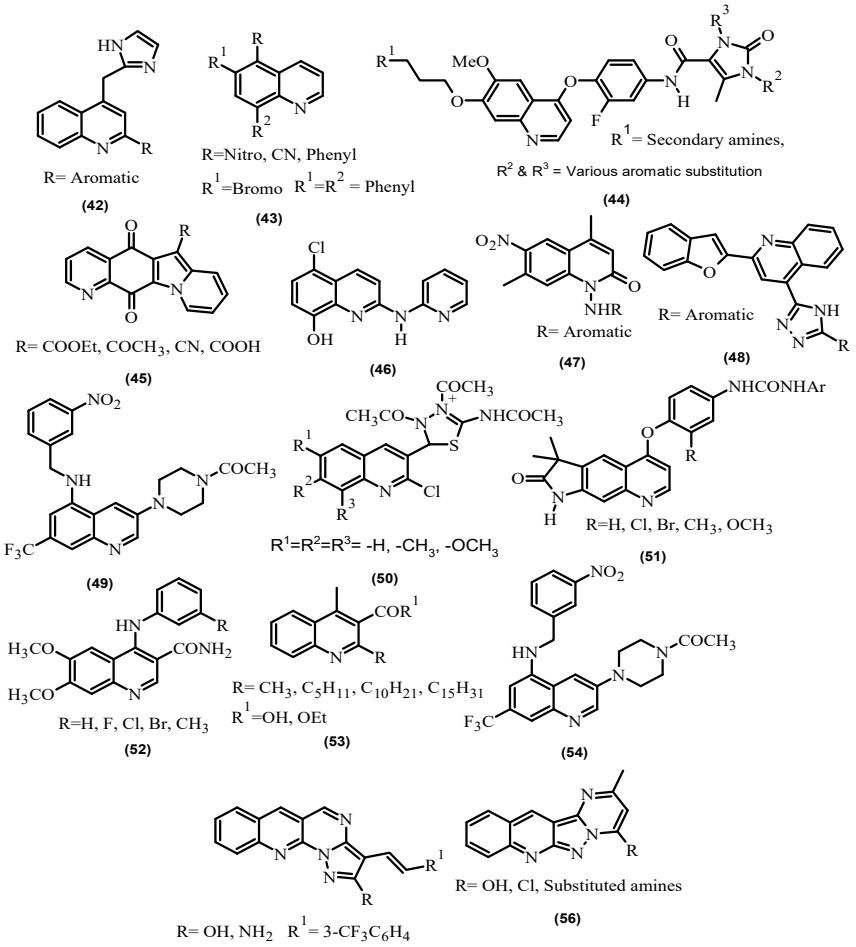


Figure 7.5b: Quinoline and its derivative structures exhibiting antineoplastic activity.

7.6 Conclusions

The quinoline motif plays a significant role in biological activity. In recent years, many quinoline compounds have been prepared and their cytotoxic properties were presented in the literature. The above said quinoline and their derivatives infatuated noteworthy antineoplastic activity because of their constitution assortment, which is accountable for their cytotoxic property. To date, several quinoline derivatives have been promoted for their anticancer activity and at present, the scientific community is also spotlighting to initiate novel quinoline molecules in the market to include foresaid activity.

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Naresh Kumar and Nidhi Goel*

8 Recent development of imidazole derivatives as potential anticancer agents

Abstract: Cancer, one of the key health problems globally, is a group of related diseases that share a number of characteristics primarily the uncontrolled growth and invasive to surrounding tissues. Chemotherapy is one of the ways for the treatment of cancer which uses one or more anticancer agents as per chemotherapy regimen. Limitations of most anticancer drugs due to a variety of reasons such as serious side effects, drug resistance, lack of sensitivity and efficacy etc. generate the necessity towards the designing of novel anticancer lead molecules. In this regard, the synthesis of biologically active heterocyclic molecules is an appealing research area. Among heterocyclic compounds, nitrogen containing heterocyclic molecules has fascinated tremendous consideration due to broad range of pharmaceutical activity. Imidazoles, extensively present in natural products as well as synthetic molecules, have two nitrogen atoms, and are five membered heterocyclic rings. Because of their countless physiological and pharmacological characteristics, medicinal chemists are enthused to design and synthesize new imidazole derivatives with improved pharmacodynamic and pharmacokinetic properties. The aim of this present chapter is to discuss the synthesis, chemistry, pharmacological activity, and scope of imidazole-based molecules in anticancer drug development. Finally, we have discussed the current challenges and future perspectives of imidazole-based derivatives in anticancer drug development.

Keywords: anticancer agents; imidazole; N-heterocyclic; synthetic derivatives.

8.1 Introduction

Cancer, an abnormal cell growth, is a serious illness around the world [133, 140]. It accounts to be the second most frequent cause of worldwide mortality. As per the World Health Organization (WHO) report, the number of cancer patients will increase up to ≥30% by the year of 2030. Carcinogenesis also known as oncogenesis or tumorigenesis is a multistep molecular changes process where normal cells undergoes to uncontrolled proliferation and become cancerous. It is a progressive disease develops in different stages with long latent period and requires 20–40 or more years thus

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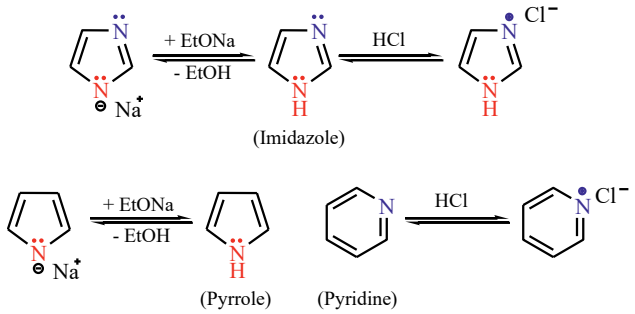
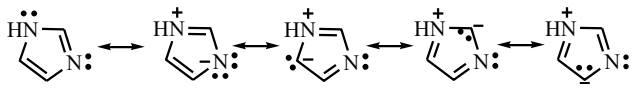
the initiation stage might passed decades before its diagnosis [125]. Cancer initiates with the mutations in DNA which is responsible for the normal functioning of cells. Usually, cells repair nearly all of these mutations but which do not get repaired cause cells to grow abnormally and becomes cancerous [1]. During initiation stage, plausible mutations in transcription factors, protein kinases, adhesion molecules and apoptotic signaling proteins genes prompt a cell to become tumorigenic. These irreversible genetic changes associated with each proliferation cell cycle and gathered in initiated cells. Afterward, a cell enters into promotion stage (second stage) which is quite long process of carcinogenesis and results in genetic as well as epigenetic alternations by continuous cell proliferation and avoids the apoptosis ability with sustained angiogenesis. Later tumor becomes malignant and cells moved towards the third stage of cancer (progression) which is not diagnosed in many cancer types. In this stage, a tumor typically requires surgery, radiotherapy and chemotherapy [26, 125]. In the strategies to control cancer, one third cases can be inhibited by controlling life style, alcohol and tobacco; another one third can be cured normally and reaming one third demands intense care and medication [28, 77, 198]. Thus, chemoprevention, an evolving field, is growing to control and reduce the occurrence of cancer. Still, we are unable to understand the cancer exactly even after spending billions of dollars. The increases in worldwide cancer incidences boost the search for novel, efficient and safer anticancer agents, aiming towards the prevention and therapy of cancer. Different combinations of chemotherapeutic drugs are used in chemotherapy but they produce numerous side effects like multi-drug resistance, less therapeutic efficacy, solubility, and poor bioavailability. Therefore, the discovery of new molecules with potential therapeutic properties and minimal toxic effects is a thoughtful challenge to medicinal chemists and researchers. In recent years, various organic compounds have been explored against several malignancies [12], but the nitrogen containing heterocyclic compounds among these organic compounds are utterly promising agents against several types of cancers. Nitrogen containing heterocycle is an essential constituent of life and exhibits many pharmacological actions [113]. Imidazole is well established nitrogen containing five membered aromatic heterocycles. They constitute a wide range of natural and synthetic products and display countless biological as well as pharmacological properties. In light of their significance, imidazole derivatives have attracted more attention, and play a crucial role in the treatment of diverse diseases. Drugs derived from imidazole have been extensively used in the treatment of numerous diseases with immense therapeutic efficacy [132]. Recently, it has been found that imidazole derivatives are auspicious scaffolds for numerous anticancer agents are being used to cure different types of cancer [6, 169]. Due to these exceptional significances, they have great therapeutic efficacy, specifically against variety of cancers. The present chapter focuses on the synthesis chemistry, pharmacological activity, and scope of diverse imidazole derivatives in anticancer drug development. Finally, we have discussed their current challenges and future perspectives in this subject area.

8.2 Structure and chemistry

1,3-diazole is the systematic name of imidazole which is rarely used in the literature. Imidazole ($C_3N_2H_4$), a 5-membered planar aromatic ring molecule, is a white or colorless solid. It contains two nitrogen atoms out of which one nitrogen atom shows the close resemblance with the nitrogen atom of pyridine (aza –N= group), while others behave like as pyrrole type nitrogen atom (amine –NH– group) as shown in Scheme 8.1 [160]. It is an amphoteric molecule and exists in two tautomeric forms which differ in the attachment of hydrogen atom with the nitrogen. It possesses six π-electrons which are responsible for its different resonance structures and aromaticity as shown in Scheme 8.2. The dipole moment of imidazole is 3.67 D and it is highly soluble in water and other polar solvents [38]. Addition as well as substitution reactions of imidazole molecules along with its derivatives is a fascinating area of research for medicinal chemist, pharmacists, and biochemist as they play key role in the designing and development of novel bioactive molecules. Imidazole ring is rich in electrons; thus, its derivatives readily bind with the various receptors and enzymes present in the biological system *via* various weak interactions such as ion–dipole, hydrogen bonds, cation–π, π–π stacking, van der Waals forces, hydrophobic effects, and many more, and exhibits wide range of biological activities [219]. Moreover, imidazole ring is a dazzling isostere to pyrazole, thiazole, thiazole, oxazole and tetrazole etc. Therefore, it is widely used in the designing of bioactive leads [91, 221, 232]. Additionally, multiple binding sites are present in imidazole ring which makes it capable to coordinate with various metal ions and organic molecules to produce the supramolecular drugs, which may exert double action mechanisms and provide support to overcome the drug resistances [231, 235].



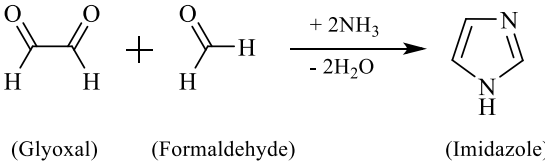
Scheme 8.1: Resemblence of imidazole with pyridine and pyrrole.



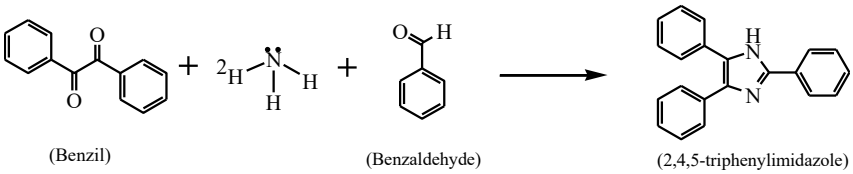
Scheme 8.2: Resonance structures of imidazole.

8.3 General methods of preparation

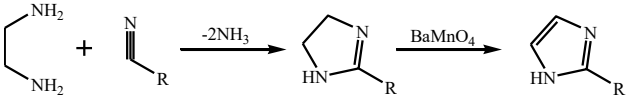
Diverse substituted imidazoles and their derivatives can be synthesized by alternating the functional groups on the reactants [13]. In 1858, Heinrich Debus synthesizes imidazole from glyoxal and formaldehyde (Scheme 8.3) for the first time [48]. Radiszewski produced 2,4,5-triphenylimidazole through the condensation reaction between benzil and benzaldehyde in the presence of ammonia as shown in Scheme 8.4 [168]. Knapp et al. reported the conversion of imidazolines, which are obtained from alkyl nitriles and 1, 2 ethanediamine on reaction with $BaMnO_4NH$, to imidazoles in presence of barium manganate (Scheme 8.5) [161]. Wallach demonstrated that a chlorine containing compound is obtained by treating *N, N*-dimethyloxamide with phosphorus pentachloride. The synthesized compound undergoes the reduction with hydroiodic acid and produce *N*-methyl imidazole (Scheme 8.6) [21]. Markwald revealed the formation of 2-mercaptoimidazoles from α-amino ketones or aldehyde and potassium thiocyanate or alkylisothiocyanates. The sulfur present in the reaction may easily be eliminated by different oxidative methods to obtain the desired imidazoles (Scheme 8.7) [127]. Imidazole derivatives are also prepared by an interaction between alpha halo ketones and an imidine (Scheme 8.8) [161]. Van Leusen and co-workers synthesized an imidazole derivative through the reaction between aldimines and tosylmethyl isocyanide (Scheme 8.9) [196]. Zhang and colleagues described the synthesis of imidazoles by Ugi four component reactions of arylglyoxals, primary amines, carboxylic acids, and isocyanide on a Wang resin as depicted in Scheme 8.10 [218]. Few researches synthesized numerous imidazole derivatives via microwave. Lupsori and co-workers synthesized (Scheme 8.11) a series of 1-hydroxymethylazoles by condensation reaction of azoles (pyrazole, imidazole, 3,5-dimethylpyrazole, 2-methylimidazole and benzimidazole) with paraformaldehyde [124]. Pathan with his colleagues reported a compound, as per Scheme 8.12, 2-substituted 2-imidazolines through the reaction of alkyl cyanide with ethylenediamine in existence of carbon disulphide [147]. Frank and co-researchers synthesized 5-substituted-2-(2-methyl-4-nitroimidazomethyl)-1,3,4-oxadiazoles by the reaction of nitroimidazole derivate with carboxylic acid as shown in Scheme 8.13 [58]. Qasim et al. described the synthesis of 2-phenylimidazo[4,5-*f*][1,10]phenanthroline derivatives under microwave assisted conditions as representing in Scheme 8.14 [154]. Zhao and co-workers prepared benzimidazole and trisubstituted imidazoles by the condensation of 1,2 phenylenediamine with carboxylic acids and acetoacetic ester as depicted in Scheme 8.15 [228]. Kawashita et al. synthesized 2-substituted imidazoles through oxidative aromatization of 2-substituted imidazolines in presence of activated carbon and molecular oxygen (Scheme 8.16) [87]. Ermolat and others developed microwave-assisted one-pot two-step protocol for the synthesis of disubstituted 2-amino-1H-imidazoles from 2-aminopyrimidines and α-bromoketones as per the (Scheme 8.17) [55]. Microwaves assisted procedure has appreciable improvements as compared to traditional methods. Few simple synthetic methods are also available in literature such as preparation of biologically active 2, 4(5)-diarylimidazoles by parallel synthesis according to Scheme 8.18 [242]. Zhu and colleagues reported a novel copper catalyzed *N*-arylation of imidazole under mild conditions as per Scheme 8.19 [240].



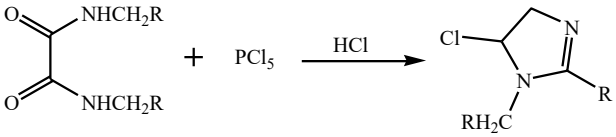
Scheme 8.3: Debus synthesis.



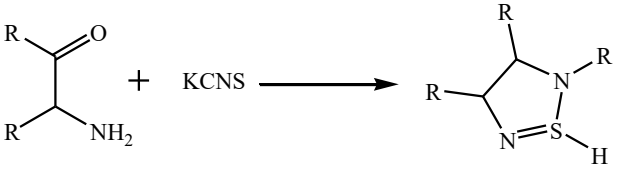
Scheme 8.4: Radiszewski synthesis.



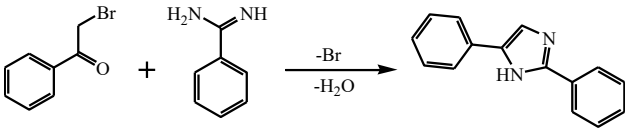
Scheme 8.5: Dehydrogenation of Imidazoline.



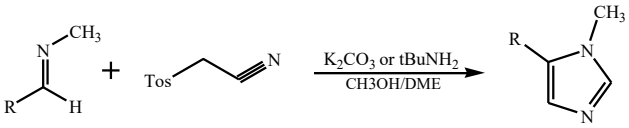
Scheme 8.6: Wallach synthesis.



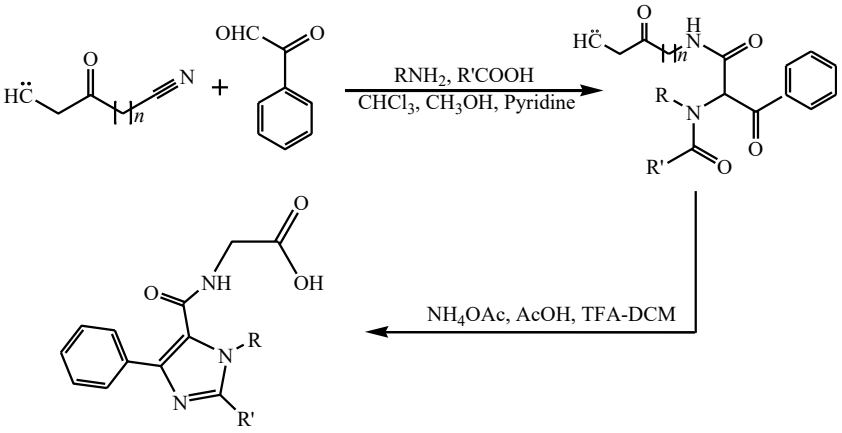
Scheme 8.7: Markwald synthesis.



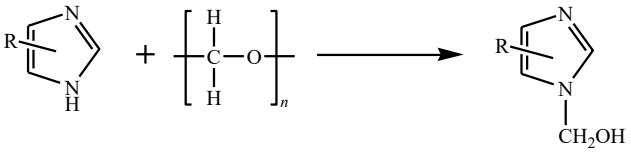
Scheme 8.8: Few general methods of preparation of imidazole derivatives.



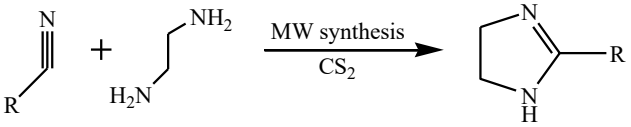
Scheme 8.9: Few general methods of preparation of imidazole derivatives.



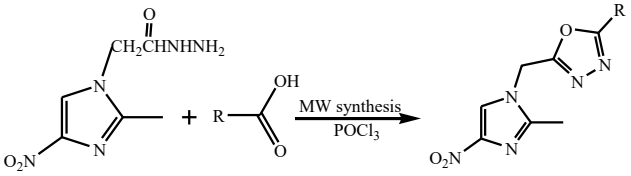
Scheme 8.10: Few general methods of preparation of imidazole derivatives.



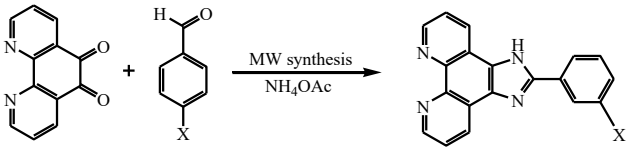
Scheme 8.11: Few general methods of preparation of imidazole derivatives.



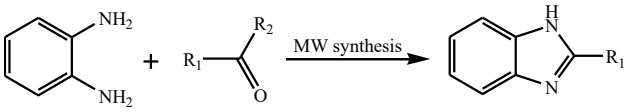
Scheme 8.12: Few general methods of preparation of imidazole derivatives.



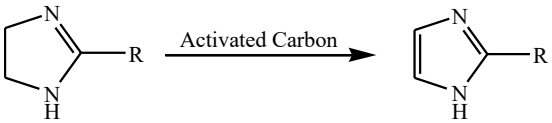
Scheme 8.13: Few general methods of preparation of imidazole derivatives.



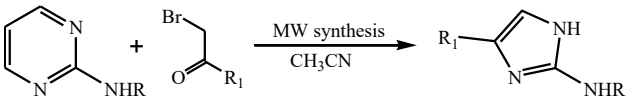
Scheme 8.14: Few general methods of preparation of imidazole derivatives.



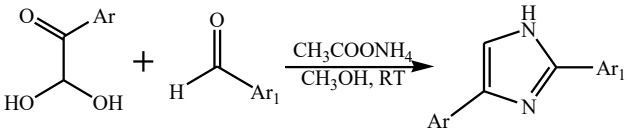
Scheme 8.15: Few general methods of preparation of imidazole derivatives.



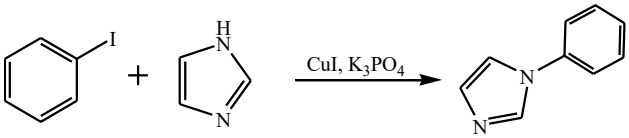
Scheme 8.16: Few general methods of preparation of imidazole derivatives.



Scheme 8.17: Few general methods of preparation of imidazole derivatives.



Scheme 8.18: Few general methods of preparation of imidazole derivatives.



Scheme 8.19: Few general methods of preparation of imidazole derivatives.

8.4 Pharmacological activity

In the medical applications, the effects (either beneficial or adverse) exhibited by a drug on the living matter are collectively termed as its pharmacological or biological activity. Imidazole ring is found in various naturally occurring bioactive molecules such as DNA, vitamin B12, biotin, histamine, and hemoglobin as well as the metabolites forms during the human metabolism which plays vital physiological role in the key biological activities [24]. As imidazole ring interacts with the various biological molecules, cations and anions, it has been commonly used to make the fluorescent

compounds as diagnostic agents and probes for the exploration of various biological processes. Due to the enormous pharmacological activities, synthesis of imidazole based novel bioactive molecules is a fascinating area for medicinal chemists with the recently developed strategies [229]. Various imidazole containing drugs have been used to treat diverse health problems such as antifungal (clotrimazole, and oxiconazole), antiparasitic (benznidazole, and secnidazole), antihistaminic (imetit, and immepip), antineuropathic (nafimidone, and fipamezole), antihypertensive (eprosartan, and olmesartan), anti-inflammatory (cimicoxib), antibacterial (metronidazole),

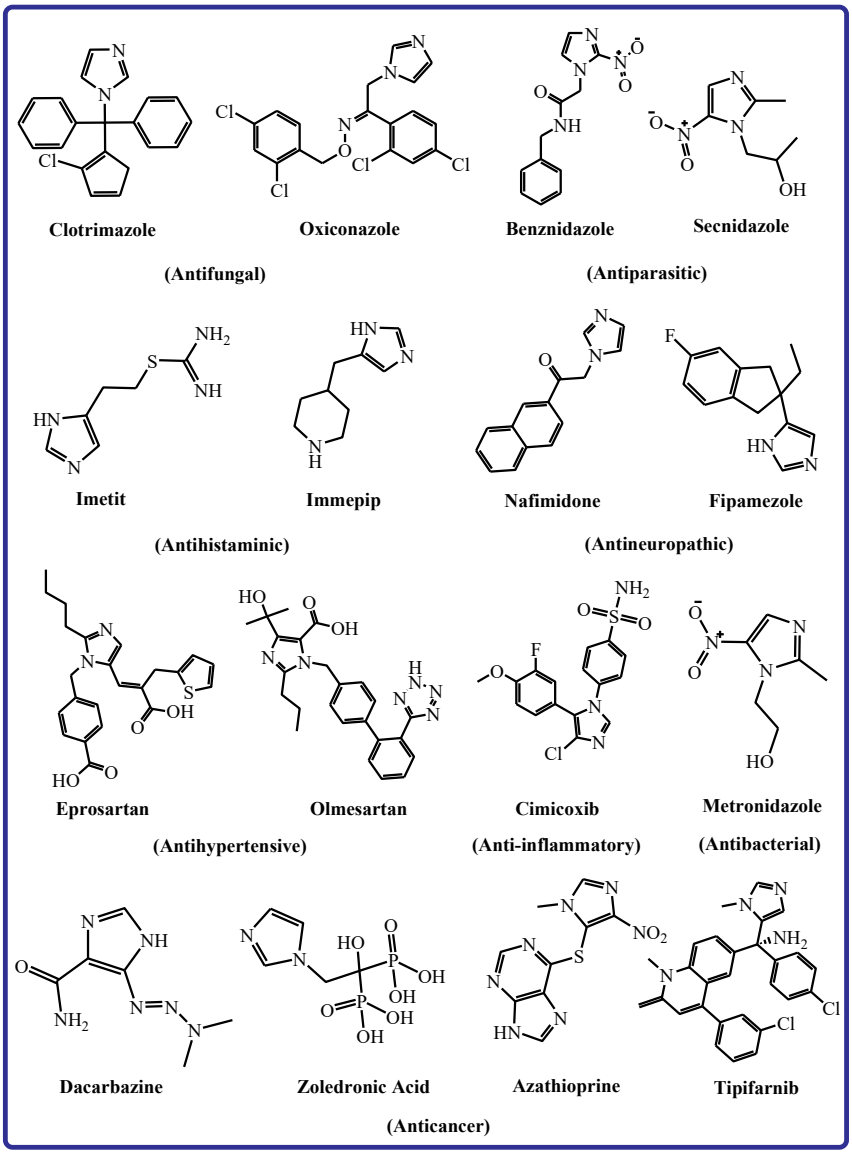


Figure 8.1: Few imidazole-based clinical drugs.

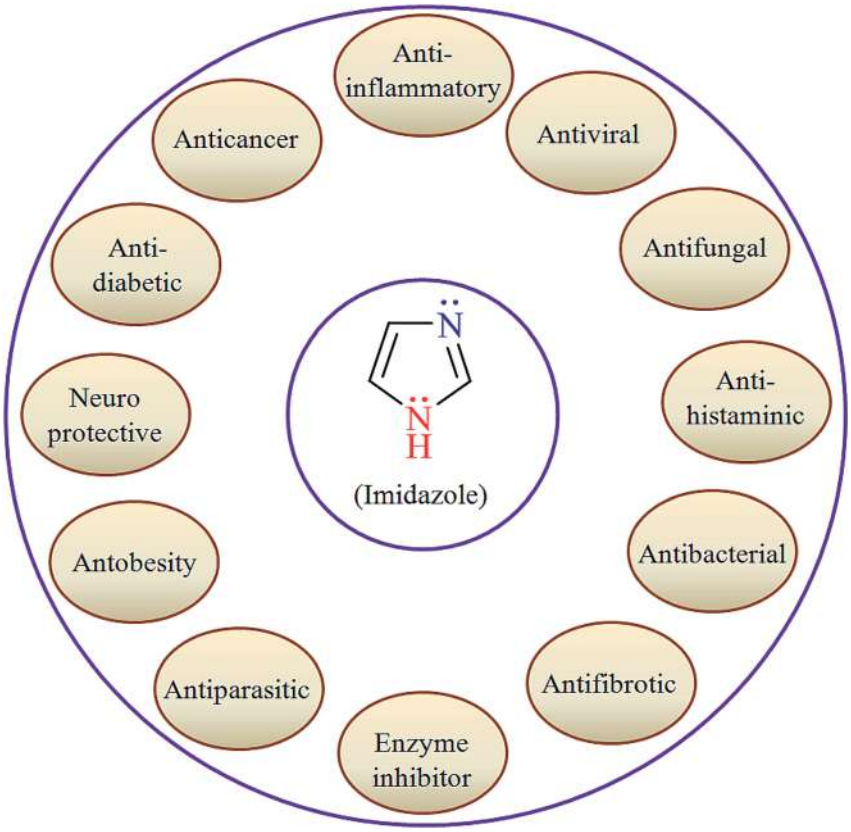


Figure 8.2: Biological activities of imidazole containing bioactive molecules.

anticancer (dacarbazine, zoledronic acid, azathioprine, and tipifarnib), and many more as representing in Figure 8.1 [178, 219]. Therefore, imidazole is an important part of many pharmaceuticals. Figure 8.2 represents the enormous biological activities of imidazole containing bioactive molecules.

8.5 Anticancer activity

In the last six to seven decades, extensive research such as radiation therapy, chemotherapy, surgery and combination of two or more therapeutics has been carried out globally with some great outputs for the development of an efficient therapeutics for cancer treatment [66]. Although, a variety of natural (viz. taxol, camptothecins) and synthetic molecules like azole derivatives (viz. fadrozole, letrozole), porphyrin drugs (viz. visudyne, photofrin), alkylating agents (viz. chlorambucil, mechlorethamin-oxide), and metal complexes (viz. carboplatin, cisplatin) showed good clinical results

[43, 193, 204, 234, 241] but the medicinal need stays unmet due to the various drawbacks such as low selectivity, high cytotoxicity, multidrug resistance, and poor curative effects. Efforts have been made towards the screening of novel tumor specific therapies with the potential of non-cross resistance and reduce metastasis [44, 95, 187]. Past and recent studies revealed the potential of imidazole derivatives as vital anticancer leads [6, 172]. Discovery of dacarbazine triggered the scientific community toward the design and development of imidazole based novel anticancer lead molecules. Many imidazole based molecules have been widely used in the treatment of cancer (Figure 8.1). These kinds of molecules possess enormous potential to conquer the various drawbacks of currently used anticancer drugs. Alkahtani and colleagues synthesized novel imidazole derivatives which showed strong anti-proliferative activity as well as induce the cancer cell apoptosis [7]. Negi et al. also developed some interesting molecules based on imidazoles which showed the promising potential (low micromolar IC_{50}) against different cancer cell lines such as A-459, H-460, and Hep-G2 [136]. Gu et al. synthesis imidazole derivatives of dehydroabietic acid and found that during *in vitro* cytotoxic assay most of the molecules exhibited visible cytotoxicity against SMMC-7721 as well as HepG cancerous cell lines while abridged cytotoxicity against was noticed against noncancerous human hepatocyte [67]. In 2018, Xiao and co-workers synthesized novel imidazole-quinoline derivatives and screened them by MTT assay against different anticancer cell lines including A549, HepG2, MCF-7, and PC-3. Results confirmed that one molecules exhibited very good anticancer activity against HepG2, A549, and PC-3 cells with the IC_{50} values as 2.42 ± 1.02 μM, 6.29 ± 0.99 μM, and 5.11 ± 1.00 μM, respectively [209]. Zhao et al., synthesized dehydroabietylamine imidazole derivatives which exhibited noticeable *in vitro* antineoplastic activities [224]. Recently, many research groups around the globe worked on the design and synthesis of novel anticancer molecules containing imidazole moiety as their core skeleton. These molecules also showed great *in vitro* anticancer activities with the good alignment to their SAR and ADMET studies against various types of cancer [4, 142, 214].

8.5.1 Imidazoles as topoisomerases inhibitors

DNA topoisomerases are a class of enzymes which catalyze the winding and unwinding of DNA strands and controls it's supercoiling. Topoisomerases (TOP) is the fascinating targets for anticancer chemotherapeutics and they play significant role in the transcription, progression, apoptosis and other cell regulations [10, 138, 152]. TOP inhibitors stabilize the cleavable DNA-enzyme complex as well as they regulate the replication and transcription in malignant tumor cells. Antitumoral drugs including amonafide, amsacrine, anthracycline (daunorubicin and doxorubicin), camptothecins, epipodophyllotoxin (etoposide and teniposide), irinotecan, mitoxanthrone, and topotecan acts as TOP inhibitors. Figure 8.3 represents some leading topoisomerase

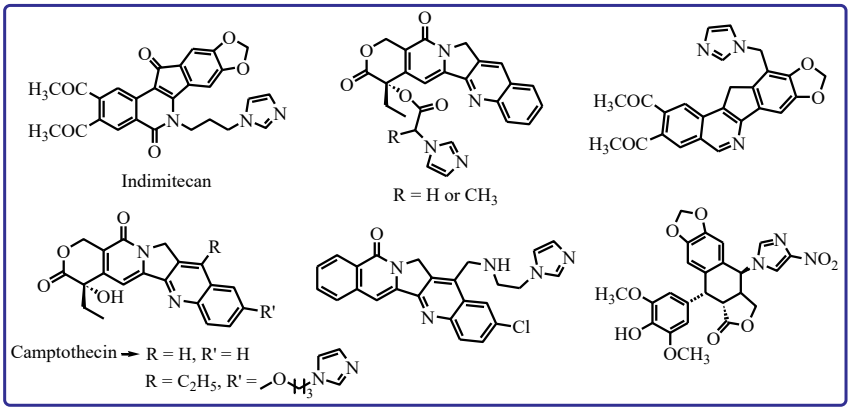


Figure 8.3: Some imidazole derivatives with topoisomerase inhibition activity.

inhibitors, which are based on imidazole scaffold. Approximately 50% of anticancer agents act as the potential TOP inhibitors though poor solubility, tiny action duration and high toxicity hampers their use. Lots of efforts have been carried out in search of an effective and safer inhibitor(s) for TOP. Imidazole, having two nitrogen atoms available for hydrogen bonding, is a suitable moiety for the synthesis of water-soluble molecules and thus used frequently for the design and development of bioactive leads. The first effective TOP-1 inhibitor was camptothecins isolated from *Camptotheca acuminate* in 1960s. It is natural secondary metabolites and known for its potential anti-tumor and immune deficiency disease resistance activities [110]. The lower water solubility of camptothecin hampers its uses. Another time, Li and co-workers alter the camptothecins by the induction of the ethyl and imidazolyl groups at 7 and 10-positions and the results conclude that both the TOP-1 inhibition and water solubility was greatly enhanced. The modified molecule showed superior IC_{50} value (0.16 and 0.06 μM) than topotecan (0.15 and 0.07 μM) against MCF-7 and HCT-8 cells [108]. Studies on the camptothecin explore that its lactone ring is unstable and generates toxic carboxylates inside the human beings. Li and co-workers amended the molecules at 20-position by the addition of imidazolyl which decreases the toxicity along with the augment the stability of lactone ring as well as superior anticancer activity on MCF-7, PC-3, and HCT-8 cancer cell lines compared to camptothecins [109]. Instability of lactone ring may be conquering by using non-camptothecin molecules with parallel potency and efficacy as TOP I inhibitors. In this regard, imidazole modified aromathecin (synthesized by the replacement of hydroxyl lactone by benzene ring) was found as interfacial inhibitor same as "camptothecin like" binding to the TOP I cleavage complex [23, 41, 153]. Imidazole derived indenoisoquinolines (without unstable hydroxyl lactone) are also used as vital non-camptothecin TOP I inhibitors. These compounds exhibited improved water solubility and enhanced affinity towards DNA as they interact with the phosphate group of DNA backbone [94]. Norindenoisoquinolines are also inhibits the

TOP I but have poor solubility. Incorporation of imidazole in norindenoisoquinolines enhanced the solubility and also increases the anticancer efficacy against MCF-7, HCT-116, IGROVI, and SN12C cancerous cell lines [182]. Amsacrine are the acridine based molecules known as DNA TIPO II inhibitors. These molecules intercalate into the DNA base pairs resulted the cell cycle arrest which lead towards the apoptosis [179]. Addition of imidazole moiety into 9-amino acridine is a booming approach for designing of novel anticancer agents. Sondhi et al. synthesized few novel acridine based molecules with promising anticancer activity against some human cancer cell lines such as A-549, COLO-205, MCF-7, HEP-2, HCT-15, and IMR-32 and these molecules also exhibited good anti-inflammatory potential [181]. Podophyllotoxin (a novel natural DNA-TOP II inhibitor) derivatives are encouraging the researchers by their clinical efficacy against different kinds of neoplasms. Imidazole derivatives of podophyllotoxin exhibit more strong anticancer activity against various human cancer cell lines such as K562, K562/ADM, and HeLa as compared to etoposide [170]. In 2011, Kundu and co-workers developed a series of imidazole based potent DNA non-intercalating topoisomerase IIα catalytic inhibitors which showed the superior *in vitro* anticancer activity than 5-fluorouracil and etoposide against breast and kidney cell lines [20]. Recently many research groups synthesized the TOP inhibitors containing imidazole moiety. Joshi and Kumar synthesized imidazole fused quinoxalines molecules which acts as dual inhibitors of human TOP and found to exhibit the anticancer potential at very low concentration (IC_{50} < 4.5 μM) in MDA-MB-231, A549, and cancer cells. These molecules were also found to amend the levels of intracellular reactive oxygen species and led to the apoptosis of cancer cells through mitochondrial independent pathway [82]. Paul's group synthesized and screened a series of derivatives containing imidazole moiety. They also worked on the binding mechanism of synthesized derivative with ct-DNA and concluded that one molecule found to inhibit the human topoisomerases IIα and also induce the apoptosis in cancerous cells [177]. Zhou and co-workers designed and synthesized a novel series of benzimidazole-chalcone hybrids molecules which inhibit the DNA relaxation mediated by topoisomerases II and antiproliferative activities against the four tumor cell lines. Two compounds from this series showed the versatile effects such as inhibition of migration and colony formation as well as promote the apoptosis in A549 cancerous cell lines [237].

8.5.2 Imidazoles as inhibitors of rapid accelerated fibrosarcoma (RAF) kinases

There are frequent oncogenic mutations have been explored in the components of mitogen-activated protein kinase (MAPK) signaling pathway especially in the rapidly accelerated fibrosarcoma (RAF) kinases resulting its association in the initiation of human cancer. Mutation in the RAF kinases leads towards the auto activation of MAPK signaling pathway and result in the uncontrolled growth and proliferation of normal

cell which turns it into the cancerous cells. Thus, the inhibition of RAF kinases (A, B and C) provides a ray of hope in the discovery of potent anticancer agents [157]. Sorafenib is a unique drug available as potent antitumor agent. It targets the Raf/Mek/Erk pathways, and inhibits the various intracellular factors like B-RAF, mutant B-RAF, C-RAF and cell surface kinases such as FLT-3, VGFR-2 & 3, PDGFR-β etc. [29]. Imidazole analogues of sorafenib (Figure 8.4) having amide linker **(a)** and urea bridge **(b)** exhibited superior anticancer activities as compared to the sorafenib (GI_{50} = 0.78 μM) against the melanoma cancer cell line (WM3629) with GI_{50} value of 0.62 and 0.65 μM, respectively. These two molecules were also found to have an outstanding C-RAF inhibition effect; mainly analogue with amide linker (Figure 8.4a) exhibited high selectivity and inhibit almost 99% activity of C-RAF at very low concentration (10 μM). The substitution of chlorine from imidazole analogue of sorafenib with urea bridge (Figure 8.4b) diminishes its bioactivities [102]. Ranjitha et al. also synthesized some imidazole derivatives of sorafenib (Figure 8.4c) with excellent RAF inhibition activity and greater anticancer activity than sorafenib against A549 and DLD-1 cancerous cell lines [156]. The molecule also showed superior *in vitro* anticancer activity as compared to sorafenib against the acute myeloid leukemia (MV4-11) cancer cell lines. During its modeling studies, it has been confirmed that the molecule occupying the ribose position of ATP binding pocket (essential for anticancer activity). Yu and co-workers also synthesized some imidazole incorporated pyrazole moieties and concluded that a molecule (Figure 8.4d) inhibit the activity of C-RAF by approximately 96% at 10 μM concentration. The molecule also showed great anticancer activities against A375P and WM3629 (human melanoma cell lines) having low GI_{50} (2.24 and 0.86 μM, respectively) in contrast to sorafenib with GI_{50}

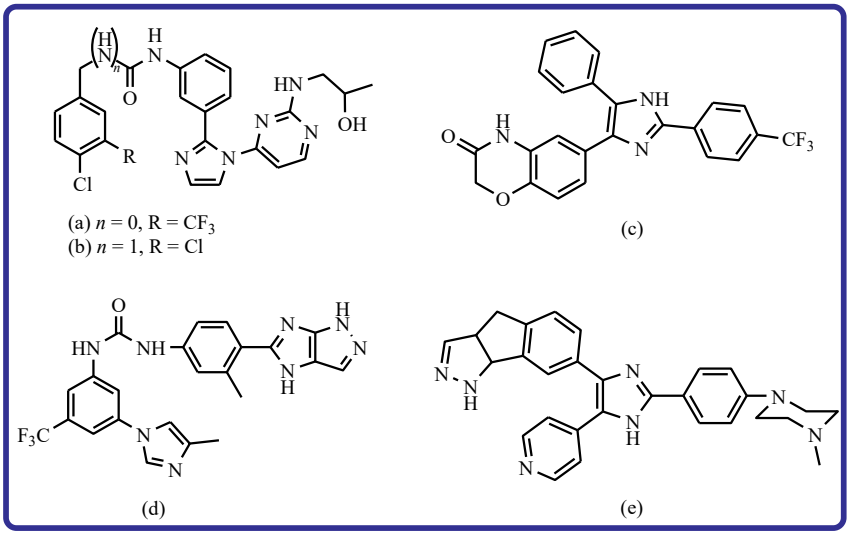


Figure 8.4: Imidazole derivatives as RAF kinase inhibitors.

value of 5.58 and 0.65 μM, respectively [216]. Niculescu-Duvaz and colleagues also reported an imidazole containing pyrazole tricyclic derivative (Figure 8.4e) as potential B-RAF inhibitor along with high quality anticancer activity with IC_{50} value of 0.24 μM against melanoma. This study also indicates the crucial role of imidazole and pyrazole moieties in the bioactivities of the molecule, and the substitution of tricyclic pyrazole by tricyclic triazoles or other six member ring diminishes its activity and B-RAF inhibition potency (Niculescu–Duvaz et al., 2010). Kim et al. synthesized a series of (4-aminobenzyl/benzoyl)-1H-imidazol-1-yl pyrimidin-2-yl derivatives and screened them for their bioactivities. Results concluded that one molecule exhibited promising anticancer activity against the A375P (human melanoma cell line) and U937 (human leukemic monocyte lymphoma cell line) with GI_{50} value of 1.82 and 2.73 μM, respectively in contrast to sorafenib (GI_{50} = 3.40 and 2.74 μM, respectively). In addition, the molecule was found to be highly selective and effective inhibitor of C-RAF as well as ^{V600}EBRAF with IC_{50} in nanomolar range (8.79 and 38.3 nM, respectively) [93]. It has been confirmed by molecular docking studies (with PDB ID: 1UWJ) that addition of carbonyl or methylene groups to the imidazole derivatives augment the selectivity as well as potency of the molecule(s). Furthermore, methylene group in between the imidazole and phenyl ring enhance the flexibility of designed derivative which help them to come closer to the secondary hydrophobic pocket of the protein (RAF) [200]. Takle et al. synthesized a novel triarylimidazole derivative (bearing a 2,3-dihydro-1H-inden-1-one oxime substituent) and it was found to be a potent and extremely selective inhibitor of B-RAF [190]. Recently, El-Damasy et al. synthesized some imidazole derivatives and one molecule from these has showed the vital B-RAF^{V600E} and C-RAF kinases inhibition activity in nanomolar range. Two molecules containing piperazine substituted phenyl ring come out as the most active members surpassing the anticancer potencies of imatinib (an FDA-approved drug) [54]. Ali and co-workers have also designed and synthesized 38 imidazole derivatives with sulphonamide moiety. Out of these two molecules exhibited highest potency against melanoma cancer cell lines and four molecules emerged as the potent $BRAF^{V600E}$ inhibitor [5].

8.5.3 Anticancer effects of imidazoles as microtubule destabilizing agents

Microtubules are filamentous, long tube shaped protein polymers and key component of cytoskeleton in all eukaryotic cells. They are composed of α-tubulin and β-tubulin heterodimers and essential for various fundamental cellular processes including the transport of mitochondria, vesicles and other necessary components, maintenance and development of cell shape, cell signaling and division [96]. Difference in some of the microtubule regulatory proteins (stathmin, survivin, TOG, MAP4, MCAK, EB1, dynactin 1, FHIT, and RAC1) and microtubule associated proteins (dynein and kinesin motor proteins) have been seen to dominate in certain cancer cells and drug resistance

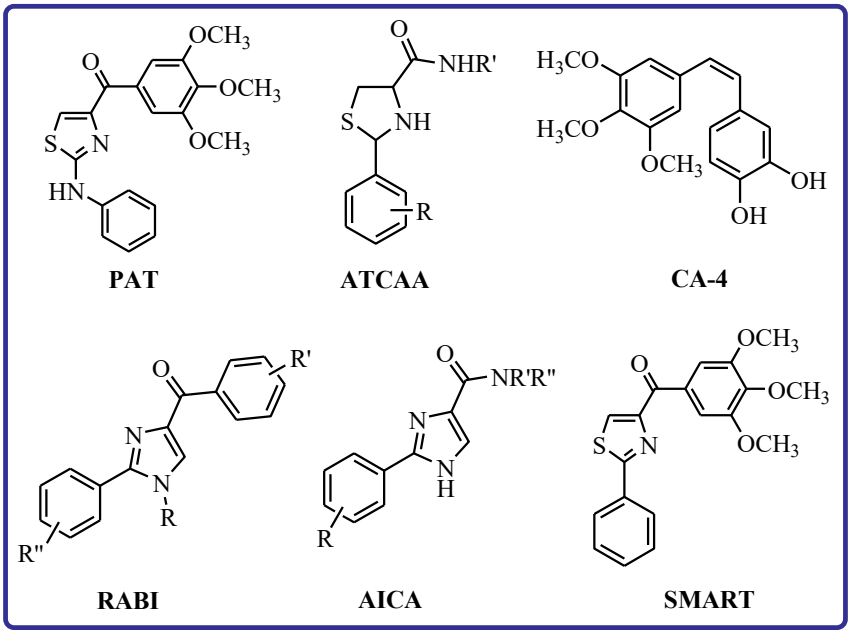


Figure 8.5: Imidazole derivatives as microtubule destabilizing agents.

conditions [59, 62, 126]. Passionate exploration is needed to understand the specific role of these changes in the tumor sensitivity towards the microtubule active agents [47, 81, 85]. Among various strategies for the development of an effective anticancer agent(s), molecule(s) that may inhibit the formation mitotic spindle fibers through the binding of tubulin is a mesmerizing area of research. Basically the anticancer chemotherapeutic agent binds to the microtubules and disorders their dynamics or inhibits the polymerization which ultimately leads to the apoptosis of cancerous cells [53]. Various tubulin binding anticancer agents such as vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, combretastatin A-4 phosphate, and epothilones, etc. exhibits great clinical effectiveness [70, 80, 85]. Four unique binding sites are available in tubulin to which vinca alkaloids, colchicine, taxanes and laulimalide bind and exhibit the anticancer activity [15, 22, 27, 206]. Microtubule targeting agents which target the binding sites of vinca alkaloids (such as vincristine and paclitaxel) and taxanes are proved as highly potent anticancer drugs and have been approved for their clinical trials [141, 171]. Though, the clinical use of the above mentioned agents has been hampered due to the variety of reasons like poor water solubility, development of MDR (multi-drug resistance), and deprived pharmacokinetics [174]. Molecules targeting the colchicine binding site in tubulin may conquer these limitations [106]. Research group of Lin and Chen have discovered numerous potential tubulin inhibitors based on imidazole (Figure 8.5) such as 2-arylthiazolidine-4- carboxylic acid amides (ATCAA), 2-aryl-4-benzoyl-imidazoles (ABI-III), 2-arylimidazole-4-carboxylic amide

(AICA), phenylaminothiazole (PAT) analogs and 4-substituted methoxybenzoyl-arylthiazoles (SMART) analogs [33–35, 105, 119–122]. Taxol (Paclitaxel) arrest the cells in G2/M phase of cell cycle thus cells are incapable in the formation of the mitotic apparatus necessary for cell division. It has an exclusive binding site on microtubule polymer which make it unique chemotherapeutic agent. Taxol have the unusual capacity to polymerize the tubulin in the absence of guanosine triphosphate and microtubule-associated proteins [205]. Like this, vincristine also destabilizes the microtubule and discontinues the chromosome separating in metaphase stage of cell division which leads towards the cell apoptosis [19]. Many research groups are interesting in synthesizing the imidazoles based novel molecules with potential microtubule destabilizing ability [32, 84, 86, 176, 236]. Li and colleagues have applied high throughput screening and discovered 2-amino-1-arylidenaminoimidazoles as an oral anticancer agent which target the microtubules for its bioactivities. These compounds showed great anticancer activity against the human gastric cancer cells (NUGC-3) and observed the disordered microtubule under fluorescence imaging through the diffused green fluorescent tubulin-immunoreactive activities. They also found that modification of the phenyl ring of arylidene also exhibited great value of anticancer activities against the NUGC-3 cancer cells [112]. In a different work, this research group synthesized a series of imidazole derivatives linked with thiazole moiety which destabilized the microtubule upon oral administration. 1-(4-phenylthiazol-2-yl)-4-(thiophen-2-yl)-1H-imidazol-2-amine was confirmed as the lead molecule from the synthesized series along with the IC_{50} value of 0.05 μM and showed oral activity as well as appreciable lifespan of leukemia mice (75% longer in contrast to the vehicle control group vinblastine 83%) [111]. Kamal et al. performed the design and synthesis of chalcone conjugates of imidazoles. They screened them against variety of human cancer cell lines including A549, DU-145, HeLa, HT-29 and MCF-7 in search of a vital microtubule inhibitor and found IC_{50} in between 0.64 and 30.9 μM. In addition, the flow cytometric analysis concluded that one molecule seize the cell cycle in G2/M phase which results in the apoptosis [83]. Stepanov and co-workers developed a series of benzoimidazolyl furazanamines as microtubule destabilizing agents in sea urchin embryo model along with various human cancer cell lines. They performed the fertilized egg test to examine the antimitotic activity after compounds treatment and observed the cleavage arrest/alteration and swimming pattern of blastulae treated after the hatching period [184]. Recently, Li and co-researchers designed and synthesized a series of 1-substituted-2-aryl imidazoles based on their earlier reported molecules (analogs of 2-aryl-4-benzoyl imidazole and combretastatin A-4) which exhibited the strong antiproliferative activities (IC_{50} in nanomolar range) against MDA-MB–231, MDA-MB-468, T47D, HCT15, HT-29, and HeLa cells. One compound among these found to arrest the cell in G2/M phase and leads the cancerous cells towards the apoptosis. The same molecule also exhibited great hope during *in silico*, *in vitro* and *in vivo* studies [107]. In the same year, Wang and colleagues synthesized (2-(1 H-Indol-3-yl)-1 H-imidazol-4-yl) (3,4,5-trimethoxyphenyl) Methanone (ABI-231) analogues which showed the specific

inhibition activity for colchicine binding site present in tubulin with low risk of potential off-target function [202]. Ren and co-workers also discovered some novel benzimidazole and indazole analogues as the potent inhibitors for tubulin polymerization along with vital anticancer potential. One molecule found its IC_{50} in nanomolar range (50 nM) which is somewhat better in contrast to the colchicine [159].

8.5.4 Farnesyltransferase inhibition potential of imidazoles

Human protein farnesyltransferase (FTase) is the member of prenyltransferase group (transfer allylic prenyl groups to acceptor molecules). FTase commands the post-translational addition of isoprenoid lipid molecules to the protein and believed as significant therapeutic agent in various diseases such as cancer [18, 78, 117, 163]. During post-translation modifications, FTase modifies the newly synthesized Ras proteins and other farnesylated proteins which exhibit the vital on the signal transduction, cell proliferation and apoptosis thus the inhibition of farnesylation is supposed to control the cancer cells growth, hence researchers are focusing on the design and development of novel inhibitors for the same [16]. Approximately in the 30% of human cancer cases, mutations of Ras proteins have been confirmed [230]. Numerous novel molecules have been developed for FTase inhibition which showed the potential anticancer activity by mediating the arrests of cell in G2/M phase and apoptosis along with the high efficacy and low toxicity [130, 201]. FTase posses zinc ions at their active site which are targeted by imidazole ring thus imidazoles presents a great contribution in the control and cure of cancer. Tipifarnib is an imidazole derivative with outstanding FTase inhibition activity in blood cancer [222]. Asoh and co-researchers designed and synthesized imidazole based novel benzofuran FTase inhibitors with potent antitumor activity. One molecule among the all newly synthesized have been found to exhibited a strong antitumor activity against human cancer xenografts in mice with IC_{50} value of 1.1 nM. Advance studies concluded that the addition of cyano group to benzofuran ring significantly enhance the potency of FTase inhibition [14]. Fletcher and colleagues identified a class of potent inhibitors for human FTase by "piggy-backing". The inhibitors were derivatized on the basis of 4-fold substitution of ethylenediamine scaffold. The lead molecule exhibited human FTase inhibition along with the 25 nM value of IC_{50} as well as 90 nM IC_{50} for whole cell H-Ras processing. Additionally, the newly synthesized molecules showed a very high selectivity towards human FTase (~333-fold) in contrast to geranylgeranyl transferase-I. In the advanced research exploration, it was found that para-nitrile phenyl moiety plays a key role in their bioactivity through $\pi-\pi$ stacking interactions [57]. Azaheptapyridines are another class of vital molecules with promising FTase inhibition potential. Zhu and co-workers described the discovery of novel C-linked imidazole azaheptapyridine bridgehead FTase inhibitors with effective cellular inhibitory activities. Synthesized molecules also found to reduce hERG capability as compared to the previously synthesized N-linked

imidazole series by the same group. A molecule from this series showed FTase inhibition activity more in its R-form (IC_{50} = 0.38 nM) as compared to S-form (IC_{50} = 12 nM). X-ray studies of one molecule confirm its binding with FTase via the interactions of imidazole 3N and zinc ion in catalytic site [238]. Duez and co-researchers also constructed new bisubstrate FTase inhibitors. Imidazole containing farnesyl analogues with the moiety of malonic acid showed most excellent FTase inhibition activity with 0.24 μM value of IC_{50}. It was also found that the removal of farnesyl chain leads to a 300-fold decrease in the FTase inhibition [52]. In 2014, Zhu and colleagues developed a series of C-imidazole bridgehead azaheptapyridine as potent FTase inhibitors. One candidate among these showed an effective enzyme and cellular activities with IC_{50} value of 1.4 and 1.3 nM, respectively along with the exceptional pharmacokinetic profiles in mice, rats, dogs and monkeys [239]. Recently, Kazi and colleagues synthesized a novel molecule which found to inhibit both farnesyl as well as geranylgeranyl transferase. The synthesized lead compound conquers the key hurdles in KRAS resistance, preventing the growth of patient derived mutant KRAS- driven xenograft of pancreatic tumors [88].

8.5.5 Imidazole based inhibitors of Transforming growth factor (TGF)-β

The immunosuppressive cytokines such as transforming growth factor (TGF)-β and interleukin (IL)-10 are secreted from tumor cells. They restrain the dendritic cells maturation and functions of T cells in tumor microenvironment [25]. TGF-β is a multifunctional cytokine amends the proliferation and differentiation of cell by activin receptor-like kinase (ALK)-5 and also has a key role in the proliferation, metastasis, and various other significant processes in the malignancy. Elevated level of TGF-β receptors has been found in various types of cancers [45, 151, 227]. TGF-β and type II receptor activates the ALK5 in the GS domain of juxtamembrane and leads to the stimulus the kinase activity. Thus, the ALK5 signals phosphorylation of Smad2 and 3, and subsequent binding of Smad4 for the formation of complexes. The Smad complexes relocate into nuclei and target the specific genes to control the production of extracellular matrix, proliferation, migration, and apoptosis of cells [49]. Dewang and Kim synthesized some novel molecules based on both imidazole and pyrazole and found that 3-(3-(6-methylpyridin-2-yl)-4-(quinolin-6-yl)-1H-pyrazole-1-carbothioamido)benzamide exhibited 96 and 93% ALK5 inhibition in the HaCaT cells (transiently transfected with p3TP-luc reporter construct) and ARE-luc reporter construct, respectively [50]. Guo conceptualize and synthesize some novel molecules based on 2-(6-methylpyridin-2-yl)-1H-imidazoles for the plausible inhibitors for ALK5. In the cell based luciferase reporter assays, on molecule showed the inhibition activity of ALK5 with the IC_{50} value of 0.24 μM which is superior to its known inhibitor (SB431542) whose IC_{50} value is 0.35 μM. *In silico* studies was found to support these experimental findings with the tight binding of this molecule in the cavity of ALK5 [69].

Past research studies showed the importance of quinoxaline ring (a heterocyclic ring complex containing both the benzene as well as pyrazine ring) for enhancing the selectivity and potency of ALK5 inhibitors. Kim and co-workers synthesized some novel benzenesulfonamide-substituted 4-(6-alkylpyridin-2-yl)-5-(quinoxalin-6-yl)imidazoles. The results of luciferase reporter assay concludes that 4-[5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide and 4-[5-(6-ethylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide exhibit >90% ALK5 inhibition at the concentration of 0.5 μM [92]. GlaxoSmithKline (GSK) developed a novel drug molecule the inhibition of receptor-like kinase (ALK) receptors for ALK4/5/7 which is known as SB-431542. This molecule does not inhibit the anaplastic lymphoma kinases which are generally known as ALK inhibitors [101]. Inman and colleagues also characterize and performed the extensive biological analysis of endogenous serine/threonine kinases for as SB-431542 small molecule ALK5 inhibitor. They concluded that the molecule shall work as a precious for the exploration of more knowledge related to the role of activin, TGF-β and nodal signaling in various biological applications [76]. In 2009, two different series of molecules (1,2,4-trisubstituted imidazoles and 1,3,5-trisubstituted pyrazoles) were synthesized and evaluated as possible in ALK4/5/7 inhibitors and cytotoxicity by Li et al. Two compounds showed the 10-fold selective inhibition of ALK5 compared to SB431542 [114]. Ciayadi and colleagues synthesized novel TGF-β1 and activin A inhibitors based on 2-aryl-4-(3-(pyridin-2-yl)-1H-pyrazol-4-yl)pyridine. It was noticed that phenyl/aromatic N-heterocyclics restrained both (TGF-β1 and activin A) type of signaling in HEK-293T cells with selective TGF-β1 inhibitor. Lead molecules possessing pyrazol-1-yl or 1H-imidazoyl-1-yl, pyridin-3-yl, pyrazol-4-yl moieties displayed the structural as well as functional aspects which found to be appropriate for the development of potential TGF-β inhibitors [40]. Thiazolyl imidazoles are another class of novel molecules with high quality of water solubility. Amada with his colleagues synthesized various molecules containing 5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1H-imidazole and screened them through cell culture based TGF-β-induced Smad2/3 phosphorylation inhibition activity for the potential inhibition of TGF-β. One molecule was found to appropriate inhibitor for TGF-β-induced Smad2/3 phosphorylation with IC_{50} value of 36 nM along with selective ALK5 inhibitor with the enzyme activity of IC_{50} value of 5.5 nM [8]. In 2012, Amada with some additional researchers synthesized a novel series of 4-thiazolylimidazoles and screened them as TGF-β inhibitor. N-{[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1H-imidazol-2-yl]methyl}butanamide showed the selectivity as well as potency towards ALK5 inhibition with fine enzyme inhibitory activity (IC_{50} = 8.2 nM) and inhibition of TGF-β-induced Smad2/3 phosphorylation with the IC_{50} value of 32 nM [9]. Galunisertib (LY2157299) is a TGF-β inhibitor developed by Eli Lilly. It showed the improvement of tumor growth inhibition with the combination of PD-L1 blockade in phase II clinical trials of hepatocellular carcinoma [71, 74]. However in January 2020, Eli Lilly discontinue the development of galunisertib. Recently, park and co-workers synthesized as series of 2,4-disubstituted-5-(6-alkylpyridin-2-yl)-1H-imidazoles as vital ALK5 inhibitors. They integrate a methylene linker and quinoxalin-6-yl moiety at 2- and 4-position of imidazole

ring along with a m-$CONH_2$ substitution of phenyl ring and developed a potent and selective inhibitor for ALK5 which is also strongly supported by molecular docking studies [146].

8.5.6 Imidazole based molecules as DNA intercalators

Intercalators are the molecules that insert between the neighboring base pairs and deform the double helical structure of DNA. Typically they contain polyaromatic moieties which stack with the bases of DNA, form H-bond and ionic interactions and ultimately hinder the replication, transcription and cell growth as well as division. Discovery of novel intercalators as anticancer agents is a robust field of research. Scientific advancement will encourage the researchers to explore this area for the design and development of novel anticancer leads with improved activity and diminish side effects [173]. Ozkay and co-workers synthesized 18 novel derivatives based on the imidazole-(benz)azole and imidazole-piperazine. Anticancer potential of these molecules were tested against HT-29 and MCF-7 carcinoma cell lines by using MTT assay, analysis of DNA synthesis along with the exploration of apoptotic DNA. Most of the synthesized molecules exhibited superior anticancer activity against HT-29 cells compared to MCF-7. The molecules showed good activity against both types of carcinoma cells were found to be poor DNA synthesis inhibitor. Three molecules showed greater DNA fragmentation in HT-29 cells [143]. Chin et al. discovered a novel triaryl-substituted imidazole (TSIZ01) with potential ligand capability towards the telomeric G-quadruplex. The discovered lead molecule also found to exhibit the 8.7 fold more selectivity for telomeric G-quadruplex in contrast to duplex DNA [37]. Satam and colleagues design and synthesized a new class of water soluble anticancer molecules based on imidazole and pyrrole-containing polyamides of low molar mass which work as selective binding agents for DNA sequence as well as have plausible role in controlling the gene also. They selected the 5′-ACGCGT-3′ sequence as target and designed Ph-ImPy*Im lead molecule. Resulted concludes the minor grove bonding of both (diamino/dicationic and monoamino/monocationic) the synthesized polyamides against the 5′-ACGCGT-3′ sequence. The diamino/dicationic polyamide showed the superior activity in SPR studies [166]. Zhao and co-workers synthesized imidazole based Schiff base derivatives and screened them against various cancer cell lines including A549, HeLa, HepG2, and MCF-7. Most of the synthesized compounds found to exhibit the great cytotoxicity against MCF-7 cells line with the value of IC_{50} between 2.5 and 15 μM. One molecule was found 180 times more effective against HepG2 cells in contrast to HUVECs cell line (normal cells derived from the endothelium of veins from umbilical cord). Two molecules (containing imidazole and leelamine) were added to the complex of ethidium bromide and DNA of salmon sperm and found decline in the fluorescence indicating the intercalation. Upon adding more DNA to these compounds showed a hypochromic shift which confirms the DNA intercalation [225]. Zhao and

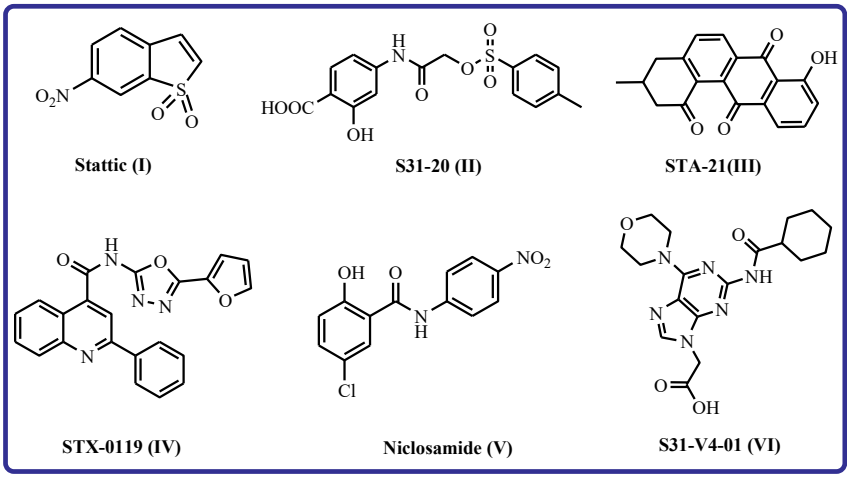


Figure 8.6: Few STAT-3 inhibitors.

colleagues further extend their work and synthesized a series of imidazole derivatives and evaluated for their anti-neoplastic potential against A549, HeLa, HepG2, and MCF-7 cancer cell lines along with a normal cell line (HUVECs). All the molecules showed superior anti-neoplastic activity and low toxicity compared to leelamine [225].

8.5.7 Imidazoles inhibiting the STAT-3

The signal transducer and activator of transcription-3 (STAT-3) is a transcription factor encoded by STAT3 gene in humans and plays vital role the proliferation, invasion, angiogenesis, metastasis and survival of cancer cells. Akira and colleagues initially identified the STAT-3 in 1994 as a DNA-binding factor in the IL-6-stimulated hepatocytes [3]. STAT-3 found to be constitutively active in most of the tumor cells but not in normal cells. Thus the STAT-3 is a key target for the design and discovery of leads which may impede the growth of cancer cells. The developed inhibitors to reduce the overexpression STAT-3 are still in the clinical research stage [213]. Figure 8.6 representing some potential inhibitors for STAT-3. Su and co-researchers synthesized a series of novel imidazopyridine molecules and screened them against PLC5 hepatoma cell line for the potential inhibitors of STAT-3. Their investigation conclude that one molecule in the series found to reduce the phospho-STAT-3 level and inhibiting the downstream signaling cascade which shrink the survival of HCC (hepatocellular carcinoma) cells [186]. Wang and co-workers synthesized a series of a series of 2,6-disubstituted purine derivatives containing imidazole moiety and screened them as believable inhibitors for STAT-3. One molecule (PD26-TL07) showed extraordinary antiproliferative potential against HCT-116 (IC_{50} = 1.77 ± 0.35), MDA-MB-231 (IC_{50} = 1.25 ± 0.38 μM), and SW480

(IC_{50} = 1.51 ± 0.19) cancer cell lines. In the advanced studies, PD26-TL07 was found to inhibit the STAT-3 phosphorylation in an efficient manner. The PD26-TL07 was found to exhibit great anticancer activity in both *in vitro* and *in vivo* studies [203].

8.5.8 Imidazoles as heat shock protein inhibitors

Heat shock proteins (HSPs) are the molecular chaperones play key role in the protein folding and protein-protein structure and function all through the cellular stress. HSPs are among the key entities for the survival of cancer cells. So the HSPs are the vital targets for cancer therapeutics [115]. In this regard, HSP90 is the most studied chaperon for the discovery and development of anticancer leads and numerous molecules for its inhibition are under clinical trials but at present there is no approved anticancer drug against HSP90 inhibition. So this is the necessity of time to develop novel molecules targeting the HSPs [165, 175]. Uno and co-researchers design and synthesized a series of HSP90 inhibitors based in the SAR optimization of a molecules containing 4-(4-(quinolin-3-yl)-1 H-indol-1-yl)benzamide structure. The pyrazolo[3,4- b]pyridine derivative (TAS-116) showed the inhibition of HSP90α as well as HSP90β proteins and confirmed the antitumor activity in an NCI-H1975 xenograft mouse during oral administration. The researchers also explored that upon the replacement of quinoline with substituted imidazole showed the best biological results [195].

8.5.9 Natural product based anticancer agents containing imidazole moiety

Natural products (obtained from plant, animals and microbes and many more) are continuously inspiring the researchers towards the novel drug discoveries for enhancing the human health [98]. They attain gigantic structural diversity which cannot correspond to any synthetic libraries and showed minimum toxicity and side effects [97]. Natural products containing imidazole moiety comprise novel chemical structures and exhibit unique mechanism of action compared to other anticancer agents. Multiple binding sites are available in imidazole ring coordinate via non-covalent as well as covalent interactions with various metal ions, enzymes and receptors of biological systems [162]. Natural imidazoles have been widely explored for their anticancer potential, though the non-fused imidazoles (except simple histamines) are used in limitations but luckily some are reported with imperative potential for drug discovery in the last decade [79, 164, 167]. A marine alkaloid naamidine A has been found to encourage the caspase-dependent apoptosis of tumor cells in tumor xenograft assay in rodents [100]. Witchard and co-workers synthesized a series of naamidine A and G analogues by using the 5-Amino-3-methylimidazolidine-2,4-dione and 1,3,5-triazine which exhibited the strong anticancer potential [208]. Bleomycin (peptide antibiotic with a non-fused imidazole moiety)

has been found to show the anticancer potential through the interaction with DNA [42]. Du and colleagues isolated four new alkaloids including two meleagrin analogs: meleagrin D and E along with the two diketopiperazines, roquefortine H and I. The D and E contain imidazole moieties and exhibited weak cytotoxicity against A-549 cancer cell line while the earlier isolated molecules (meleagrin and meleagrin B) from the same strain of Penicillium sp. Were found the arrest the cell in G2/M phase and apoptosis of HL-60 cell line, respectively [51]. Hu and co-researchers designed a series of novel molecules based on the artemisinin-indole/imidazole hybrids and screened them against A549, HepG2, MCF-7, and MDA-MB-231 cancer cell lines along with a normal LO2 cell line. Almost all the newly synthesized molecules showed good anticancer activity against A549 and MCF-7 cell line with 20 μM average IC_{50} value compared to artemisinin and dihydroartimisinin (IC_{50} = 34–51 μM) [75]. On the basis of potential anticancer activity of neoplanocin A and cyclopentenyl, Yoon and colleagues synthesized a novel series of fluorocyclopentenyl purines and pyrimidines and evaluated them against A549, HCT-116, MDA-MB-231, SK-Hep-1, SNU-638 and PC-3 cell lines. Pyrimidine derivatives showed the IC_{50} value between 0.18 and 0.76 μM. The purine derivatives (fluorocyclopentenyl analogues) exhibited a potent cytotoxicity against the six screened cancer cell lines [215]. Ceratamine A (an anti-mitotic heterocyclic alkaloid) isolated from *Pseudoceratina* sp. was found to display a strong anticancer activity [128, 139]. Pan and co-researchers synthesized a series of imidazoazepine derivatives based on the anticancer potential of ceratamine A. Construction of imidazoazepine core through the intramolecular Heck reaction was the crucial step in the synthesis of these derivatives. All the molecules were screened for the plausible anticancer activity against carious cancer cell lines including A549, A2780, BGC-823, HCT-116, and HepG2. A lead molecule containing methoxy group at C-14, 15 and 16 was exhibit the best cytotoxicity against all the cancer cell lines (IC_{50} = 8.56–12.9 μM) and superior to ceratamine A also. Removal of benzyloxymethyl group from imidazole ring leads to magnificent reduction in the anticancer potency of the molecules [145]. Figure 8.1 summarizes some potential natural anticancer molecules containing imidazole moiety in their structure.

8.5.10 Imidazole based supramolecules as potential anticancer agents

Supramolecules are the aggregates generally synthesized by one or more metal/non-metal compounds with one or more organic/inorganic molecules through the coordination bonds. Development of anticancer agents based on supramolecular complexes is a fascinating interdisciplinary area of research [234, 235]. Cisplatin ($Pt(NH_3)_2Cl_2$, a well known anticancer drug) is an inorganic supramolecular drug. The redox potentiality of this kind of metal compounds may cooperate well with the redox state of the cells and alter the cell viability in a significant manner. Imidazole moiety owned multiple binding sites including carbon or electron donating nitrogen thus able to

interact with various metal ions as well as organic molecules to produce the supramolecular drugs. At present, platinum complexes are among the extensively used metal supramolecular as anticancer drugs [30, 135]. However, anticancer therapy by platinum containing complexes has some problems including drug resistance. Nitrogen atoms of imidazole moiety in clotrimazole (an earliest imidazole antifungal agent) offer the promising interaction with metal ions. Two novel *trans*-platinum complexes of clotrimazole were synthesized and evaluated for their possible interaction with DNA and anticancer activity. These complexes were found to exhibit superior activity in contrast to transplatin but less compared to cisplatin against the six screened tumor cells lines including B16/BL6, HT-29, LoVo, MCF-7, PC-3, SKBR-3 [158]. Imidazole complex with organoplatinum(II) exhibit feeble anticancer activity against the Raji (IC_{50} = 14 μg/mL) and Jurkat leukemia (IC_{50} = 12 μg/mL) cancer cell lines [60, 73, 118, 123, 134, 137, 155, 207, 220, 226, 233]. Ruthenium complexes are also used as potential anticancer drugs as they provide superior water solubility and less toxicity compared to cisplatin as well as they can mimic the iron due to their chemical similarity and binding potential with transferrin [104]. Ruthenium complexes were prepared with imidazole and found to exhibit good *in vitro* anti-proliferative activity against MDA-MB-435S with approximately 5 and 15 μmol/L IC_{50} value compared to cisplatin (IC_{50} ~35 μmol/L) [90]. Imidazole based Ru(III) complex was synthesized and showed a high activity with precise selectivity against the metastasis of solid tumors and enters into the clinical trial phase [36]. Bis-imidazole-Ru(III) complex was synthesized in DMSO and screened for inhibition of cyclin dependent kinase. The complex showed the IC_{50} between 564.4 and 750.4 μM against HeLa, HepG2, MCF-7, and 95-D cancer cell lines and found that it inhibit the metastasis [191]. Furthermore, another complex of Ru(II) with three bis-imidazoles also showed the greater efficiency (IC_{50} = 18 mM) compared to cisplatin (IC_{50} = 35 mM) against the MDA-MB-45S cell lines [89]. Kunz and co-workers synthesized some novel derivatives of 2-isopropylimidazol-4(5)yl-diphenylphosphane (4-MIP(iPr)) and tris(2-isopropylimidazol-4(5)yl)phosphane (4-TIP(iPr)) with gold(I) and evaluated them against seven ovarian and two leukemia cancer cell lines. Result showed that all molecules showed the expected anticancer activity [99]. Two imidazole complex of Ag(I) were synthesized and displayed a great value of IC_{50} with 3.2 and 3.3 μM, respectively as compared to cisplatin (IC_{50} = 3.3 μM), while the substitution of imidazole carbine with 4,5-dichlorine imidazole carbene or benzimidazole carbene decreases the potency by 8 and 11-fold, respectively [148, 149]. Copper based supramolecules have been explored extensively as anticancer agents due to their fewer side effects [189, 194, 199, 210]. Nitroimidazoles (a radiosensitizers used in the treatment of cancer) and their Cu(II) complexes were found to exhibit an increase in the sensibility towards the cancer cells [116, 217, 223]. Cobalt (component of Vitamin B12) complexes are also found to exhibit the attractive environment against hypoxic cells and may provide the opportunities for novel drug discovery. Co(III) supramolecule containing two imidazolyl ligands was confirmed as a selective and efficient anticancer agent by reducing into Cu(II) under hypoxic conditions and exhibit potential cytotoxicity

(IC_{50} = 0.50 mM) compared to cisplatin (IC_{50} = 0.60 mM) under *Saccharomyces cerevisiae* cells [183]. A novel molecule (tridentate ^{99}mTc(I)-tricarbonyl nitroimidazole) has been synthesized and confirmed the favorable ratio of tumour/muscle and noticeable cytotoxicity in hypoxia imaging in tumors [56]. Moreover, organometallic complexes were found to generate a synergistic effect and also offering the rationality for coupling the various anticancer agents but further exploration is needed for general rule.

8.5.11 Imidazoles based CYP26A1 inhibitors

The physiological metabolites of vitamin A (all trans retinoic acids, ATRAs) have been used in the treatment of various diseases including skin, autoimmune, neurodegenerative disorders and cancer predominantly against acute promyelocytic leukemia (APL) etc. [68, 103, 180, 192]. These metabolites were found to amend the condition of APL from fetal to greatly curable [11, 129, 144]. During the clinical phase, CYP26A1 (a metabolic enzyme) converts the ASTRAs into 4-hydroxyl-RA and hinders their anticancer potential. Hence, inhibition of CYP26A1 opens a novel strategy towards the design and discovery of specific anticancer leads [39]. Various molecules, known as retinoic acid metabolism blocking agents (RAMBAs), targeting the CYP26A1 have been explored from generation to generation. First generation anticancer imidazoles acting as retinoic acid metabolism blocking agents includes ketoconazole, liarozole and miconazole [2, 46]. However, lack of specificity for CYP26 isoform and exploration of structure activity relationship of imidazoles encourage the researchers towards the second generation inhibitors of CYP26A1 [63, 72, 131, 185]. In the advance studies such as clinical trials, elevated potency and improved specificity against the CYP26A1 were recorded by these second generations RAMBAs [61, 197]. Sun and co-workers synthesized 15 imidazole based novel molecules as retinoic acid metabolism blocking agents. All the 15 molecules exhibited CYP26A1 inhibition activities with the half-maximal inhibitory concentration between 0.22 and 1.11 μM. Furthermore, two molecules (IC_{50} = 0.22 and 0.46 μM) were found to be selective against CYP26A1 in comparison to CYP2D6, and CYP3A4 [188]. Synthesis of novel 3-(1H-imidazol- and triazol-1-yl)-2,2-dimethyl-3-(4-(naphthalen-2-ylamino)phenyl)propyl derivatives as the potent CYP26A1 inhibitors were carried out by MCF-7 CYP26A1 microsomal assay. All the molecules displayed superior activity than R116010, as well as aligned induction correlation with the inhibition date for CYP26 inhibition. The results emphasize the promising CYP26 inhibition activity and recommend this novel imidazole based derivatives as an appropriate lead for future development [64]. Gomaa and colleagues also made efforts for the synthesis of imidazole derivatives as CYP26 inhibitors and evaluated them by using a cell-free microsomal assay and compared with the R116010 (IC_{50} = 10 nM) and liarozole (IC_{50} = 540 nM). Two imidazole derivatives including 3-[4-(6-bromopyridin-3-ylamino)-phenyl]-3-imidazol-1-yl-2,2-dimethylpropionic acid methyl ester and 3-[4-(benzoij1,3]dioxol-5-ylamino)-phenyl]-3-imidazol-1-yl-2,2-dimethyl-propionic acid methyl ester showed a strong CYP26 inhibition [65].

8.6 Future scope of imidazoles based anticancer leads

There are enormous advancement of science have been carried out in recent decades but the cancer remains a major challenge and one of the leading cause of worldwide deaths [17]. The uses of current available anticancer drugs are limited as their high cost and side effects which results in the necessity to develop an effective anticancer drug with quick action, high selectivity and bioavailability, minimum side effects and off course less cost. Imidazole, possessing various molecular properties as anticancer lead, provides a valuable core to design and develop an effective anticancer agent. In the modern science, various simulation programmes like molecular operating environment, density functional theory [212] and experimental techniques such as multinuclear polarization transfer NMR, high resolution electrospray mass spectrometry advances the understanding of chemical as well as biochemical reactivates and establishment of a great structure activity relationship for a lead molecule. Furthermore, the emergence of nanotechnology has changed the pharmaceuticals in a revolutionary way and resolves the numerous concerns of anticancer agents [31]. Nanocapsules containing imidazoles are through to be an effective way to replace their conventional ways [6, 150, 211]. In brief, imidazole based drugs with anticancer potential are having a bright future.

8.7 Conclusions

Imidazole is five-membered nitrogen containing heterocyclic compound, and has played a significant role in drug discovery and development. Moreover, imidazole has the structural similarity with histamine and histidine. Therefore, it can bind with the protein easily and display the remarkable pharmacological activities such as anticancer, antibacterial, antifungal, antineuropathic, antihypertensive, antihistaminic, antiparasitic, and so on. Thus, their derivatives have been extensively used in clinic to prevent the numerous diseases with low toxicity, high bioavailability, and good biocompatibility. This chapter highlights few synthesized imidazoles derivatives, which display the selectivity towards various targets and exhibit promising anticancer activities that emphasize the efficacy of imidazole moiety in present pharmaceutical field. Additionally, we hope that this chapter would provide an excellent insight into the synthesis and modification of imidazole ring, which would open rooms for this research field by highlighting new perspectives based on the new findings reported by the experienced researchers around the world.

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Sasadhar Majhi*

9 Recent developments in the synthesis and anti-cancer activity of acridine and xanthine-based molecules

Abstract: Cancer is the uncontrolled growth and development of abnormal cells which is a major cause of death in both advanced and emerging countries. Although currently chemotherapy is most broadly used among an extensive range of anti-cancer therapies, it includes many demerits, such as highly toxic, side-effects, expensive and partial lack of targeting specificity. So the design and synthesis of new molecules that perform specifically on target proteins in tumor cells is a focus of contemporary research. So many researchers aim for new drugs that will be more efficient, more selective, and less toxic. Because of the interesting structures and significant biological profile, naturally occurring acridines and xanthines as well as their analogues have attracted considerable interest in researchers and technologists. Natural and synthetic acridine derivatives form a significant category of heterocycles having nitrogen that is of considerable interest for organic chemists and biological communities due to their attractive anti-cancer activity. Another important class of therapeutic agents with diverse biological properties including cytotoxic effects is xanthine derivatives which are collectively called xanthines (a group of alkaloids). Among many significant molecules based on the structure of the purine, there is a group of natural xanthines, involving theobromine, caffeine, and theophylline and analogues of xanthine display anti-cancer activity. Hence the present chapter wishes to concentrate the attention on the synthesis and anti-cancer activity of acridine and xanthine-based compounds brilliantly.

Keywords: acridine and xanthine-based compounds; anti-cancer activity; drug discovery; synthetic analogues.

9.1 Introduction

Acridine (also known as 10-azaanthracene or dibenzopyridine or 2,3,5,6-dibenzopyridine) including π-conjugated planar structure and its analogues is the wealthiest pharmacophore in medicinal chemistry with divergent applications [1]; they have been widely explored as powerful therapeutic agents for the treatment of several diseases including cancer, bacterial and protozoan infections as well as Alzheimer's [2, 3]. A nitrogen

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heterocycle acridine bearing molecular formula $C_{13}H_9N$ is structurally linked to anthracene (tricyclic aromatic hydrocarbon comprising three fused benzene rings) with one of the central CH groups substituted by nitrogen. In 1870, Carl Gräbe and Heinrich Caro in Germany isolated the polycyclic alkaloid acridine for the first time from a high boiling fraction of coal tar [4]. Presently, various methods have been reported for the syntheses of acridines and their derivative; however, Bernthsen reported the primary synthesis of acridine by the condensation between diphenylamine and carboxylic acids in the presence of zinc chloride at high temperature [5]. In 1961, the validation of intercalation action of promising acridines was carried out for the first time [6]. In another study, the structure of the DNA-acridine complex was demonstrated in 1962 [7]. These studies disclosed that intercalation arises in a damage of the structure and the stability of deoxyribonucleic acid by enlarging its size [8] together with reducing the linear charge density [9], a broadly exploited quality to grow new anti-cancer treatments.

Xanthine (1*H*-purine-2,6(3*H*,7*H*)-dione or 3,7-dihydropurine-2,6-dione or 3,7-dihydro-1*H*-purine-2,6-dione) and its natural as well as synthetic derivatives represent a significant class of therapeutic agents with manifold biological properties including anti-cancer activity [10, 11]. Natural xanthine derivatives such as theophylline and caffeine were observed to induce apoptosis and raise cytotoxicity induced by doxorubicin [12]. In 1817, German chemist Emil Fisher first discovered xanthine, a natural heterocyclic alkaloid, and later in 1899 the name 'xanthine' was coined [13]. Xanthine includes a similar skeleton as that of purines which gives the building blocks of a unit of life such as deoxyribonucleotides (DNA) and ribonucleotides (RNA). The structural similarity with two of the valuable purine derivatives adenine and guanine; provides xanthine a good therapeutic compound in the current research [14].

Cancer is the second major reason for mortality worldwide; it is a serious problem influencing the health of all human societies and it represents more than 277 different classes of cancer disease on this planet [15]. Mother Nature is an indispensable source of bioactive secondary metabolites and historically, natural products and their derivatives have played a crucial role in drug discovery and drug development, especially for cancer and infectious diseases [16–22]. Although presently chemotherapy is most extensively used among an extensive range of anti-cancer therapies, it includes many disadvantages, such as highly toxic, side-effects, expensive and partial lack of targeting specificity. So the design and synthesis of new compounds that perform specifically on target proteins in tumor cells is a focus of recent research. So many researchers aim for new drugs that will be better selective, a little toxic as well as more potent. Synthetic together with the biological community was attracted in syntheses of promising molecules such as acridine and xanthine as well as their derivatives owing to their interesting architectures and anti-cancer activities. Hence, the primary goal of the present chapter is to cover modern progress in the synthesis and anti-cancer properties of acridine and xanthine-based molecules elegantly.

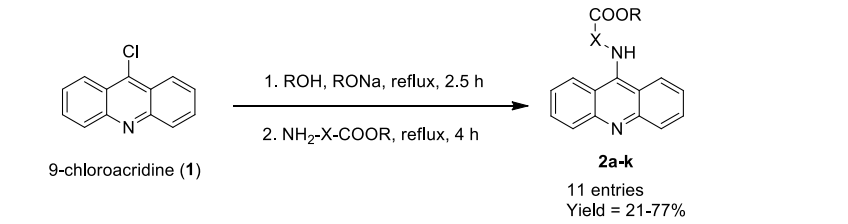
9.2 Synthetic analogues of acridine and xanthine

This section tries to deal with the syntheses of acridine and xanthine-based compounds handsomely.

9.2.1 Synthetic analogues of acridine

9.2.1.1 Synthesis of novel 9-acridinyl amino acid derivatives

It has been investigated that several acridinyl amino acid analogues exhibited better antitumor properties compared to readily available 9-aminoacridine along with *m*-amsacrine, probably because of the improvement of biologically significant chelating activities providing to the development of the superior DNA damaging reactive species [23]. Recently, Dobricic et al. [24] synthesized a new series of some derivatives of 9-acridinyl amino acid having improved anticancer property as well as lower toxicity than that of the amsacrine involving a two-step procedure in 2020 (Figure 9.1). In the first step, 9-chloroacridine (**1**) was treated with a solution of alkoxide in alcohol for 2.5 h under reflux conditions. Secondly, the targeted 9-acridinyl amino acid derivatives (**2a–k**) were prepared from the corresponding amino acid when amino acid was mixed into the reaction mixture under reflux conditions for 4 h. To avoid transesterification, a solution containing alkoxide in alcohol additionally (sodium alkoxides in corresponding alcohols such as methanol, ethanol, or propanol) was synthesized under the amino acid ester employed in the final



R = CH_3, C_2H_5, C_3H_7
X = Corresponding amino acids such as L-phenyalanine methyl ester hydrochloride, L-histidine methyl ester dihydrochloride, 1-methyl-L-tryptophan, 4-aminobutyric acid propyl ester hydrochloride etc.

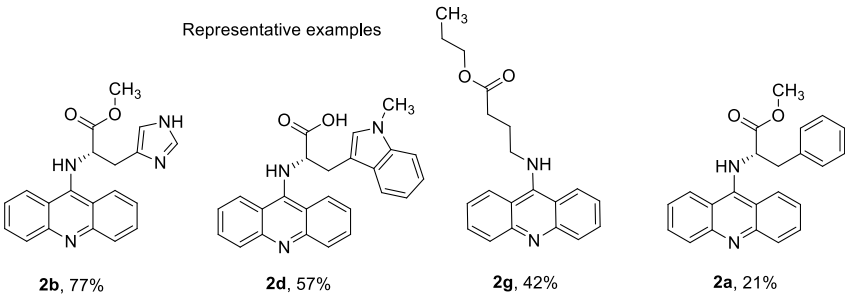


Figure 9.1: Synthesis of novel 9-acridinyl amino acid derivatives.

step. This methodology was used because desired yields were derived in better amounts than that of the direct transformation between 9-chloroacridine (**1**) and an amino acid in the basic condition.

9.2.1.2 Synthesis of new acridine derivatives of thiazolidine moieties

We need more efficient anticancer drugs as cancer has become a vital cause of mortality [25]. Lately, Prasad VVS et al. [26] accomplished synthesis together with anticancer activity of some new acridine analogues using a condensation reaction as a key step in 2020 (Figure 9.2). *o*-Chlorobenzoic acid (**3**) in amyl alcohol was reacted with aniline or substituted

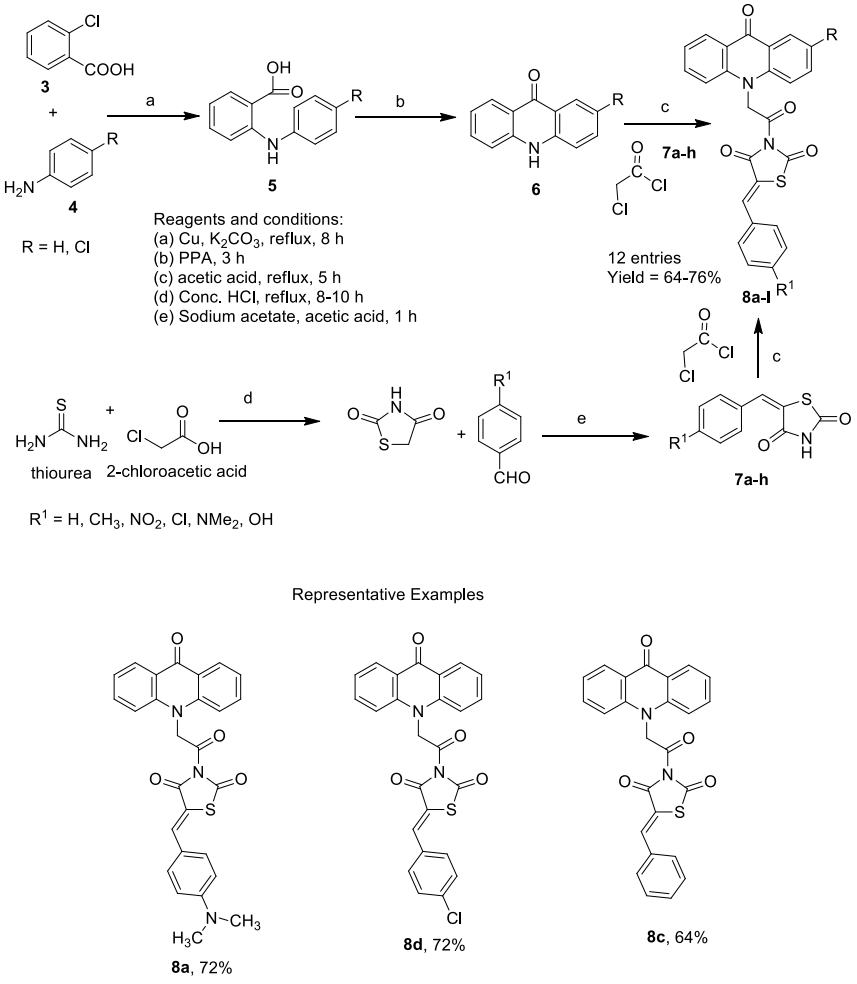


Figure 9.2: Synthesis of new acridine derivatives of thiazolidine moieties.

aniline (**4**) in the presence of the metallic copper and K_2CO_3 at 135–140 °C for 8 h to deliver *N*-phenyl anthracitic acid (**5**). Next, cyclization of *N*-phenyl anthracitic acid (**5**) took place at 100 °C for 3 h in the presence of the polyphosphoric acid (PPA) to provide acridone (**6**) as the desired product. Twelve novel acridine derivatives (**8a–l**) were synthesized by the action of acridone (**6**) with chloroacetyl chloride, and various 5-substituted-1,3-thiazolidine-2,4-dione (**7a–h**) in the presence of the glacial acetic acid in DMF. The same group was also able to prepare the desired derivatives (**8a–l**) from thiourea and chloroacetic acid using the same intermediate thiazolidine-2,4-dione (**7a–h**) in another way [26].

9.2.1.3 Synthesis of acridine thiosemicarbazides derivatives

Since the planar structure of acridine strongly binds to DNA so it acts as a potential molecule for developing novel anticancer drugs [27]. However, some adverse effects related to the application of acridines have been observed. In this context, hybrid compounds may decrease the side effects as well as increase the beneficial properties because of synergistic activity. Acridine and thiourea analogues have received attention in medicinal chemistry because of their various biological activities including anticancer activity [28]. In 2019, Huo and co-workers [29] synthesized novel hybrids of acridine thiosemicarbazides derivatives and the same group also evaluated their anticancer activity successfully (Figure 9.3). At first, 2-bromo benzoic acid (**9**) was treated with naphthalen-1-amine (**10**) in the presence of the copper and potassium carbonate to yield 2-(naphthalen-1-ylamino)benzoic acid (**11**). Next, aminobenzoic acid (**11**) was reacted with phosphorus oxychloride ($POCl_3$) to afford naphtho-fused acridinyl derivative (**12**) elegantly through the classic synthetic methods of acridine. Isothiocyanate (**13**) was prepared from naphtho-fused acridinyl derivative (**12**) using NaNCS via nucleophilic substitution. Finally, treatment of isothiocyanate (**13**) in ethanol with aryl hydrazides to furnish the desired compounds (**14a–e**) involving condensation as a key step.

9.2.1.4 Synthesis of acridine-based imidazolium salts

The development of novel anticancer drugs as well as effective treatment strategies for cancer is of very important because it is a major cause of death worldwide, accounting for about 10 million deaths in 2020 [30, 31]. Acridine-based natural molecules and synthetic analogues of 9-chloroacridine along with *N*-substituted imidazole derivatives have attracted major attention as antitumor agents recently [32]. In 2018, Sharhan and co-workers [33] completed the synthesis of a novel series of acridine-based imidazolium salts with better yields (74–95%) as well as easy purification by crystallization (Figure 9.4). Aryl aminobenzoic acids (**5**, **17**) were obtained by the reaction of 2-chlorobenzoic acids (**3**, **15**) and anilines (**4**, **16**) in the presence of the K_2CO_3, KI, and Cu through Ullmann condensation under reflux conditions. Subsequent cyclization of

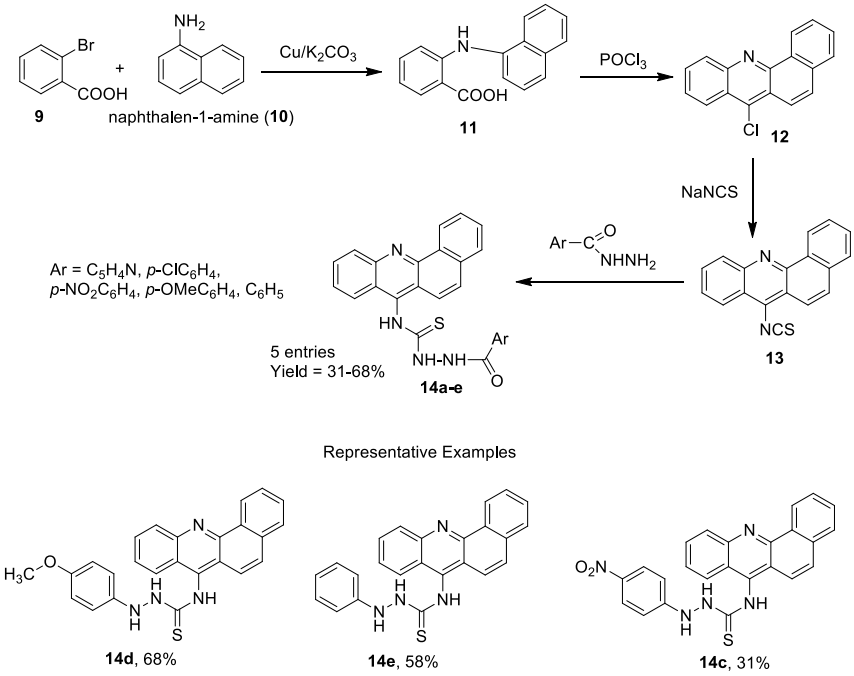


Figure 9.3: Synthesis of acridine thiosemicarbazides derivatives.

the aminobenzoic acids (**5**, **17a,b**) occurred to deliver 9-chloroacridines (**1**, and **18a,b**) in the presence of the $POCl_3$ at 135 °C in 70–85% yields. Next, 9-chloroacridines (**1**, and **18a,b**) were treated with 1*H*-imidazole (**19**) to produce derivatives of imidazolylacridine (**20a,b**). Finally, derivatives (**20a,b**) were transformed into the targeted acridine-based imidazolium salts (**21a–p**) using alkyl halide in CH_3CN. The investigators used two alternative approaches to synthesize acridine-based imidazolium salts (**21a–p**), varying in a pattern of alkylation of an important imidazole building block [33].

9.2.1.5 Synthesis of new oxazine substituted 9-anilinoacridines

Since oxazine derivatives exhibit several biological activities including anticancer property [34] so a series of oxazine substituted 9-anilinoacridines were prepared by Kalirajan et al. [35] from 9-chloroacridine (**1**) in 2018. 1-(4-(acridine-9-ylamino)phenylethanone (**24**) was synthesized by the reaction of 9-chloroacridine (**1**) with *p*-amino acetophenone (**23**) in the presence of the 2-butanol at 130–140 °C for 3 h (Figure 9.5). Then, phenylethanone derivatives (**24**) in ethanol was treated with various aldehydes in the presence of the sodium hydroxide to provide various chalcone substituted 9-anilinoacridines (**25a–k**) at room temperature as a green protocol for 8 h. Chalcone

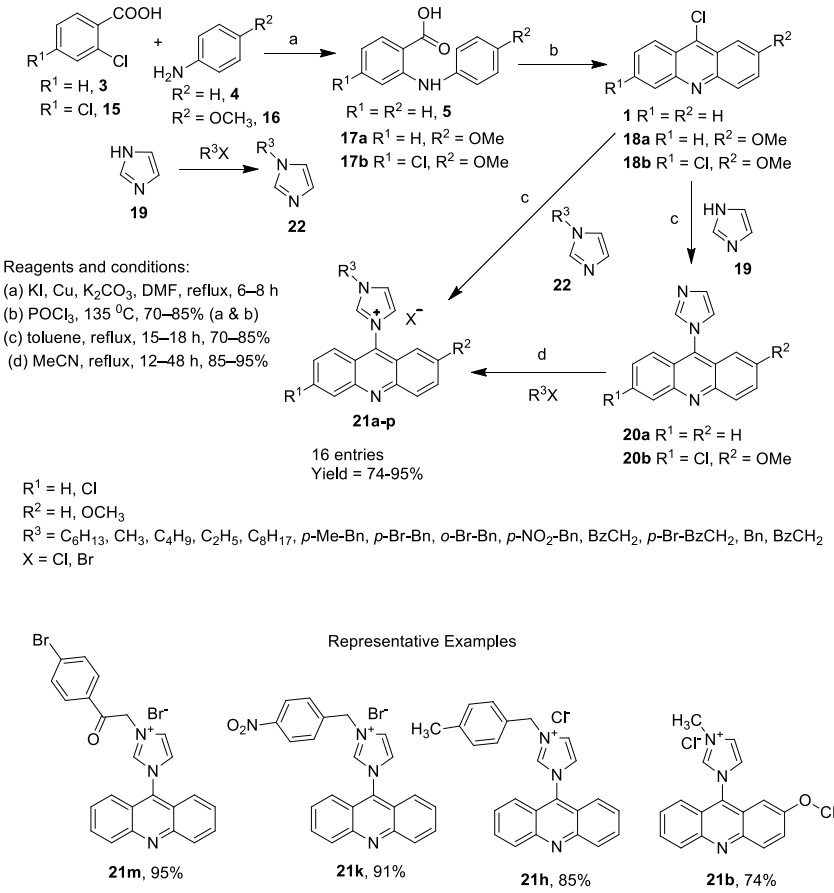
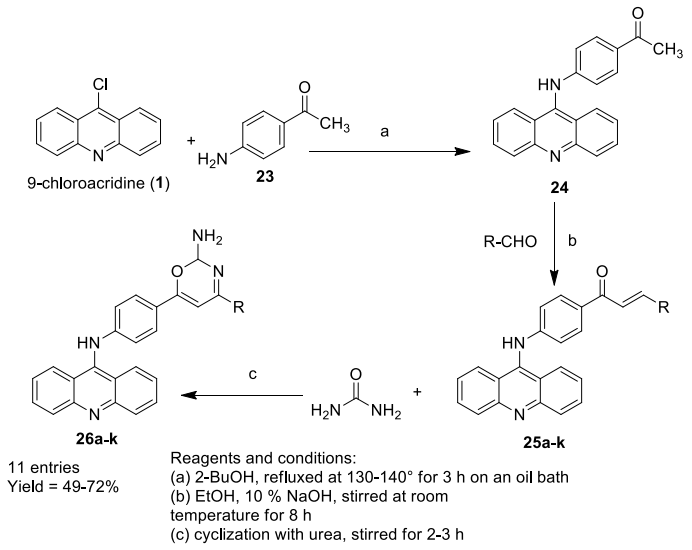


Figure 9.4: Synthesis of acridine-based imidazolium salts.

derivatives (**25a–k**) underwent cyclization reaction with urea to generate the corresponding oxazine substituted 9-anilinoacridines (**26a–k**) finally.

9.2.1.6 Synthesis of 2-methyl-9-substituted acridines

The most serious threat to human health is the malignant tumor on the planet. 9-anilinoacridines play a key role in the area of antitumor DNA-intercalating agents because of their antiproliferative activities [36]. Kumar et al. [37] reported syntheses of various 2-methyl-9 substituted acridines (**30a–h**) involving the nucleophilic substitution of 2-methyl-9 chloroacridine (**29**) as a key step (Figure 9.6). At first, *o*-chlorobenzoic acid (**3**) was treated with *p*-toluidine (**27**) in DMF in the presence of sodium acetate, copper powder, copper oxide at 160–170 °C for 2 h to afford 2-(*p*-tolylamino) benzoic acid (**28**). Next, cyclization of 2-(*p*-tolylamino) benzoic acid (**28**) took place to



R = 4-OHC_6H_4, 4-$OMeC_6H_4$, 3,4-$(OCH_3)_2C_6H_3$, 3-OMe,4-OHC_6H_3, 2-furyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, CH_3, C_2H_5, CH_3-CH=CH-

Representative Examples

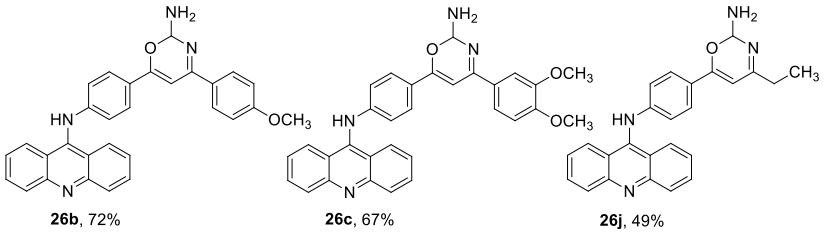


Figure 9.5: Synthesis of new oxazine substituted 9-anilinoacridines.

generate 2-methyl-9-chloroacridine with the freshly distilled phosphorous oxychloride. Finally, 2-methyl-9-chloroacridine (**29**) in methanol was heated with different substituted aromatic amines to provide desired 2-methyl-9 substituted acridines (**30a–h**).

9.2.1.7 Synthesis of *para*-phenylene diamine linked acridine derivative

Tuberculosis (TB) is normally caused by pathogenic bacteria *Mycobacterium tuberculosis* (MTB) bacillus and it is an air-borne contagious disease that usually attacks our lungs. The World Health Organization (WHO) global TB reports (2015) disclosed that about nine million people get infected with this bacterium, nearly1.5 million died from tuberculosis. Bedaquiline (SIRTURO/TMC-207) was the only drug from the past 40 years which has been approved by US-FDA; this drug exhibited adverse effects such as

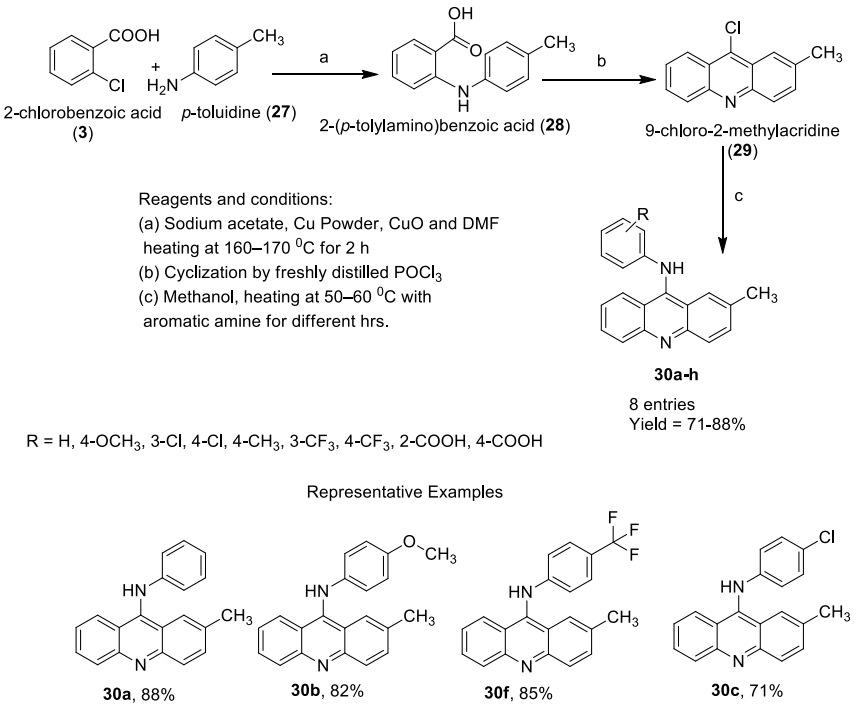


Figure 9.6: Synthesis of 2-methyl-9-substituted acridines.

QT prolongation and enhanced mortality [38]. So we need new antimycobacterial agents with lower side effects. Hence, Sriram and co-workers [39] designed *p*-phenylene diamine related acridine analogue that selectively inhibits quinolone-aminopiperidine hybrid MTB DNA gyrase enzyme with aiming lower side effects namely less cardiotoxicity. The investigators started four-step syntheses of acridine-*p*-phenylene diamine based DNA gyrase inhibitors with simple 2-chlorobenzoic acid (**3**) (Figure 9.7). At first, the starting material (**3**) was treated with various *para*-substituted anilines to furnish intermediates amino aromatic acid analogues (**31a–c**) in the presence of the copper powder and *N*,*N*-dimethylformamide at 140 °C for 6 h. Further these key intermediates (**31a–c**) undergo cyclization reaction with phosphorousoxy chloride ($POCl_3$) to deliver 9-chloro-2-substituted acridine derivatives (**32a–c**) for 6 h under reflux conditions. Next, acridine derivatives (**32a–c**) were treated with *para*-phenylenediamine in methanol using a catalyst PTSA (*para*-toluenesulphonic acid) under microwave irradiation as a green tool for 1 h to afford N^1-(2-methyl/2-chloro/simple acridin-9-yl)benzene-1,4-diamine analogues (**33a–c**) successfully. Finally, these important amine intermediates were treated with several substituted aryl sulfonylchorides and aryl isothio/iso cyanates to deliver targeted sulphonamide (**34a–l**) and thio/urea (**35a–l** and **36a–l**) analogues.

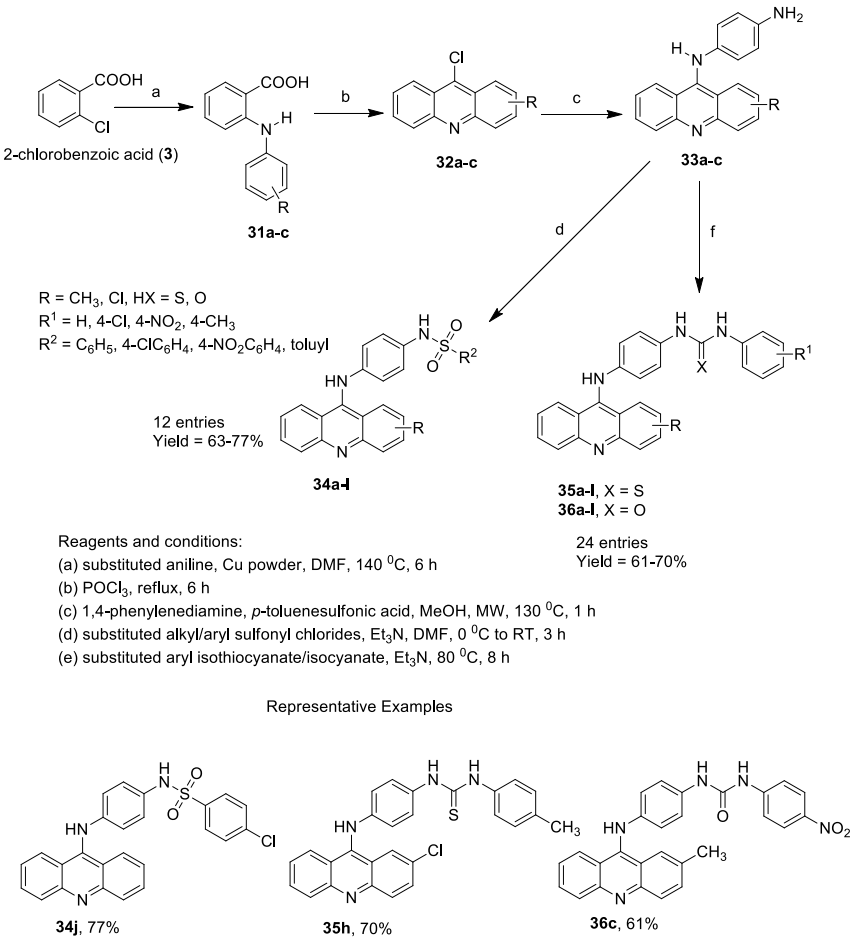


Figure 9.7: Synthesis of *para*-phenylene diamine linked acridine derivative.

9.2.1.8 Synthesis of new acridine bis-sulfonamides derivatives

The sulfonamides and their isosteres namely the sulfamides and sulfamates are in clinical application for the treatment of various diseases such as glaucoma, epilepsy, obesity, and more for nearly 70 years as carbonic anhydrase inhibitors [40]. However, the main demerit linked to the use of carbonic anhydrase inhibitors is described by the lack of selectivity in inhibiting several isoforms, thus resulting in an excess of adverse effects [41]. Thus, various efforts have been made for the development of efficient specific carbonic anhydrase inhibitors with lower side effects. Hence, Kaya et al. [42] disclosed syntheses of a novel class of acridine bis-sulfonamides using multicomponent reactions as a central step with efficient inhibitory activity (Figure 9.8). In the first

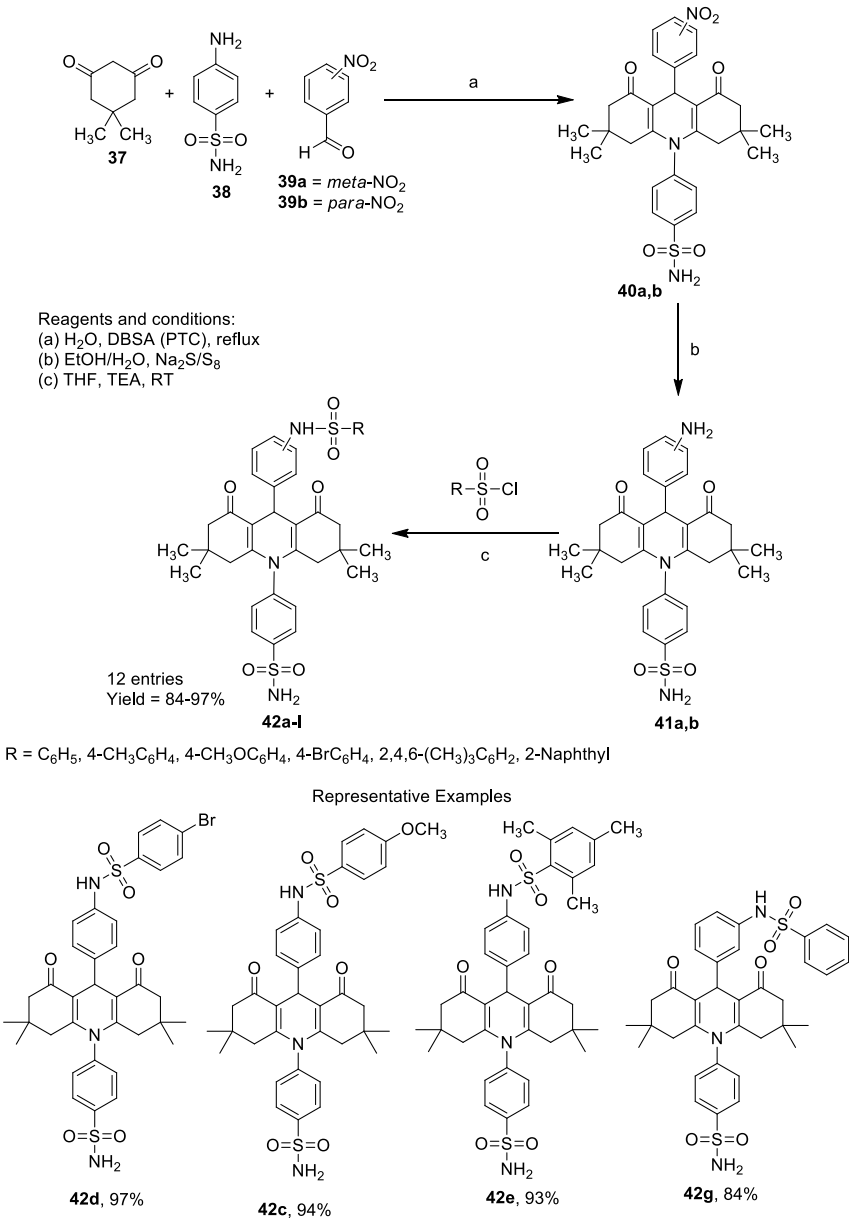


Figure 9.8: Synthesis of new acridine bis-sulfonamides derivatives.

step, on the treatment of dimedone (**37**) in water with 4-aminobenzenesulfonamide (**38**) and nitroaromatic aldehydes (**39a** and **39b**) employing the phase transfer catalyst DBSA under reflux conditions yielded nitro acridine derivatives (**40a** and **40b**) in a

one-pot reaction. For the second step, nitro acridine compounds (**40a** and **40b**) were able to provide novel amino acridine analogues (**41a** and **41b**) involving excessive reduction reactive-aqueous sodium polysulfide under reflux conditions with high yields (respectively, 87 and 82%). For the final step, new amino acridine derivatives (**41a** and **41b**) in THF were treated with several sulfonyl chlorides in the presence of triethylamine (TEA) to give new acridine bis-sulfonamide derivatives (**42a–l**) in good to excellent yields (84–97%).

9.2.1.9 Synthesis of 1,8-dioxoacridine derivatives

Acridine and its several derivatives such as dioxoacridine analogues are among the most extensively studied heterocyclic molecules because of their various applications in medicine [43]. Kaya et al. [44] disclosed syntheses of novel 1,8-dioxoacridine derivatives from cyclic-1,3-diketones as starting materials (Figure 9.9). Tetraketone intermediates (**45a** and **45b**) were prepared through condensation of cyclic-1,3-diketones (**37** and **43**) namely dimedone (**37**) with *p*-methoxy benzaldehyde (**44**). The targeted new dioxoacridine analogues (**46a–l**) were obtained by reactions of key tetraketone intermediates (**45a** and **45b**) and primary aromatic amines employing the Dean–Stark

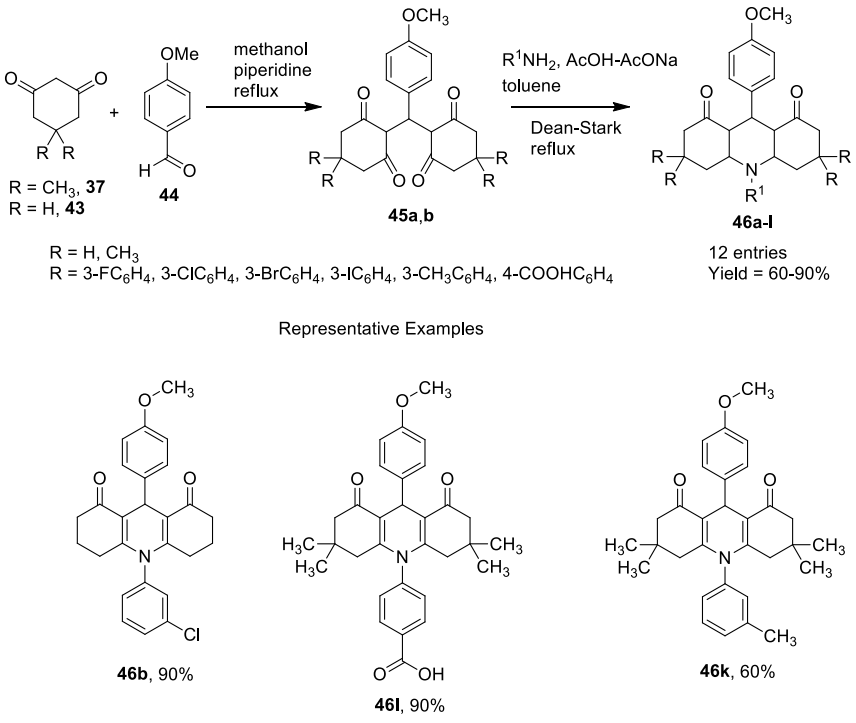


Figure 9.9: Synthesis of 1,8-dioxoacridine derivatives.

apparatus with a toluene–acetic acid mixture (1:1) in the presence of the sodium acetate under reflux conditions.

9.2.1.10 Synthesis of substituted acridine derivatives

Acridines as an important type of heteroaromatic molecules have attracted considerable interest due to their broad range of biological properties including anticancer activity, and industrial applications [45]. In 2014, Mu and co-workers [46] reported an efficient method for the synthesis of a wide range of acridine analogues by $ZnCl_2$-promoted intramolecular cyclization reaction of *o*-arylaminophenyl Schiff bases as a central step (Figure 9.10). *Ortho*-fluorobenzaldehyde (**47**) reacted with aniline (**4**) in the presence of the anhydrous $MgSO_4$ in *n*-hexane to produce *ortho*-fluoroimine compound (**48**) at room temperature as a green methodology for 2 h. A solution of *n*-BuLi in *n*-hexane was mixed to aniline (**4**) in THF at −78 °C; the resulting solution was added into a solution of the obtained *ortho*-fluoroimine compound (**48**) at 25 °C for 24 h to

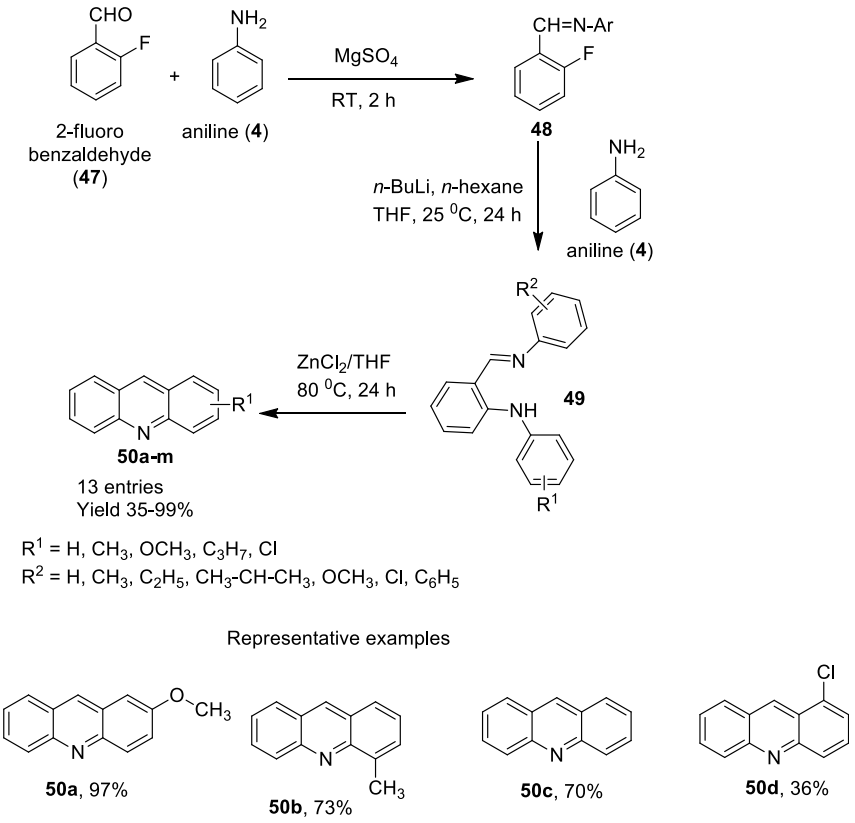


Figure 9.10: Synthesis of substituted acridine derivatives.

afford targeted *ortho*-arylamino-phenyl Schiff base compounds (**49**). A wide range of acridine derivatives (**50a–m**) was obtained from arylamino-phenyl Schiff base compounds (**49**) by the $ZnCl_2$-promoted cyclization reaction at 80 °C for 24 h.

9.2.1.11 Synthesis of novel acridine-based derivatives

For efficient treatment of cancers, we need new DNA binding agents against topoisomerases. Although a variety of bis-acridine analogues have been reported to enhance the DNA binding affinity, the substitution of benzene ring by naphthalene or heterocycles namely pyridine has gained little attention [47]. Hence, Jiang and coworkers [48] achieved a series of novel acridine-based derivatives with $-NHCH_2-$ or $-NHCH_2CH_2-$ linker as topoisomerase I inhibitors (Figure 9.11). Initially, 2-chloro-benzoic acid (**2**) undergoes a Ullmann coupling reaction with aniline (**4**) in DMF in the presence of the K_2CO_3 and copper as a catalyst at 130 °C to yield key acid (**5**). Then, acid (**5**) was treated with $POCl_3$ at 105 °C to furnish the 9-chloroacridine (**1**). The important

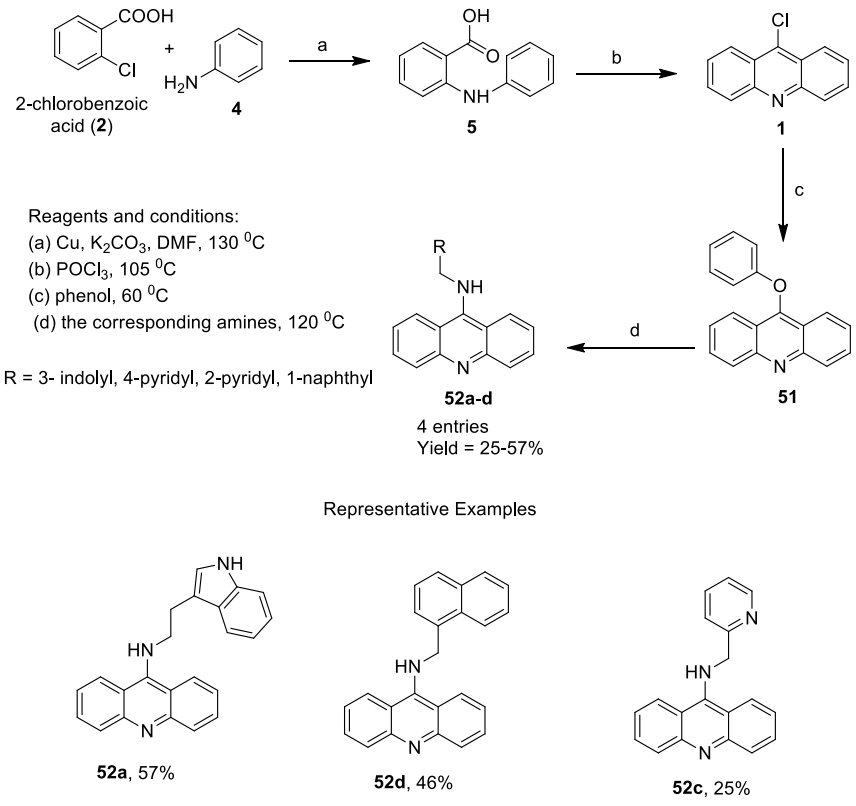


Figure 9.11: Synthesis of novel acridine-based derivatives.

intermediate (**51**) was obtained from 9-chloroacridine (**1**) in the presence of the phenol at 60 °C. Finally, the key intermediate (**51**) was treated with the corresponding amines to deliver the targeted acridines (**52a–d**) under reflux conditions.

9.2.1.12 Synthesis of acridine-3-carboxylates

The synthesis of cyclohexane derivatives makes it an attractive synthetic target because of a variety of medical effects such as a number of their analogues possess antitumor and fungicidal activities [49]. Nearly 2.5 billion people have been infected with dengue vector which has been estimated by the WHO; no effective drug or vaccine for the dengue viral diseases is accessible as yet. However, acridin-1(2*H*)-one analogues and acridine-3-carboxylates comprise good larvicidal activity [50]. Hence, Roopan and co-workers [51] synthesized a new series of acridine-3-carboxylates (**55a–e**) via sodium hydroxide (NaOH) base mediated cyclocondensation reaction under reflux conditions (Figure 9.12). The cyclocondensations took place between (*E*)-7-chloro-dihydroacridin-1(2*H*)-ones derivatives (**53a–e**) and EAA (ethyl acetoacetate) (**54**) using ethanolic NaOH (10%) for 5–6 h to afford acridine-3-carboxylate derivatives (**55a–e**) in good yields.

9.2.2 Synthetic analogues of xanthine

9.2.2.1 Synthesis of 8-substituted xanthine derivatives

Uracil derivatives are attracting heterocycles comprising a broad spectrum of pharmaceutical as well as biological importance [52]. Among uracil derivatives, xanthines

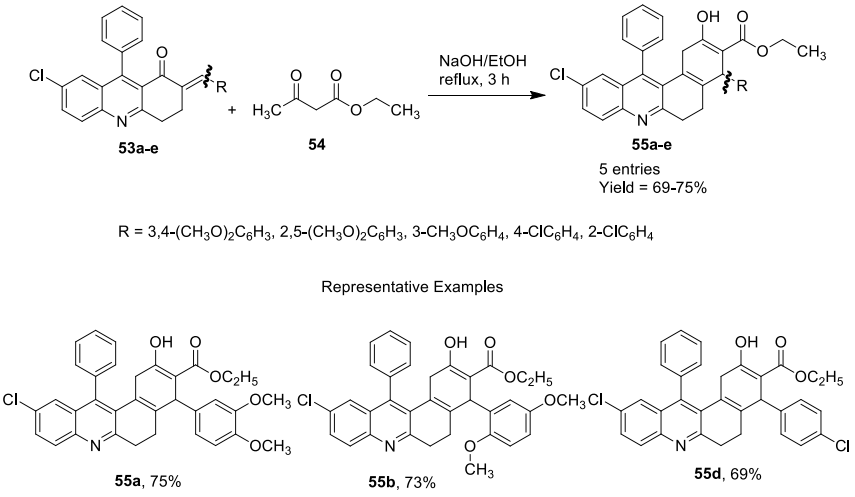


Figure 9.12: Synthesis of acridine-3-carboxylates.

have attracted great attention because of a wide range of bioactivities including anticancer property [53] and various biological activities [14]. It has been investigated that eliminating the hydrogen atom at C-8 in xanthine with a suitable *N*-substitution resulted in enhancing both affinities along with selectivity toward ARs as antagonists. Hence, very recently, Sadek and co-workers [54] disclosed a green and efficient one-pot synthesis of 8-substituted xanthines using microwave irradiation as a green tool in 2021 (Figure 9.13). 1,3-dimethyl-5,6-diaminouracil (**56**) was heated with 2-(4 methoxybenzylidene)malononitrile (**57a**) in the presence of the pyridine under conventional heating earlier for 3 h led to deliver the targeted product (**60a**) in 60% yield only through the formations of key (**58** and **59**). However, when 1,3-dimethyl-5,6-diaminouracil (**56**) was treated with malononitrile (**57a**) in pyridine using microwave irradiation as a green technology at 120 °C the yield of the desired product (**60a**) was increased up to 92% for short time (20 min only). The role of the pyridine was significant to afford desired 8-substituted xanthines products (**60a–e**) because it acts as a solvent as well as a basic catalyst to decrease the number of synthetic steps [54]. Moreover, the investigators extended the scope of their work to the reaction of 1,3-dimethyl-5,6-diaminouracil (**56**) with phenyl isothiocyanate in acetone, and the transformation was promoted by microwave heating as a non-polluting source of energy at 70 °C for 5 min to produce the xanthine derivative (**62**) as colorless needles in 88% yield through the formation of the intermediate (**61**).

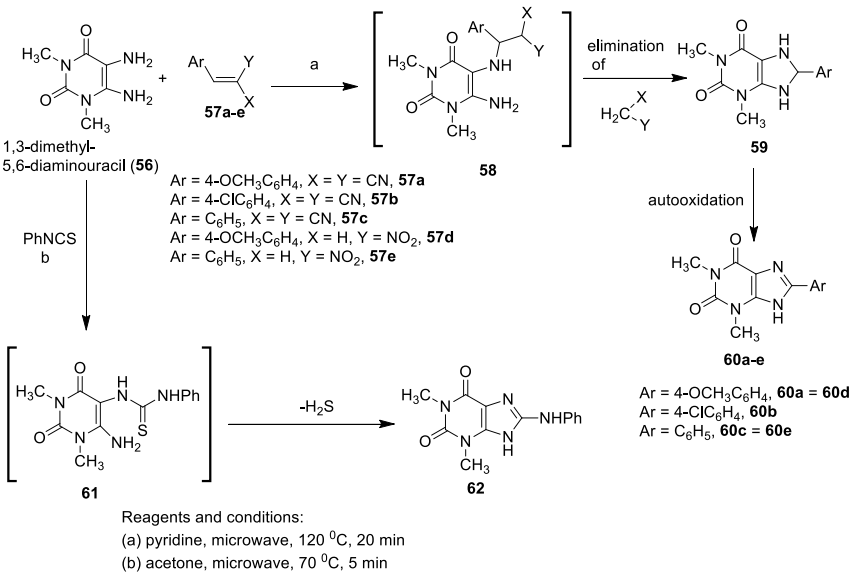


Figure 9.13: Synthesis of 8-substituted xanthine derivatives.

9.2.2.2 Synthesis of 1,3,8-trisubstituted or 1,8-disubstituted xanthine derivatives

Xanthine derivatives represent a potential type of therapeutic agent having diverse biological activities comprising antitumor activity [55]. A new series of 1-methyl or 1,3-dimethyl-8-alkylthioxanthine derivatives, as well as 1,3,8-trisubstituted or 1,8-disubstituted xanthine analogues, were designed and synthesized by Youssif and co-workers [56] in 2019 (Figure 9.14). Firstly, compounds (**70a,b**) were prepared using condensation, nitrosation, and cyclization as central steps. *N*,*N*′-dimethylurea (**63**) condensed with cyanoacetic acid (**64**), followed by stirring in the presence of the sodium hydroxide solution provided the targeted compound (**66a**) through the formation of 1-(2-cyanoacetyl)-1,3-dimethylurea (**65**). 6-Amino-3-methyluracil (**66b**) was synthesized from 6-aminouracil (**67**) by refluxing it in hexamethyldisilazane (HMDS), followed by the addition of methyl iodide. Nitrosation of synthesized compounds (**66a,b**) was performed in the presence of the sodium nitrite in 50% aqueous acetic acid to provide compounds (**68a,b**). Compounds (**69a,b**) were obtained by the reduction of the compounds (**68a,b**) with sodium dithionite in an aqueous ammonia solution. Direct cyclization of 5,6-diamino-1,3-dimethyluracil (**69a**) occurred to generate compound (**70a**) in the presence of the carbon disulfide in DMF under reflux for 5 h; on the other hand, compound (**70b**) was synthesized through cyclization of 5,6-diamino-1,3-dimethyluracil (**69b**) using carbon disulfide in an ethanolic solution of KOH under reflux for 4 h [56].

The authors extended their study to prepare the desired hybrid ketone intermediates (**72a,b**) and (**73a–h**) as well as their corresponding final oximes (**74a,b**) and (**75a–h**) successfully. Hence, *p*-amino acetophenone (**23**) was treated with bromoacetyl bromide to deliver *N*-(4-acetylphenyl)-2-bromoacetamide (**71**). Next, compounds (**70a,b**) were reacted with 2-bromoacetamide (**71**) to generate compounds (**72a,b**) in good yields through an alkylation reaction. Besides, compounds (**70a,b**) underwent a Schotten-Baumann reaction with *p*-(un)substituted phenacyl bromides to produce compounds (**73a–h**). Finally, ketones derivatives (**72a,b**) and (**73a–h**) were transformed into their corresponding oximes (**74a,b**) and (**75a–h**) in the presence of the hydroxylamine HCl in absolute ethanol under reflux conditions [56].

9.2.2.3 Synthesis of xanthine derivatives employing the FAD-based drug design strategy

A simple purine base is the xanthine molecule it is widely distributed in human body tissues together with in other organisms [57]. Several biologically important xanthine derivatives were employed as a drug such as xanthinol, GS-6201, cipamfylline, etc. [58]. Yu and co-workers reported [59] the synthesis of xanthine derivatives and evaluated these derivatives as LSD1/KDM1A inhibitors in 2018 (Figure 9.15). 6-Amino-1-methylpyrimidine-2,4(1*H*, 3*H*)-dione (**76**) was treated with bromine and sodium hydrogen carbonate in methanol to provide bromo compound (**77**). Next, bromo

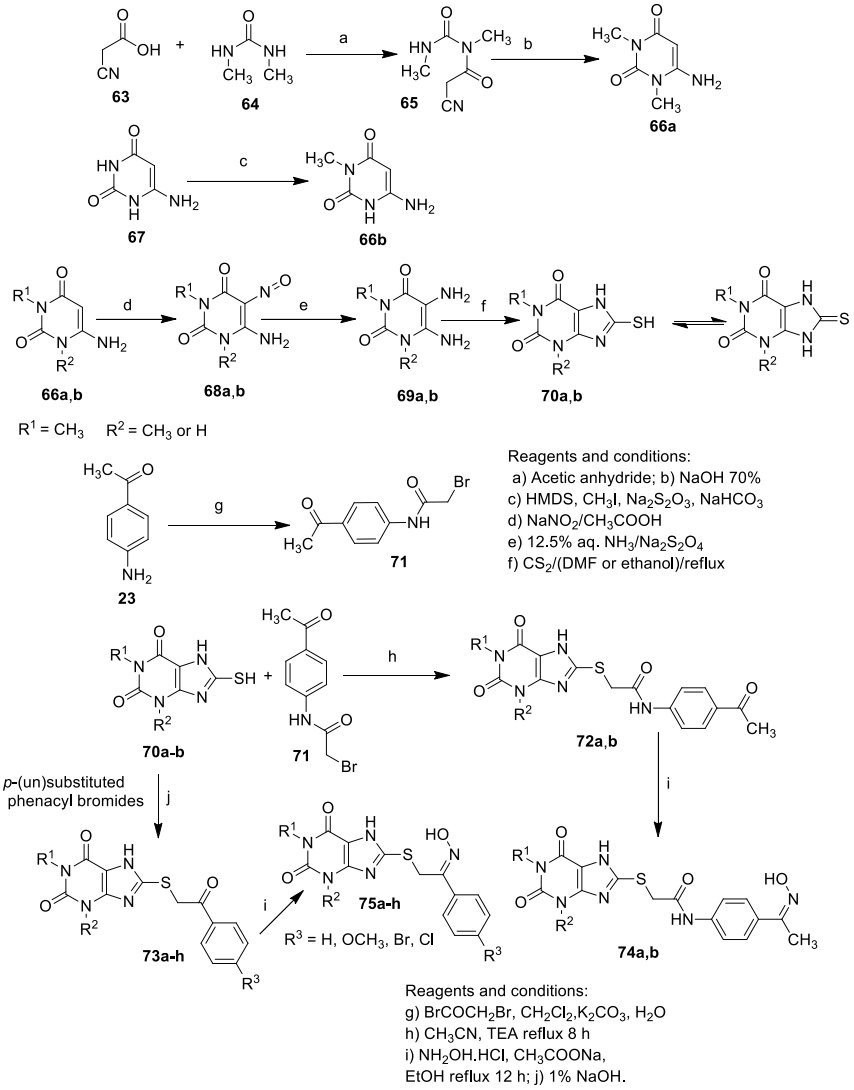


Figure 9.14: Synthesis of 1,3,8-trisubstituted or 1,8-disubstituted xanthine derivatives.

compound (**77**) was reacted with *n*-propylamine (also act as the solvent) to produce the corresponding product (**78**) under reflux overnight through amination reaction. The synthesized compound (**78**) was converted to 8-mercapto compound (**79**) with potassium ethylxanthate in DMF at 100 °C for 8 h. To compound (**80**), *tert*-butyl bromoacetate and NaH in DMF were added to generate *tert*-butyl purin-1-yl acetate ester (**81**) and hydrolysis of compound (**81**) occurred in the presence of the trifluoroacetic acid to yield acid (**82**).

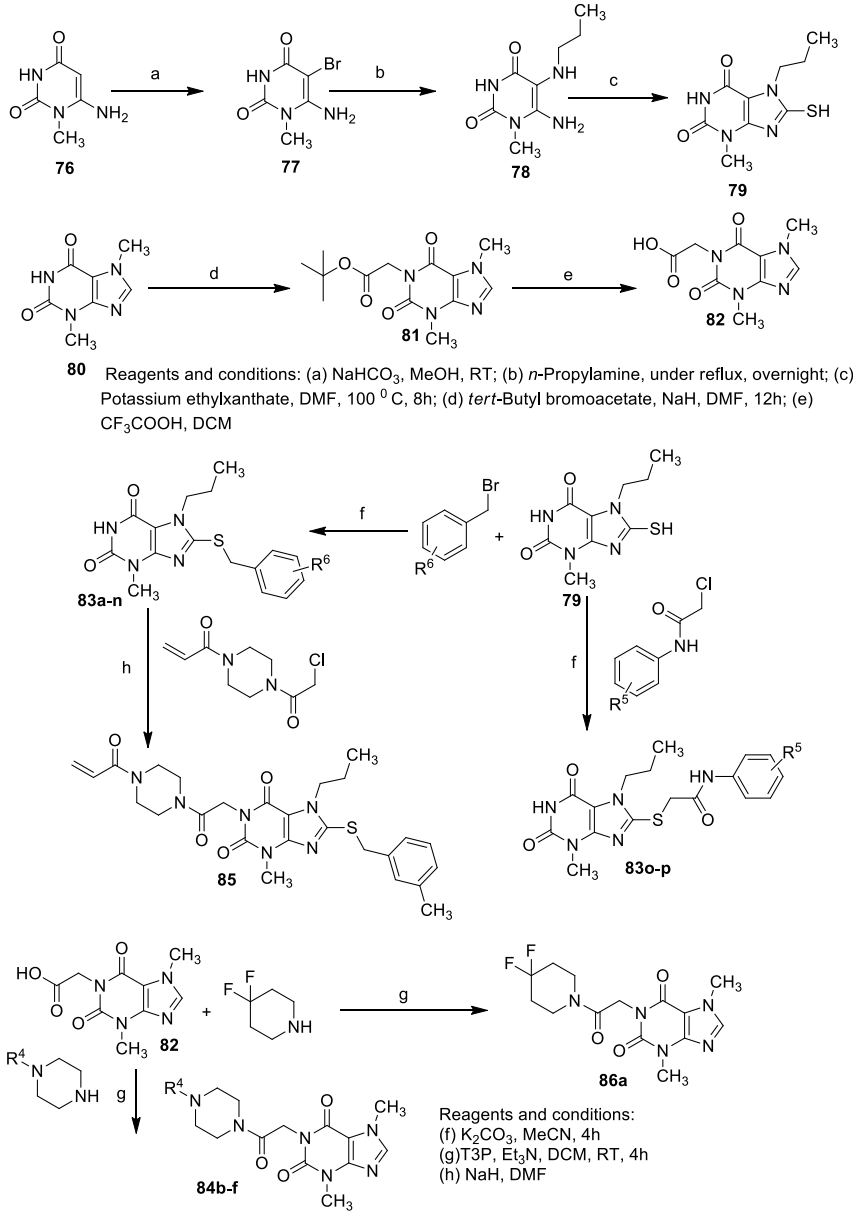


Figure 9.15: Synthesis of xanthine derivatives employing the FAD-based drug design strategy.

With the crucial intermediates mercapto compound (**79**) and acid (**82**) in hand, the investigators were able to prepare a focused library of xanthine derivatives via different substituents linked to the position 1 or 8 of the core scaffold. Two types of common

substituents (benzyl and acetamide) were inserted to obtain targeted compounds (**83a–p**) in 45–83% yields to position 8 through the base-promoted nucleophilic substitution transformations for the purpose of the primary structure-activity relationships (SARs) studies. Besides, based on the central intermediate (**82**), the investigators also prepared compounds (**84a–f**) by inserting different cyclic amines with propylphoshonic anhydride (T3P) in triethylamine at room temperature, aiming to explore the effects of substituents associated with position 1 on the activity. Moreover, the active compound (**83c**) (LSD1 IC_{50} = 8.89 μM) was converted to compound (**85**) in the presence of the NaH in DMF to further study the significant effect of such substituent on the activity [59].

9.2.2.4 Synthesis of 8-substituted 3,7-dimethylxanthines

The pharmacologically potential xanthines are usually employed for their activities as adenosine receptor antagonists, bronchodilators, stimulants, and phosphodiesterase inhibitors [60]. Kowalska and co-workers [61] disclosed a synthesis of 8-aminobutynylthioxanthines and evaluated anticancer activity of 8-monoaminobutynylthio derivatives in 2018 (Figure 9.16). At first, compound (**87**) was brominated with bromine in nitrobenzene to furnish 8-bromo compound (**88**) under reflux conditions for 5 h. Next, 8-bromo compound (**88**) was converted into 8-xanthinethione (**89**) in the presence of the sodium hydrosulfide; then 8-xanthinethione (**89**) was treated with propargyl bromide in DMF at room temperature using potassium *tert*-butoxide to provide compound (**90**) containing

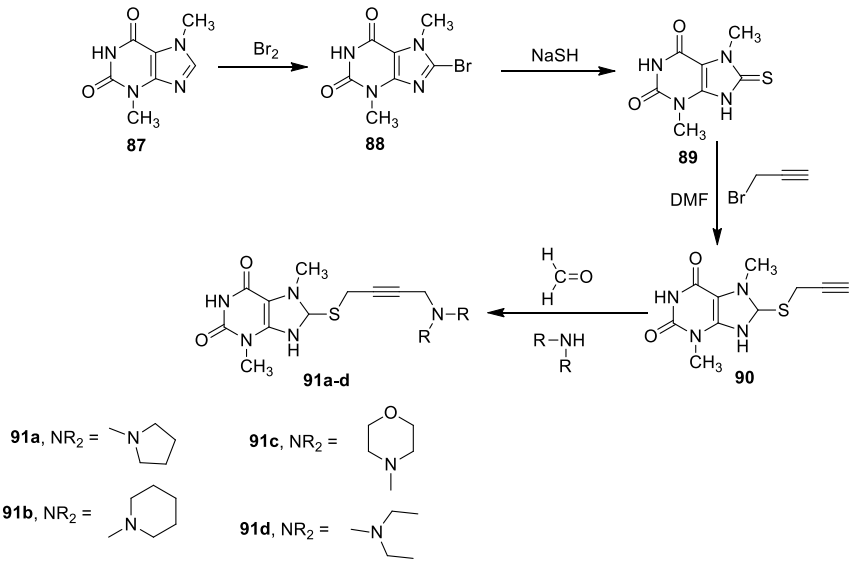


Figure 9.16: Synthesis of 8-substituted 3,7-dimethylxanthines.

terminal triple bond through S-alkylation reaction. Compound (**90**) underwent a Mannich reaction with paraformaldehyde in the presence of secondary amine such as pyrrolidine, piperidine, morpholine, and diethylamine in dry dioxane using CuCl catalyst to afford targeted 8-aminobutynylthioxanthines (**91a–d**) finally.

9.2.2.5 Synthesis of new xanthine derivatives as powerful and selective A_{2B} adenosine receptor antagonists

The weak together with non-selective AdoR antagonists are alkylxanthines such as theophylline (1,3-dimethylxanthine), enprofylline which are employed for the therapy of asthma for several decades [62]. A novel series of xanthine analogues was developed by Basu and co-workers [63]; their work aimed to recognize and expand new, active together with selective A2BAdoR antagonists incorporated with better aqueous solubility as well as enhancing *in vivo* half-life (Figures 9.17–9.20). The diaminouracil (**92**) was able to couple with several carboxylic acids (**93a–d**) using EDCI in methanol at room temperature as a green methodology to afford the corresponding derivatives of carboxamide (**94a–d**) firstly. 8-Substituted xanthine derivatives (**95a–d**) were obtained from the resulting derivatives (**94a–d**) through the cyclization in the presence of the 10% sodium hydroxide in methanol under reflux. Next, compounds (**96a–d**) were originated from (**95a–d**) via the protection of the *N*-7 position using (2-(trimethylsilyl)ethoxymethylchloride) and K_2CO_3 in DMF; on debenzylation of (**96a–d**) employing hydrogen and 10% Pd/C furnished the crucial intermediates (**97a–c**) having "O" and (**97d**) bearing "N" as a handle for further chemical modification [64].

The obtained compounds underwent alkylation reaction with several substituted (3-bromo-prop-1-ynyl)-benzene derivatives in the presence of the K_2CO_3 in acetone to generate the silyl protected xanthine analogues under reflux conditions which on silyl deprotection by 2 N hydrochloric acid yielded the desired products (**98–126**) in good to excellent yields (28–99%) [63].

In a similar way, the key intermediates (**128a** or **129b**) were synthesized by starting from 5,6-diaminouracil compounds (**127a** or **127b**) to follow the synthetic routes as presented in Figure 9.17 [65]. The crucial intermediates (**128a** or **128b**) underwent alkylation reaction with several phenyl substituted propargyl bromides in the presence of the potassium carbonate in acetone at 50 °C; after the event, *N*-7 silyl deprotection in the presence of the 2N HCl provided another targeted compounds (**129–135** and **136–142**) in good yields (11–85%) (Figure 9.18) [63].

The alkylation of intermediate (**97d**) took place with corresponding bromo compounds in the presence of the K_2CO_3 in acetone at under reflux, subsequently, *N*-7 deprotection of the synthesized silyl protected derivatives (**143** and **144**) using 2 N HCl in ethanol at reflux condition to furnish propargyl along with 2-butyne xanthine compounds (**145** and **146**) ultimately (Figure 9.19) [63].

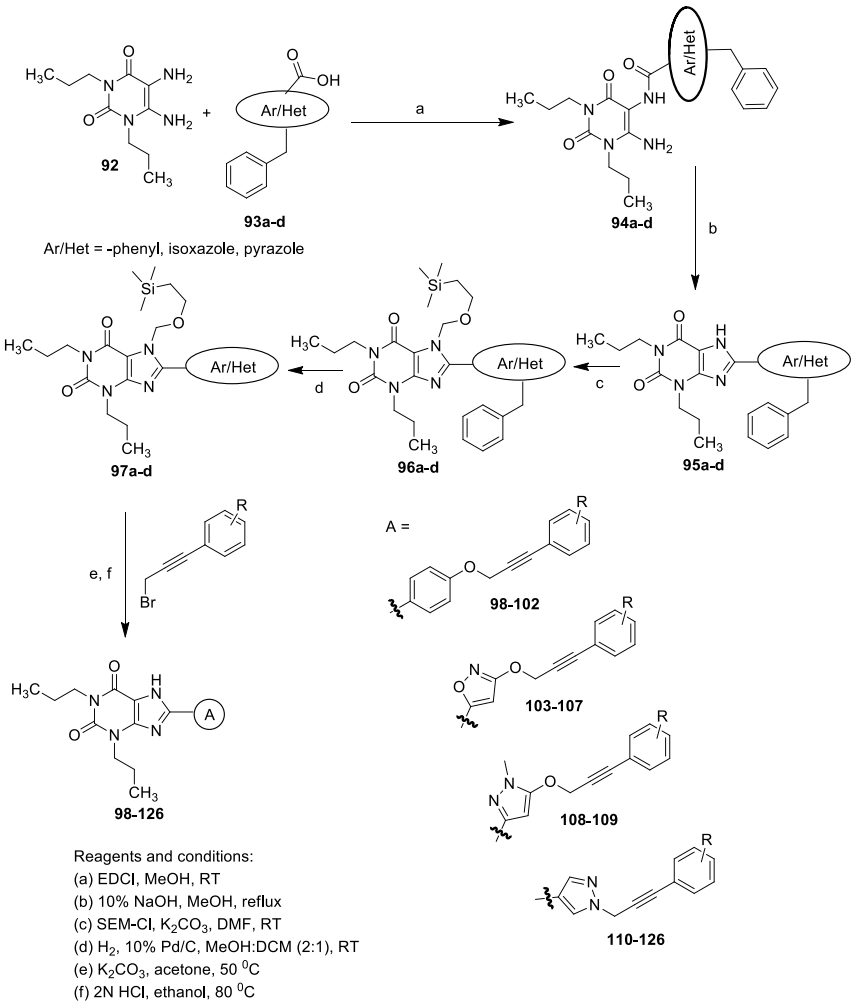


Figure 9.17: Synthesis of 8-(4-((3-phenylprop-2-yn-1-yl)oxy)phenyl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione derivatives, 8-(1-methyl-5-((3-phenylprop-2-yn-1-yl)oxy)-1*H*-pyrazol-3-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione derivatives.

The synthesized compound (**143**) was reacted with 37% solution of formaldehyde and heterocyclic or acyclic amines using CuI in DMSO at room temperature as a green methodology, followed by *N*-7 silyl deprotection with 2 N hydrochloric acid in ethanol to produce derivatives substituted by basic amino functionality on terminal acetylene (**147–154**). The desired xanthine derivatives (**153** and **154**) were synthesized from the intermediate (**143**) when it was treated with cyclopentanone or cyclohexanone using lithium hexamethyl disilazide in THF at low temperatures, subsequently *N*-7 silyl deprotection with 2 N HCl in ethanol (Figure 9.20) [63].

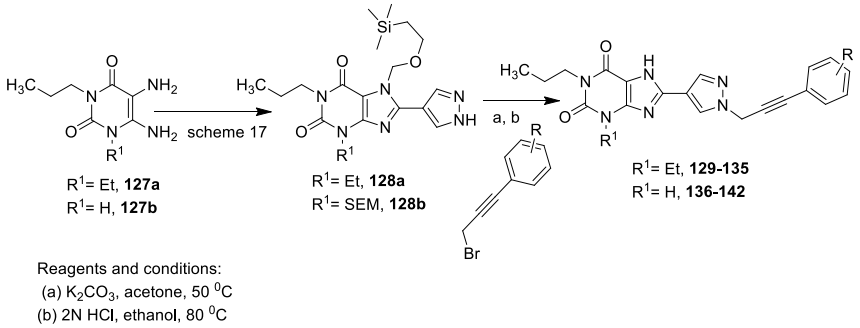


Figure 9.18: Synthesis of 3-ethyl-8-(1-(3-phenylprop-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione and 8-(1-(3-phenylprop-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione derivatives.

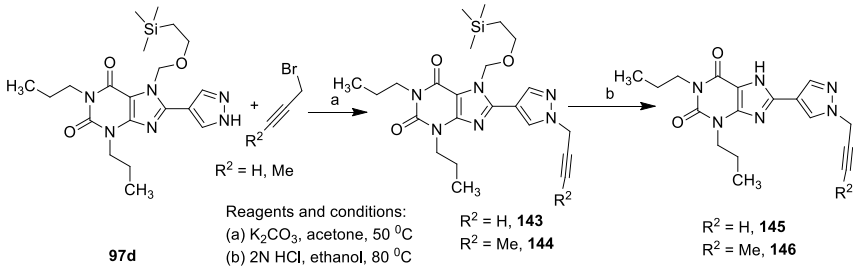


Figure 9.19: Synthesis of 8-(1-(prop-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione and 8-(1-(but-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione.

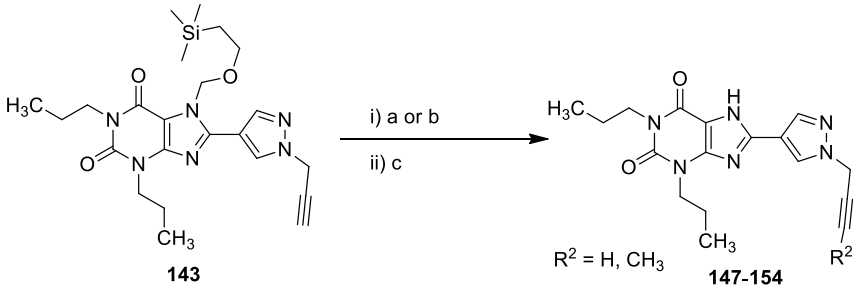


Figure 9.20: Synthesis of 8-(1-(prop-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione and 8-(1-(but-2-yn-1-yl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione.

9.2.2.6 Synthesis of novel 1,3-dimethylxanthine derivatives with thiazolidine-4-one scaffold

Profire and co-workers [66] completed a synthesis and biological evaluation of the novel 1,3-dimethylxanthine derivatives from theophylline (**155**) using condensation reaction as a key step (Figure 9.21). Theophylline (**155**) was converted to its sodium salt (**156**) at room temperature in a quantitative yield using sodium methoxide as a base. The salt (**156**) in EtOH/DMF (4:1.5) was treated with ethyl chloroacetate to afford ester (**157**) under reflux overnight. The ester (**157**) in ethanol was treated with hydrazine hydrate to provide theophylline hydrazide (**158**) in a very good yield under reflux for 6 h. The compound (**158**) underwent a condensation reaction with different aromatic aldehydes to deliver the corresponding hydrazones (**159a–k**) in satisfying yields. The cyclization of hydrazones (**159a–k**) in toluene occurred to generate desired thiazolidine-4-one derivatives (**160a–k**) in the presence of the mercaptoacetic acid in moderate to excellent yields.

9.2.2.7 Synthesis of 1,3,7,8-tetrasubstituted xanthine derivatives

Lee et al. [67] developed a traceless solid-phase synthetic protocol for the preparation of 1,3,7,8-tetrasubstituted xanthine derivatives; starting on a solid supported *N′*-cyano-*N*-substituted carbamimidothioate, which was synthesized from cyanamide, isothiocyanate, and Merrifield resin successfully (Figure 9.22). Hence, polymer bound (**163**) was obtained from sodium *N′*-cyano-*N*-substituted-carbamimidothioate (**161**) and Merrifield resin (**162**) using DMF at 60 °C. Polymer bound (**163**) was treated with ethyl 2-bromoacetate to afford corresponding resin (**164**) in the presence of the K_2CO_3 in DMF

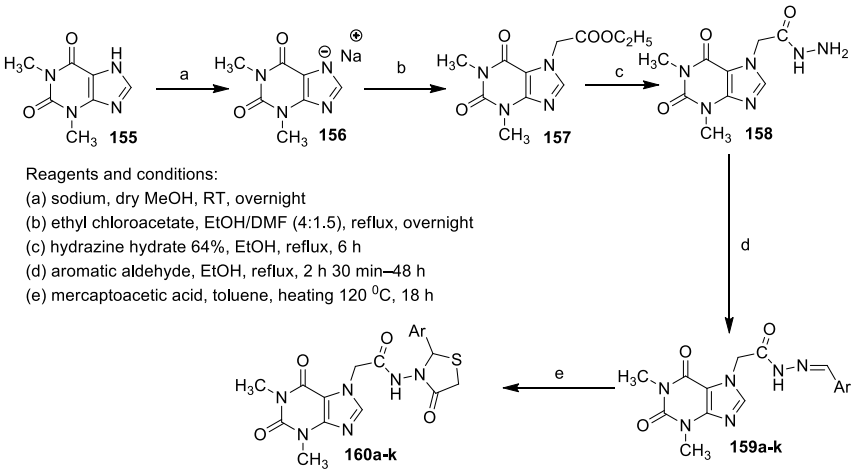


Figure 9.21: Synthesis of novel 1,3-dimethylxanthine derivatives with thiazolidine-4-one scaffold.

at 45 °C. Thorpe–Ziegler-type imidazole construction of resin (**164**) was flourished by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in acetone and led to the formation of the targeted imidazole ring bearing resin (**165**). Resin in toluene was treated with a selected isocyanate under a Lewis acid ($SnCl_2{\cdot}2H_2O$) condition at 60 °C to deliver corresponding urea resin (**166**). The intramolecular cyclization of (**166**) took place to yield resin (**167**) in DMF; the solvent was altered to DMF due to the low swelling effect of ethanol. Resin (**167**) was treated with an alkyl halide in the presence of the K_2CO_3 in DMF to give resin-bound 1,3,7-trisubstituted xanthine (**168**). The sulfide in resin (**168**) was converted to the sulfone on the resin (**169**) through oxidation using *m*-CPBA in DCM. The sulfone group in resin (**169**) was substituted with the corresponding nucleophiles (*n*-propyl thiol) through a desulfonative substitution reaction to afford the desired 1,3,7,8-tetrasubstituted xanthine (**170**) (83% yield over eight steps from Merrifield resin **162**); the desired product was also obtained under microwave irradiation in DMSO by the properly substituted amines finally.

9.2.2.8 Synthesis of annelated xanthine derivatives

It is well documented that caffeine, a methylxanthine class molecule, generated intrinsic antinociceptive effects in various rodent models [68]. Zygmunt et al. [69] presented the synthesis of a series of annelated derivatives of xanthine and analgesic properties of these derivatives in experimental models in rodents (Figure 9.23). Bromo compound (**171**) was reacted with 1-bromo-3-chloropropane to afford chloro compound

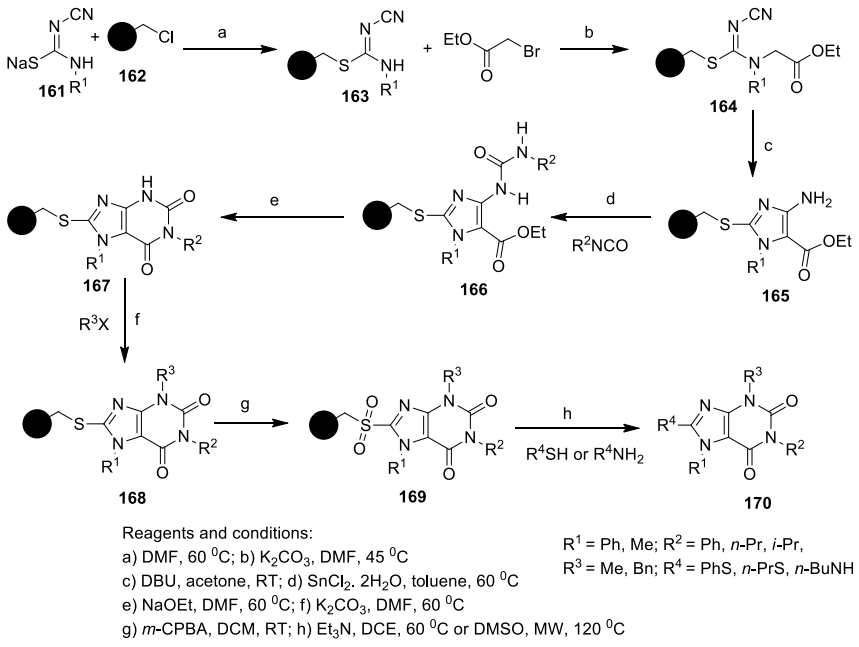


Figure 9.22: Synthesis of 1,3,7,8-tetrasubstituted xanthine derivatives.

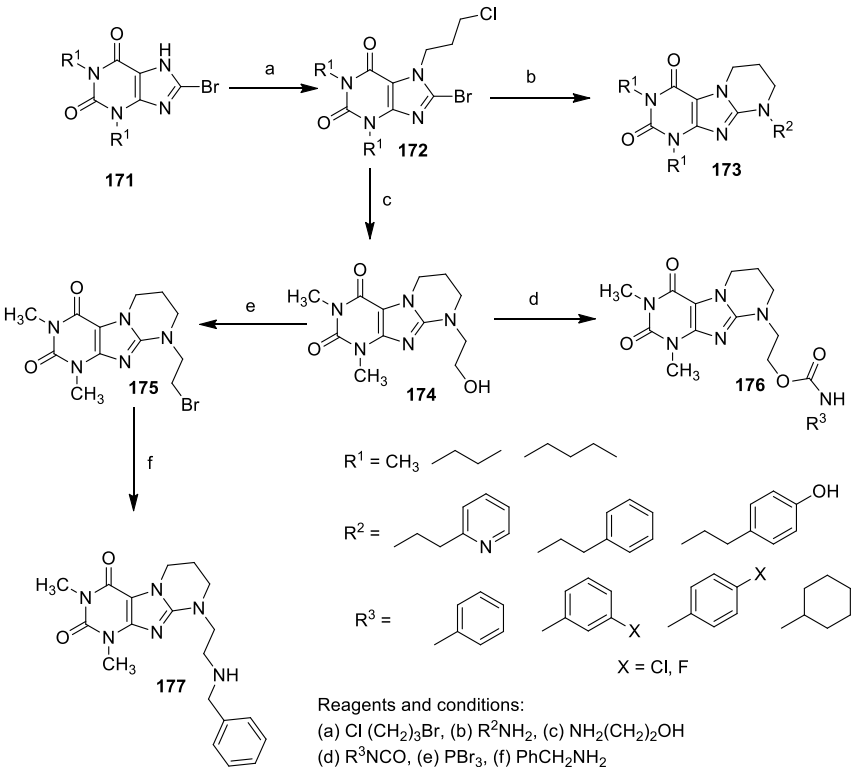


Figure 9.23: Synthesis of annelated xanthine derivatives.

(**172**), cyclization of chloro compound (**172**) by substituted amines yielding compound (**173**). The synthesized compound (**172**) was again treated with 2-aminoethanol to provide key intermediate (**174**) through cyclization reaction. Methylcarbamate derivative (**176**) was obtained from crucial intermediate (**174**) by isocyanato compound and intermediate (**174**) was also converted into bromoethyl phylline compound (**175**) in the presence of the phosphorus tribromide (PBr_3) through substitution reaction. Finally, bromoethyl compound (**175**) was transformed into the targeted compound amine (**177**) using phenylmethanamine successfully [69].

9.3 Modernization at acridine and xanthine-based molecules with anti-cancer activity

9.3.1 Anticancer activity of acridine-based molecules

Dobricic et al. [24] aimed to synthesize a new series of 11 derivatives of 9-acridinyl amino acid with the improved anticancer property as well as smaller toxicity than that

of the chemotherapeutic agent amsacrine in 2020. By applying the MTT assay, cytotoxicity of amino acid derivatives was conducted on the normal diploid cell line MRC5, A549 together with K562. It has been observed that compounds (**2f**, **2g**, **2h**, and **2i**) were the best active viability inhibitors in cancer cells having the half-maximal inhibitory concentration (IC_{50}) values comparable to or lower compare to amsacrine, especially compounds (**2h** and **2i**) in the A549 lung epithelial carcinoma cell line ($IC_{50} \approx 6$ μM) (Figure 9.1). Cell cycle analysis disclosed that derivatives (**2g** and **2i**) created G2/M block in A549 cells, while molecules (**2f** and **2h**) induced apoptotic cell death separately of the cell cycle regulation. Although only compound (**2g**) was responsible for DNA intercalation properties, compounds (**2f**, **2g**, **2h**, and **2i**) displayed similar inhibitory potential towards topoisomerase Iiα than that of amsacrine. It was also evident that in contrast chemotherapeutic agents to amsacrine, compounds (**2f**, **2g**, **2h**, and **2i**) exhibited a lack of toxicity in the direction of unstimulated normal human leucocytes [24].

Prasad VVS and co-workers [26] executed the preparation of some novel acridine analogues as well as evaluated anticancer activity of the newly synthesized derivatives using MTT assay in 2020. It has been observed that the synthesized derivatives (**8a**, **8f**, and **8h**) displayed good anticancer properties against MCF-7 (human breast cancer cells) and SKVO3 (human ovarian carcinoma cell lines) cancer cell lines at a concentration of 0.5 mg/mL, with doxorubicin as standard (Figure 9.2). It has been also investigated that synthesized derivatives (**8a–l**) exhibited IC_{50} values in the range of 18.8–49.15 μM against SKVO3 cell line and 17.98–72.72 μM against MCF-7 cell line.

Huo et al. [29] demonstrated the synthesis of acridine thiosemicarbazides derivatives and evaluated the anticancer activity of novel hybrids of acridine thiosemicarbazides analogues in 2019. In anticancer studies, the synthesized compounds (**14a**, **14d**, and **14e**) displayed potent cytotoxicity against the MT-4 cell line, having IC_{50} values of 18.42 ± 1.18, 10.96 ± 0.62, and 11.63 ± 0.11 μM, respectively (Figure 9.3). The results for the study of the synthesized compound (**14d**) in MT-4 cells disclosed that this molecule exhibited clear cell apoptosis-inducing effects and cell-cycle analysis also revealed that molecule (**14d**) could arrest MT-4 cells in the G1 stage. The test of anticancer effects and the related mechanism disclosed that the anticancer properties may be linked to topoisomerase I inhibitory property, apoptosis, and cell-cycle.

In 2018, Sharhan et al. [33] reported a novel series of acridine-based imidazolium salts and evaluated all resulted analogues of substituted acridine-imidazolium salt for *in vitro* cytotoxicity against a panel of human cancer cell lines (MCF-7, CAOV-3, PC-3, T1074, and MCF-10a) using an MTT assay. The panel comprised of MCF-7 (breast cancer cells), CAOV-3 (ovarian cancer cells), PC-3 (prostate adenocarcinoma cells), MCF-10a (non-tumorigenic breast cell line), and T1074 (non-tumorigenic ovarian cell line). The reference drugs tamoxifen and paclitaxel were employed for positive control; unlike the reference drugs, the therapeutic activity of acridine-derived imidazolium salts is very specific for definite cancer types. In fact, unproblematic cell-toxicity was exhibited by all acridine-derived imidazolium salts against non-cancer cell lines having a half maximal inhibitory concentration (IC_{50}) values above 50 mg/mL which indicates that the molecules can be

carefully applied for cancer treatment. Although compound (**21b**) was practically inactive against breast and prostate cancer, it exhibited more potential against ovarian cancer, surpassing paclitaxel as well as practically matching tamoxifen (Figure 9.4). Besides, significant breast cancer activity was shown by compounds (**21a**) and especially (**21n**), which were more active compare to any of the positive controls [33].

Kalirajan and co-workers [35] presented the synthesis of a series of oxazine substituted 9-anilinoacridines and the main objective of this work was to evaluate the significant antitumor activities of oxazine substituted 9-anilinoacridine analogues (**26a–k**) against Daltons lymphoma ascites cells in 2018 (Figure 9.5). Prominent cytotoxic activities were displayed by all the synthesized compounds (**26a–k**); compounds (**26b**, **26c**, **26e**, and **26j**) were found to show the most cytotoxic with a common toxicity criteria 50 (CTC_{50}) value of 96.5–190 μg/mL (0.125–0.352 μM). *In vivo* antitumor activities of selected compounds were performed by DLA tumor model in mice; compounds (**26b** and **26h**) were selected for *in vivo* antitumor activity whereas all the oxazine substituted 9-anilinoacridine analogues (**26a–k**) was screened for a short term *in vitro* antitumor property against DLA cells [35].

In 2017, 2-methyl-9 substituted (**30a–h**) acridines were synthesized by Kumar and co-workers [37] and three compounds were tested for antiproliferative activity against A-549 cell lines by the MTT assay (Figure 9.6). It has been found that compound (**30c**) displayed better *in vitro* cytotoxic properties against A-549 (Human, small cell lung carcinoma) and MCF-7 (Human, breast cancer) cancer cell lines having CTC_{50} 187.5 and 212.5 μg/mL respectively. It was also evident that the cancer cell cytotoxicity of acridines against A-549 cell line was more potent than that of the MCF-7 cell line. It was interesting to be noted that aromatic amine having an electron-withdrawing group on *m*-position was effective to display antiproliferative activity compared to electron releasing group; also replacement of Cl group on *m*-position of 9-position of acridine ring was more potent compare to CF_3 group on the same position [37].

Jiang et al. [48] conducted the preparation of new acridine-based derivatives as well as they tested the DNA binding ability and topoisomerase I assisted relaxation of plasmid pBR322 DNA successfully. Thus the synthesized acridine-based analogues (**52a–d**) were evaluated for the antiproliferative property against K562 and HepG-2 cell lines (Figure 9.11). It has been observed that distortion of the DNA structure took place when the synthesized compound (**52c**) can interact with DNA brilliantly and it exhibited excellent topoisomerase I inhibitory activity at about 1 mmol/L. The DNA-binding affinity of the synthesized compound (**52c**) was tested by UV–vis absorption spectra along with fluorescence emission spectra [48].

9.3.2 Anticancer activity of xanthine-based molecules

Hayallah and co-workers [56] carried out the biological evaluation of new xanthine derivatives as powerful apoptotic antitumor agents in 2019. It has been found that the

non-oxime congeners (**72a,b**) and (**73a–h**) were less active as antiproliferative agents than that of the oxime-containing compounds (**74a,b**) and (**75a–h**) (Figure 9.14). The authors also revealed that compounds (**75a–h**) bearing hydroxyimino-phenethyl scaffold exhibited higher activity compared to the hydroxyimino-ethyl phenyl acetamide (**74a,b**) analogues. The synthesized compounds (**75b–d**) and (**75f–h**) displayed inhibition of EGFR with the half-maximal inhibitory concentration (IC_{50}) ranging from 0.32 to 2.88 μM and these compounds (**75b–d**) and (**75f–h**) enhanced the level of active caspase 3 by 4–8 folds than that of the doxorubicin as a reference drug. The most caspase-3 inducers were compounds (**75b**, **75f**, and **75g**); the work also disclosed that compounds (**75f**) and (**75g**) enhanced the levels of caspase-8 and 9 showing activation of both intrinsic and extrinsic pathways. Ultimately, a molecular docking study was carried out at the EGFR active site to indicate their possible binding mode during their study [56].

In 2018, Kowalska et al. [61] studied the anti-cancer activity of 8-substituted 3,7 dimethylxanthines carrying a triple bond against human adenocarcinoma MDA-MB-231, human glioblastoma SNB-19, and melanoma C-32 cell lines; the nature of the substituent and its position in the xanthine framework are related to the anti-cancer property. Xanthine derivatives (**91d** and **91c**) having the diethylaminobutynylthio and morpholinylbutynylthio groups displayed potent activity against the SBN-19 line ($IC_{50} < 1.7$ μg/mL) (Figure 9.16). All the synthesized compounds were evaluated for their cytotoxicity on the normal human fibroblasts (HFF-1) and it has been observed that derivatives (**91c,d**) along with cisplatin also turned out to be cytotoxic for fibroblasts [61].

Very recently in 2021, Rafraf et al. [70] carried out a study to assess the chemopreventive effects of natural theanine and theobromine, alone or together, on dimethylhydrazine (DMH)- induced colon cancer. The results of the work revealed that these phytochemicals alone or together decreased the number of cancerous and precancerous lesions, the volume of tumors, immunostaining of Ki-67, and the expression of Akt/mTOR as well as JAK2/STAT3 oncogenic pathways. It was interesting to note that the inhibition of the Ki-67 along with Akt/mTOR expression was more manifested by the theobromine treatment compared to theanine.

In a more recent study, Sheng et al. [71] reported that 1,3,7-trimethylxanthine caffeine inhibits the anticancer property of paclitaxel through down-regulation of α-tubulin acetylation in 2020. The findings of this study revealed that xanthine alkaloid caffeine raised the growth of cancer cells treated with paclitaxel. Moreover, caffeine increased migration capability, inhibited apoptosis, and reduced the acetylation of α-tubulin in paclitaxel-treated cancer cells. Besides, natural caffeine reduced the inhibitory activity of paclitaxel on tumor growth via down-regulation of α-tubulin acetylation *in vivo*.

Sadzuka et al. [72] disclosed the mechanism through which caffeine behaves as a biochemical modulator of adriamycin, and the investigators studied several methylxanthine derivatives to decide whether they would be of utility as biochemical

modulators. It has been observed that theophylline, pentoxifylline, and theobromine inhibited the efflux, while on the contrary, the methylxanthine alkaloid caffeine metabolites are not able during in an *in vitro* examination of adriamycin efflux in Ehrlich ascites carcinoma cells which suggests that pentoxifylline and theobromine would be of benefit as biochemical modulators of adriamycin. The effects of various methylxanthine analogues on the antitumor property of adriamycin and adriamycin concentration in tissue were also investigated in CDF_1 tumor-carrying mice. A bitter alkaloid of the cacao plant theobromine, which inhibited adriamycin efflux *in vitro*, enhanced the antitumor property of adriamycin as well as the concentration of adriamycin in tumors.

Tang et al. [73] aimed to investigate the anticancer activities of natural stimulant caffeine on gastric cancer (GC) cells (MGC-803 and SGC-7901) *in vitro*, along with ascertaining whether the apoptosis-linked caspase-9/-3 pathway is related to these effects. In this work, the sustained antiproliferative activities of caffeine on gastric cancer were also examined. Flow cytometry was carried out to assess cell cycle dynamics and apoptosis along Western blot analysis was performed to identify the effect of the caspase-9/-3 pathway. The results of the work suggested that caffeine treatment remarkably suppressed GC cell growth and viability as well as induced apoptosis through activating the caspase-9/-3 pathway. Caffeine may behave as a sustained anticancer candidate by operating the caspase-9/-3 pathway, which suggests that it may be effective as a therapeutic agent in gastric cancer.

Huang et al. [74] reported that methylxanthine theophylline displays anti-cancer activity through suppressing SRSF3 in cervical as well as breast cancer cell lines. The study expressed that theophylline induced cellular apoptosis, senescence, together with reduced colony construction. The results of the work provided that theophylline could be reused as an antitumor leading molecule through the downregulation of splicing factor SRSF3 along with its target genes.

In a study by Rangappa et al. [75] a series of theophylline methyl 1,3,4-oxadiazole small compounds were synthesized and evaluated cytotoxic effect in three different cancer lines. Several compounds exhibited good cytotoxic activity among 10 synthesized oxadiazole derivatives; especially the compound 7-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione, 7-((5-(2-Bromophenyl)-1,3,4-oxadiazol-2-yl)-methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione displayed a significant effect compared to other compounds in this series and these two compounds displayed 6.25–8.6 fold better cytotoxicity compared to SCR7 in T cell leukemic cell lines, CEM.

Sugimoto and co-workers [76] examined the chronic administration of a xanthine alkaloid theobromine inhibits the mTOR signal in rats in 2019. In this study, the level of phosphorylated mTOR was employed as an index of mTOR activity; male Wistar rats were classified into two types such as the theobromine group was fed a diet supplemented having 0.05% theobromine for 40 days, while the control group was fed a usual diet. It has been observed that the body weights together with tissue weights, food along with water intake, and blood count as determined was not influenced by the

orally administered theobromine compared to the levels in rats that were fed standard chow. Theobromine was identified in the liver, plasma, and brain-derived from the theobromine group rats, but was not identified in tissues derived from the control group rats. The phosphorylated mTOR levels in the brain, as well as liver, were remarkably lower in the theobromine group rats compared to the control group rats, indicating that oral natural theobromine inhibits mTOR signaling *in vivo*.

9.4 Conclusions

Cancer is the uncontrolled growth and development of abnormal cells which is a major source of death in both advanced along with emerging countries and it represents in the wider sense to more than 277 different classes of cancer disease. Although currently chemotherapy is most broadly used among an extensive range of anti-cancer therapies, it includes many demerits, such as highly toxic, side-effects, expensive and partial lack of targeting specificity. So, we need new molecules that perform specifically on target proteins in tumor cells is a focus of contemporary research. Naturally occurring molecules acridine and xanthine as well as their derivatives are of great interest to researchers and technologists owing to their anti-cancer activities. The present review covered various syntheses as well as anti-cancer activity of acridine and xanthine-based compounds elegantly. Their capabilities to interact with the biomolecules of interest provide the acridine and xanthine-based nucleus an efficient functionality in the improvement of target-derived drug delivery treatment for generating the hit to lead compounds.

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10 Synthesis of new horizons in benzothiazole scaffold and used in anticancer drug development

Abstract: Benzothiazole scaffolds exhibit exciting medicinal properties including anticancer. In recent time most complicated job for every researcher is to discover a novel drug that can treat cancer with minimal side effects. Some heterocyclic anticancer drugs including daunorubicin, 5-flourouracil, doxorubicin, methotrexate, etc. are markedly available. In addition, few natural products such as vincristine alongwith vinblastine are used as anticancer drugs. More than 90% of the novel drugs bearing heterocyclic moieties have always been main portions in the development of anticancer drugs. Heterocyclic compounds containing benzothiazole moiety show a superior pharmaceutical effect than non-nitrogen compounds. These N-/S-containing benzothiazole compounds, the heart of drug discovery, present a significant and valuable group of molecules that play a chief and vital role in our living cells. This chapter recites the weightage of benzothiazole nuclei in the progress of anticancer drugs.

Keywords: anticancer agent; benzothiazole; drugs development; molecular docking studies; synthesis.

10.1 Introduction

The forefronts of most of the scientists around the world are finding remedies of the serious worldwide health disease "cancer". The global research endeavors in this field are focused on the finding of novel biological targets and also development of novel potent anti-neoplastic agents (medications used to treat cancer). From the literature study cancer is the main foremost cause in come down patient health and affecting a well person.

Bearing in mind the growing the cancer cases and death rate, heterocyclic molecules play an important role in the progress of therapeutically potent anticancer compounds due to their enormous cytotoxicity against quite a lot of cancer cell lines. The heterocyclic compounds have proven a helpful core in the searching of novel anticancer substances [1]. Benzothiazoles and its derivatives are accounted as suitable

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scaffolds and hold an important position within the several heterocycles as an effective cytotoxic agent and exhibit remarkable and prevalent biological, pharmacological and pharmaceutical activities [2].

The heterocycle Benzothiazole compounds containing nitrogen (*N*) andsulfur (*S*) heteroatom. The benzothiazole analogs and its derivatives contain a significant function particularly in synthetic, pharmaceutical, and medicinal chemistry owing to their pharmacological and biological activities.

Particularly in the previous few years, a small number of benzothiazoles have found important application in medicinal chemistry, bio-organic and in the development of clinical drugs for instance lubeluzole, pramipexole, probenazole, ethoxzolamide, bentaluron and zopolrestat etc.

Besides anticancer potential, benzothiazoles have a wide range of biological activities and are trace in numerous potent biologically active molecules. Benzothiazole skimmed in proceedings with frame-up as result of their several natural activities for example anticancer [3], antimicrobial [4], anticonvulsant [5], antiviral [6a], anti-HIV [6b], antitubercular [7], antimalarial [8], antihelmintic [9], analgesic [10], anti-inflammatory [11], antidiabetic [12] and fungicidal activities [13] (Figure 10.1).

10.1.1 Anticancer agents

The novel heterocycle Benzothiazole compounds are efficient to fight against different type of cancers. Assess the anticancer activity of benzothiazole derivatives by the

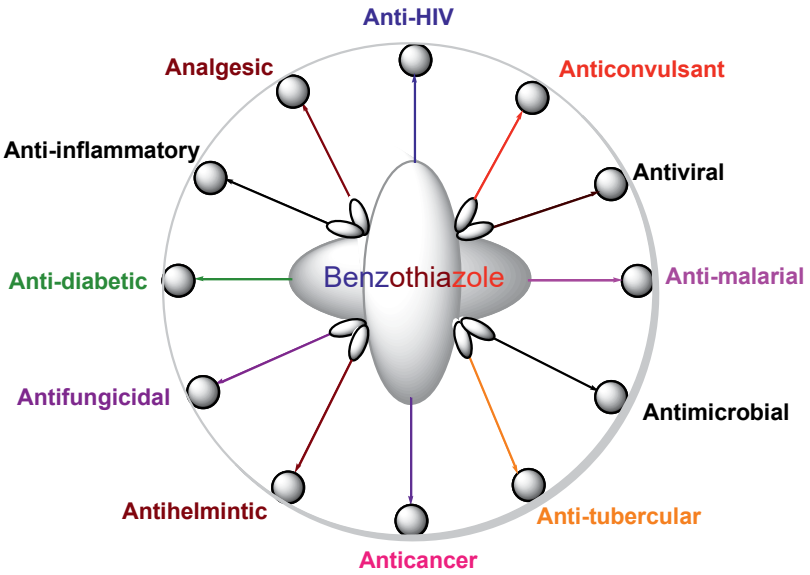


Figure 10.1: Several biological activities of benzothiazoles.

effects of different types of substitutions in the ring. The core structure of the benzothiazole skeleton for antineoplastic activities are shown (Figure 10.2).

Several biologically active benzothiazole derivatives are shown (Figure 10.3). Within these, frentizole (A) is an immunosuppressive agent and nontoxic antiviral used clinically in systemic lupus erythematosus and rheumatoid arthritis. Likewise, substituted benzothiazoles such as 2-(3,4- dimethoxyphenyl) - 5-fluoro benzothiazole (*PMX 610*) (B) were screened by National Cancer Institute (*NCI*), for *in vitro* antitumor activities against 60 human cancer cell lines for example colon, non small-cell lung, and breast cancer subpanels etc.

Another benzothiazoles for example 2-(4-amino-3-methylphenyl) benzothiazole (*DF 203*) (C) and the 2-(4-amino-3-methylphenyl)-5-fluoro benzothiazole (*5F 203*) (D) have been found to actuate the Aryl hydrocarbon Receptor (*AhR*) *through* translocation to the nucleus from the cytosol and organelles. The induced cytochrome (*P450 CYP1A1*) enzyme activity has been destined in these, which afterwards led to the production of a reactive chemical intermediate accountable for the formation of DNA adducts only in sensorial tumor cell types (e.g., ovarian and mammary tumor cell lines). Another substituted benzothiazole compound is 4- hydroxylcyclohexadieneone (*AW 464*) (E) is the prototype of a novel series of "quinols" with effective antitumor activity against colon and renal

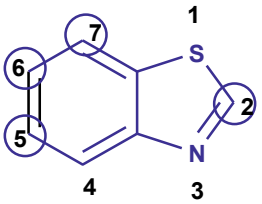


Figure 10.2: Core structure of benzothiazole skeleton and antineoplastic activity are shown at 2-positions.

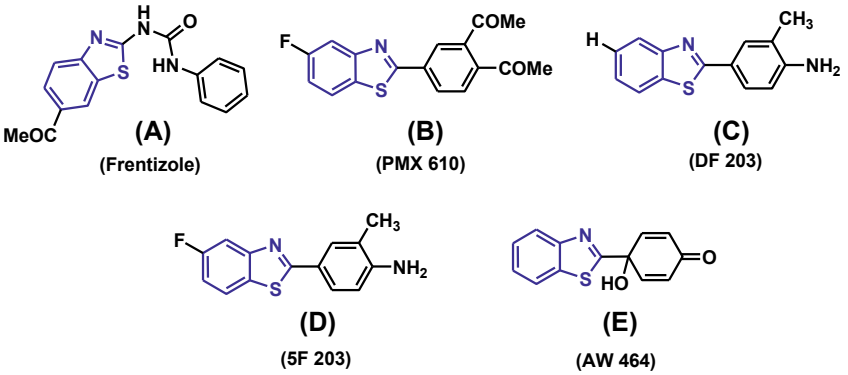


Figure 10.3: Structures of the benzothiazole derivatives including anticancer activity.

cancer cell lines that affects on vital cell-signaling actions downstream of the redox regulatory protein thioredoxin.

10.1.1.1 Imidazole ring based benzothiazole derivatives

Yurttas et al. has founded 2-(4-aminophenyl) benzothiazole derivatives substituted with several heterocyclic rings and examined their antitumor activity against sixty (60) human cancer cell lines. The benzothiazole derivatives (N-(4-(benzo[*d*]thiazol-2-yl) phenyl)-2-(1-phenyl-1H-benzo[d]imi-dazol-2-yl-thio)-acetamide) (**1**) and N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzimidazole-2-yl)thio]acetamide (**2**) were shown remarkable antitumor potential with various cancer cell lines [16] (Figure 10.4).

Compound (1) and (2) avert the growth of cancer cell with nanomolar scale in opposition to a big panel of human cancer cell lines mainly against colon, ovarian, and breast cell lines in *in vitro* anticancer were screened by National Cancer Institute (*NCI*). Compound (1) was found to occupy selective *in vitro* activity against tumor cell line like ovarian cancer (OC, growth percentages 72.63%) and prostate cancer (PC, growth percentages 74.22%) with mean $\log_{10}$GI50 values for (OC, –4.90) and (PC, >–4.00) [16]. Whereas, the compound (2) was found to occupy selective *in vitro* potential against tumor cell line like ovarian cancer (OC, growth percentages 10.92%) and prostate cancer (PC, growth percentages 26.28%) with mean $\log_{10}$GI50 values for (OC, –5.55) and (PC, 5.55) [16].

Singh et al. has reported the preparation of imidazole based benzothiazoles through the reaction of substituted anilines with KSCN which gives benzothiazole derivative (**3**) has shown excellent anticancer potential on **MCF-7** cancer cell line using "**Doxorubicin**" as standard representing its activities as anticancer agent. [17] (Figure 10.5).

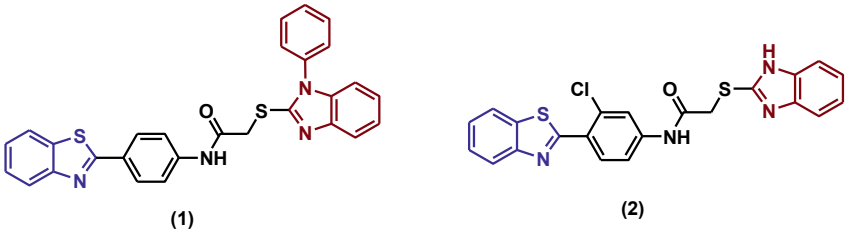


Figure 10.4: Imidazole ring based benzothiazoles derivatives.

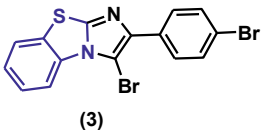


Figure 10.5: Imidazole based benzothiazole derivative.

10.1.1.2 Piperazine ring based benzothiazole derivatives

Al-Soud et al. has described the benzothiazole derivatives conjoining piperazino-arylsulfonamide, sulphonamide moieties such as (*N*-(2–(4-(benzo[d]thiazol-2-yl) piperazin-1-yl)-2-oxoethyl)-4-chloro benzenesulfonodithioamide) (4) showed anti-proliferative potential in opposition to human derived such as DU-145 cell line [human prostate cancer (HPC) cell lines elicit androgen receptor] whereas compound (5) (*N*-(2–(4-(benzo[*d*]thiazol-2-yl)piperazin-1-yl)-2-oxoethyl)-2,5-dichlorobenzenesul-fonodithio-amide) exhibited notable activities against different human cell lines for example human liver cancer (HepG2) and DU-145 cell lines [18] (Figure 10.6).

10.1.1.3 Oxadiazole based benzothiazole

Akhtar et al. [19] has synthesized benzothiazole derivatives such as (N-(benzo[d]thiazol-2-yl)-2–(5-(1–(3,4-dichlorophenoxy)ethyl)-1,3,4-oxadiaz- ol-2-ylthio)acetamide) (**13**) and (*N*-(benzo[*d*]thiazol-2-yl)-2-(5-(1-(2-chlorophenoxy)propyl)-1,3,4-oxadiazol-2-ylthio) acetamide) (**14**) (Figure 10.7) exhibited significant activities in opposition to a panel of tumor cell lines building of CD4 human T-cells having an integrated form of leukaemia (CCRF-CEM) (CC_{50} = 12 ± 2 μmol L^{-1}, 8 ± 1 μmol L^{-1} respectively).

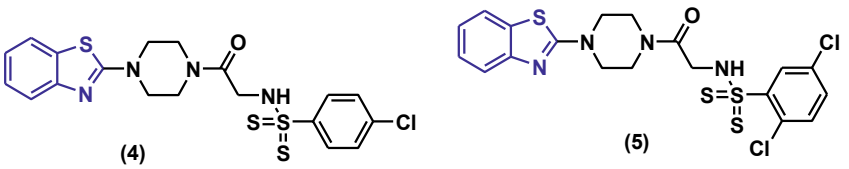


Figure 10.6: Di-/monochlorobenzenesulfonamide ring based piperazine benzothiazoles compounds.

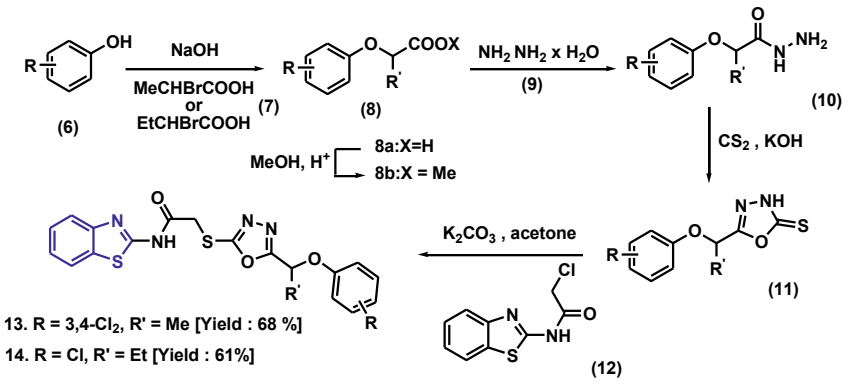


Figure 10.7: Synthesis of oxadiazole ring based acetamide benzothiazole compounds.

In the synthetic methodologies, 2-(3,4-dichlorophenoxy)propanoic methyl ester (**8a**) and 2-(*o*-chlorophenoxy)butanoic methyl ester (**8b**) compounds were prepared by the reaction of 2-bromopropanoic acid or 2-bromobutanoic acid (**7**) with substituted phenols (3,4-dichlorophenol and *o*-chlorophenol) (**6**) in aqueous NaOH. To a solution of (**8**) in EtOH, hydrazine hydrate (**9**) was added and heated under reflux for 3–4 h to afford 3,4-dichlorophenoxypropionic acid hydrazide or 3,4-dichlorophenoxy butyric acid hydrazide (**10**). The compounds (**10**) was added to a stirred mother solution of KOH in methyl alcohol, followed by addition of carbon disulfide (CS_2) to give 5- 1-(3,4-dichlorophenoxy)methyl -1,3,4-oxadiazole-2-thione and 5- 1-(*o*-chlorophenoxy) propyl -1,3,4-oxadiazole-2-thione (**11**). Finally the compounds (**13**) and (**14**) were synthesized by the treatment of compounds (**11**) with benzothiazolyl chloroacetamide (**12**) in appearance of anhydrous K_2CO_3 at 23 °C for 5 h (Figure 10.7).

10.1.1.4 Morpholine-thiourea based benzothiazole derivatives

Saeed. et al. have established an efficient procedure for the synthesis of benzothiazole based thiourea derivatives. This benzothiazole derivative was examined for anticancer activities with cancer cell lines HeLa cells and MCF-7. The thiophene ring based acetamide benzothiazole compounds (**21a**), morpholine based thiourea aminobenzothiazole compounds (**21b**), and morpholine linked thiourea bromobenzothiazole (**21c**) in (Figure 10.8) are potent anticancer agents against HeLa cells lines (human cervical) and MCF-7 cell line (human breast adenocarcinoma) respectively [20].

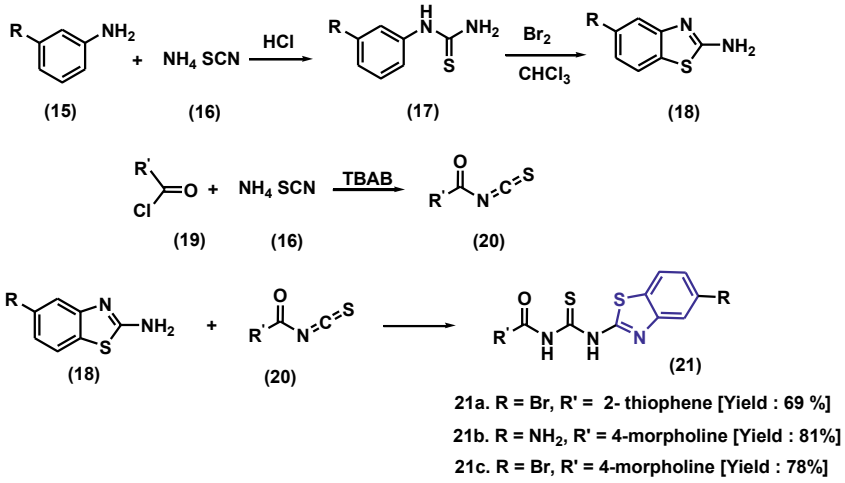


Figure 10.8: Preparation of benzothiazole based thiourea derivatives.

Saeed and his co-workers have been demonstrated the reaction of substituted aniline (**15**) with ammonium thiocyanate (**16**) in appearance of concentrated hydrochloric acid (HCl) to provide substituted phenylthiourea (**17**), which is brominated by using bromine solution in chloroform to get a desired product Substituted 2-amino benzothiazole (**18**) in reproducibly good yield.

In the second step, the substituted carbonyl chloride (**19**) reacts with ammonium thiocyanate (**16**) in presence of TBAB in acetone to give compounds (**20**). Finally, the substituted 2-amino benzothiazole (**18**) undergoes addition reaction with compounds (**20**) in acetone under refluxing condition for 1.5 h to give our desired compounds (**21a–c**) in good yield (69–81%) (Figure 10.8).

Lei et al. [21] has synthesized the morpholine-linked acetamide benzothiazole compound (**27**) (Figure 10.9) investigated its anticancer potential against HCC (human hepatocellular carcinoma) cell lines like as Bel7402 and HepG2.

The morpholine-4-carbonyl chloride compound (**24**) was synthesized by using of morpholine (**22**) and 2-chloroacetyl chloride (**23**) at 25 °C temperature. The compound (**24**) reacts with 6-aminobenzo[d]thiazole-2-thiol (**25**) in presence of K_2CO_3 in THF to provide compound (**26**). Finally, the morpholine based acetamide benzothiazole compound (**27**) was obtained by the treatment of a mixture of compound (**26**) and 2-chloroacetyl chloride (**23**) in presence of K_2CO_3 in dichloromethane (Figure 10.9).

10.1.1.5 Thiadiazole ring based benzothiazole derivatives

Sekar et al. have disclosed the preparation of benzothiazole derivatives and their anticancer activities. The substituted thiadiazole flourobenzothiazole (**31a**) and methoxybenzothiazole (**31b**) (Figure 10.10) have shown the excellent anti-cancer activity owing to the presence of strong electronegative and electron-donating pharmacophores [22].

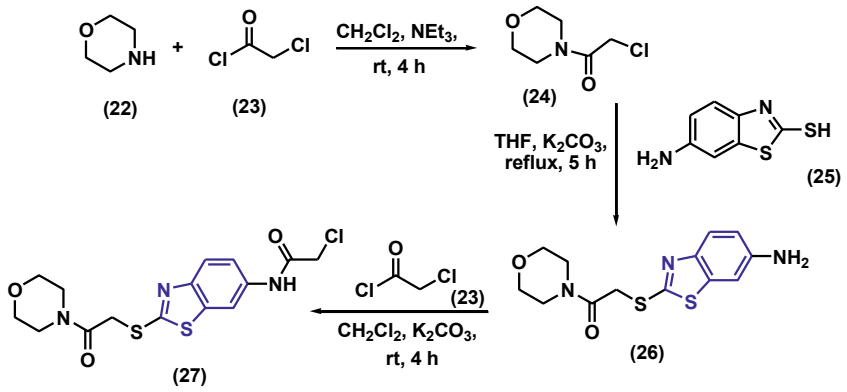


Figure 10.9: Preparation of morpholine based acetamide benzothiazole compound.

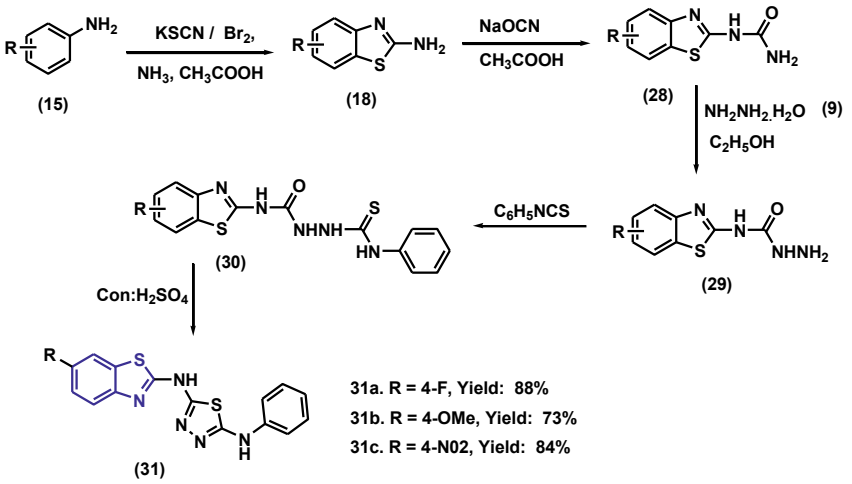


Figure 10.10: Synthesis of substituted thiadiazole based benzothiazole deri-vatives.

Sekar and his research group have demonstrated that multistep approach for the synthesis of N-(6-fluoro-1,3- benzothiazol-2-yI)-N′-phenyI-1,3,4- thiadiazole-2,5-diamine and N-(6-methoxy-1,3- benzothiazol-2-yI)-N′-phenyI-1,3,4- thiadiazole-2,5-diamine respectively. In the first step, a mixture of substituted aniline (**15**) and potassium thiocyanate (KSCN) in glacial CH_3COOH was stirred. This reaction mixture was coupled with Br_2 and aqueous solution of ammonia to give 6-substituted-1, 3-benzothiazol-2-amine (**18**). In the second step, compound (**18**) was heated with solution of NaOCN in glacial acetic acid to provide solid compound (**28**).In the third step, a solution of compound (**28**) in EtOH was heated with hydrazine hydrate under reflux condition for 6 h to give the compound (**29**).In the next step, the addition of Phenyl isothiocyanate to the solution of the compound (**29**) and refluxed this mixture for 6 h to produced compound (**30**).

In the final step, the concentrated H_2SO_4 was added to solution of compound (**30**) and stirring at below 0 °C temperature to give the desired product substituted thiadiazole flourobenzothiazole (**31a**) and methoxybenzothiazole (**31b**) respectively (Figure 10.10).

10.1.1.6 Substituted pyridine based benzothiazole compounds

Shi and Wang have synthesized the substituted bromopyridineacetamide benzothiazole derivative (**35**) (Figure 10.11). It has demonstrated strong antitumor activity against SW620, A549, SKRB-3, and HepG2 cell lines respectively [23].

Shi and his co-workers have discussed how the benzothiazole-2-thiol derivatives coupled with heterocyclic rings were planned and synthetic pathway shown (Figure 10.11). Markedly accessible 2-amino-5-bromopyridine (**32**) was first reacts with

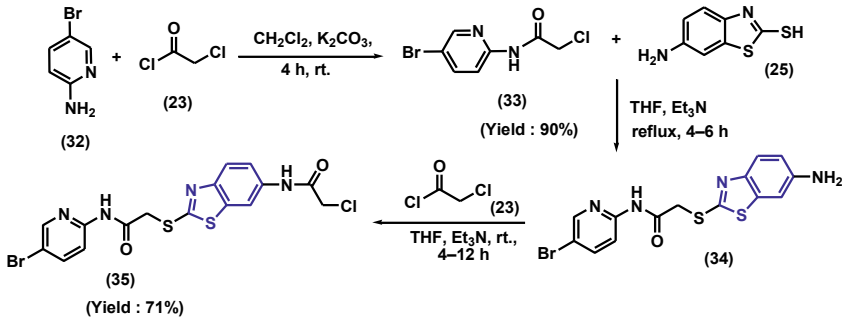


Figure 10.11: Synthesis of substituted bromopyridine ring based acetamide benzothiazole compound.

2-chloroacetyl chloride (**23**) in dichloromethane and K_2CO_3 to provide the compound (**33**). The compound (**33**) reacts with 6-aminobenzothiazole-2-thiol (**25**) in the presence of base triethylamine (TEA) in tetrahydrofuran (THF) under reflux to afford (**34**). In the presence triethylamine (TEA), the compounds (**34**) were treated with (2-chloroacetyl chloride) (**23**) to give desired products (**35**) in good yield (**71%**).

10.1.1.7 Pyrimidine ring based benzothiazole derivatives

Kambhare et al. has disclosed the pyrimidine ring based benzothiazole derivative (**43**) (Figure 10.12) contain good anticancer activity against U937, MCF-7, A549 and colo205 cell lines [24].

In appearance of 4-dimethylaminopyridine (5 mol %), the pyrimidin-2-amine (36) was treated with 1-isothiocyanato benzene (37) in DMF to give 1-phenyl-3-(pyrimidin-

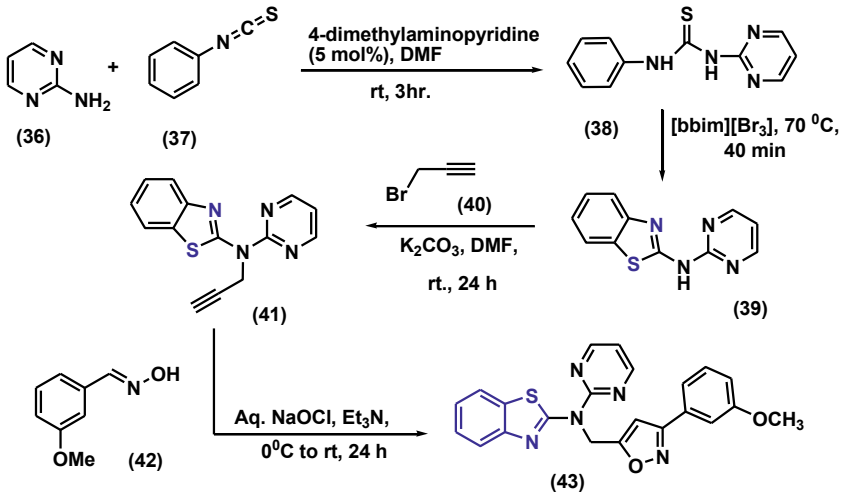


Figure 10.12: Preparation of pyrimidine based benzothiazole derivative.

2-yl)thiourea (**38**). The compound (**38**) was undergoes catalytic conversion into N-(pyrimidin-2-yl)benzo [d] thiazol-2-amine (**39**) in presence of [bbim][Br_3] at 70 °C. The compound (**39**) was further treated 3-bromoprop-1-yne (**40**) in the presence of mild basic medium (K_2CO_3) to produce *N*-(prop-2-ynyl)-N-(pyrimidin-2-yl)benzo[d]thiazol-2-amine (**41**). In the final step, the desired product (**43**) was obtained by the completion the reaction between compound (41) with compound (42) under aqueous NaOCl and Et_3N in high yield (Figure 10.12).

10.1.1.8 Piperidine ring based benzothiazole derivatives

Osmaniye et al. [25] has synthesized the piperidine based acetohydrazide derivative (**52**) (Figure 10.13) showed modest anticancer activities against A549 (human lung adenocarcinoma epithelial), HT-29 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), C6 (rat brain glioma), and NIH_3T_3 (mouse embryo fibroblast) cell lines.

Osmaniye and his co-workers have been demonstrated that synthesis of 2-((5-Chlorobenzothiazol-2-yl)thio)-N′-(4-(4-methylpiperidin-1-yl)benzyli-dene)acetohydrazide (**53**) as an anticancer agent in (Figure 10.13). In preliminary stage, 4-fluoro benzaldehyde (**44**) and 4-methyl piperidine (45) was refluxed in presence of K_2CO_3 in DMF for 10 h to give 4-substitute benzaldehyde derivative (**46**). In continuation, preparation of ethyl 2-(5-chlorobenzothiazol-2-yl-thio) acetate (**49**) by successfully conduct the reaction between 5-chlorobenzothiazol-2-thiol (**47**) and ethyl 2-chloroacetate (**48**) in presence of K_2CO_3 was refluxed in acetone for 2 h.

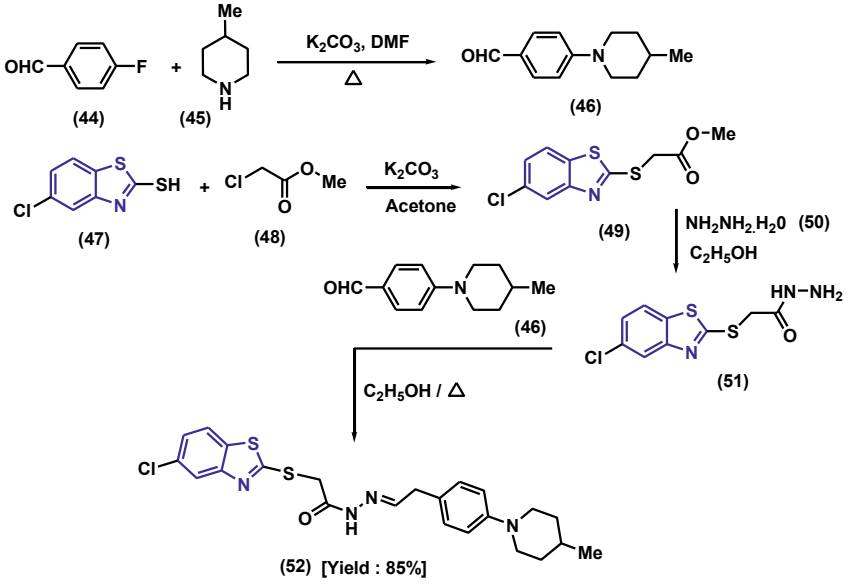


Figure 10.13: Synthesis of piperidine based acetohydrazide benzothiazole derivative.

Uremis et al. [26] has prepared 2-substituted benzothiazole compounds and measured their anticancer potential against pancreatic cancer cell. Uremis's group have found the compound 2-((1S, 2S)2-((E)-4-florostyryl) cyclopent-3-en-1-yl) benzo [*d*] thiazole (**57**) and 2-((1S, 2S)2-((E)-4- nitrostyryl) cyclopent-3-en-1-yl) benzo [*d*] thiazole (**58**) possess good anticancer potentiality (Figure 10.14).

Uremis and his coworker have been accounted a proficient and convenient procedure for the synthesis of 2-substituted benzothiazole derivatives (**57**) and (**58**) respectively. The chalcone derivatives (55) was synthesized by the reaction between cis-bicyclo [3.2.0]hept-2-en-6-one (**53**) and p-halobenzaldehyde (**54**) in presence of NaOH at 25 °C. In the next step, the compound (**55**) was treated with 2-amino-thiophenol(**56**) and p-TsOH in ethanol under reflux condition to give the corresponding 2-((1S, 2S)2-((E)-4-florostyryl) cyclopent-3-en-1-yl) benzo [*d*] thiazole (**57**) and 2-((1S, 2S)2-((E)-4- nitrostyryl) cyclopent-3-en-1-yl) benzo [*d*] thiazole (**58**) possess good yield (80–82%) (Figure 10.14).

Kini et al. [27] have discussed the newly synthesized 2-(benzo[*d*]thiazol-2-yl)-*N*, *N*-bis(2-chloroethyl)-5-fluoroaniline (**62**) (Figure 10.15) compound and measured the anticancer activity against Human Cervical Cancer cell lines.

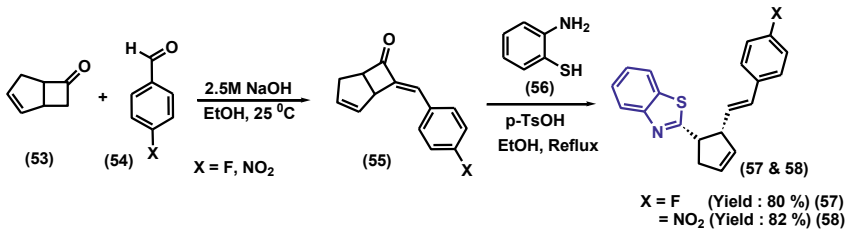


Figure 10.14: Preparation of (E)-NO_2/F – styryl based cyclopenten benzothiazole derivatives.

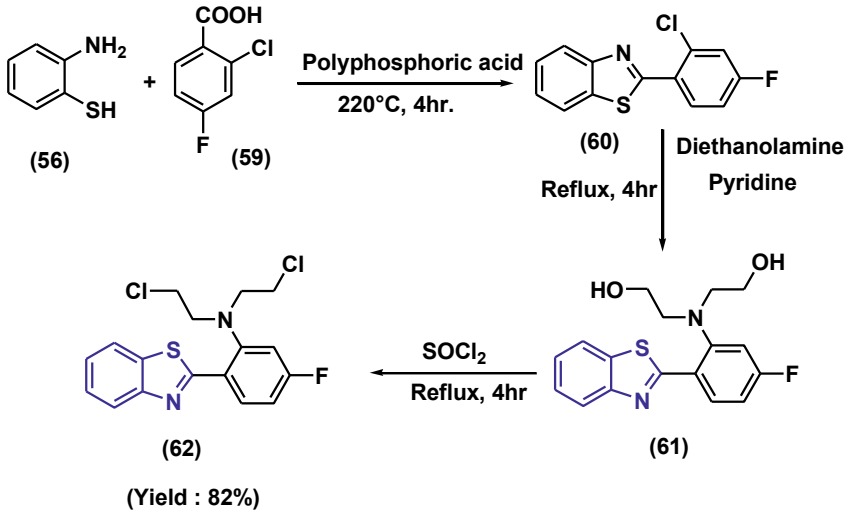


Figure 10.15: Synthesis of 2-(benzo[*d*]thiazol-2-yl)-*N*, *N*-bis (2-chloroethyl) – 5- fluoroaniline.

Kini and Swain have disclosed a proficient and suitable technique for the synthesis of 2-[2-(*N*, *N*-dichloroethylamino)-4-fluorophenyl)- benzothiazole **(SBNCF)**. In the first step, 2-aminothiophenol (**56**) is reacts with 2-chloro-4-fluorobenzoic acid (**59**) and polyphosphoric acid under reflux condition at 220 °C temperature to obtain 2-(2-chloro-4-fluorophenyl)benzo[d]thiazole (**60**). The compound (**60**) reacts with diethanolamine in presence of pyridine and refluxed for 4hr to get 2-[2-(N,N-dihydroxyethylamino)-4-fluorophenyl) benzothiazole (**61**). In the final step, the desired product (**62**) was obtained by the reaction between the compounds (**61**) with $SOCl_2$ under reflux condition in good yield (82%) (Figure 10.15).

10.2 Drugs development

10.2.1 Analysis of structure-activity relationship (SAR) regarding anti-cancer activity

SAR study has disclosed that benzothiazole nuclei as being important for potent activity and show that changes of the substituent group at C-2 position gives an outcome in the modify of its biological activity. Along with this substitution, halogen-substituted compounds offer a significant attention owing to their strong bioactivities (Figure 10.16).

The following observations guided by SAR studies:

- Anticancer potentiality has been detected to be influenced by substitution in the phenyl ring with an alkyl group or a halogen atom, with improved strength in the breast cancer panel and thus elongated the *in vitro* spectrum of potency to include particular lung, colon, renal, and human ovarian cell lines [28].
- The appearance of lipophillic moieties such as hydroxyl, amino, and chloro group of the molecule are important for the cytotoxic potential of benzothiazole compounds (**63**) against cell lines of cancer [29] (Figure 10.17).

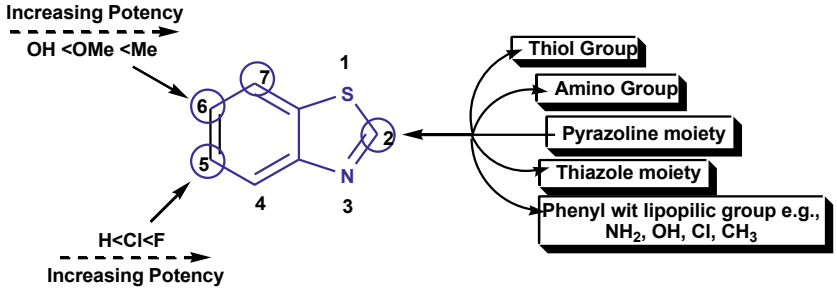
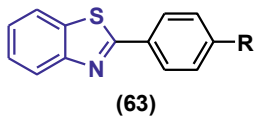


Figure 10.16: Structure-activity relationship (SAR) of benzothiazole moiety.

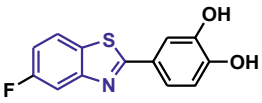


(63)

R = -NH_2 , -OH, -Cl

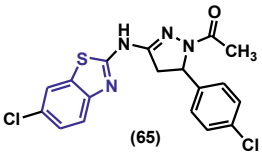
Figure 10.17: Chemical structure of benzothiazole derivatives.

- Taking away of fluro(-F) group or its substitution with several halogens, some modification of dihydroxyphenyl group (**64**) have a rising dyschemotherapeuitic effect as to *in vitro* inhibitory activity of cancer cell growth [30] (Figure 10.18).
- Taking away of fluro(-F) group or its substitution with several halogens, some modification of dihydroxyphenyl group (**64**) have a rising dyschemotherapeuitic effect as to *in vitro* inhibitory activity of cancer cell growth [30] (Figure 10.18).
- Chloro (-Cl) substituted amino benzothiazoles (**65**) has been detected to have an important susceptibility to cancer cell compared to fluro(-F) substituted benzothiazole derivatives (Figure 10.19) [31].
- Anticancer inhibition depend on the types of the substitution on the side chains (**region 2**) and chloro methyl show a vital function in this class of benzothiazole-2-thiol derivatives (**66**) (Figure 10.20) [32].



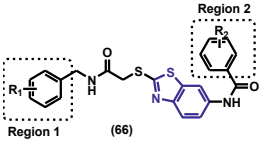
(64)

Figure 10.18: Dyschemotherapeuitic effect of 2-(3′, 4′dihydroxyphenyl)-5-fluro-benzothiazoles.



(65)

Figure 10.19: Anticancer activity of chloro substituted amino benzothiazoles derivatives.



(66)

Figure 10.20: Anticancer inhibition activity of benzothiazole-2-thiol derivatives.

10.2.2 Anticancer drug development processes

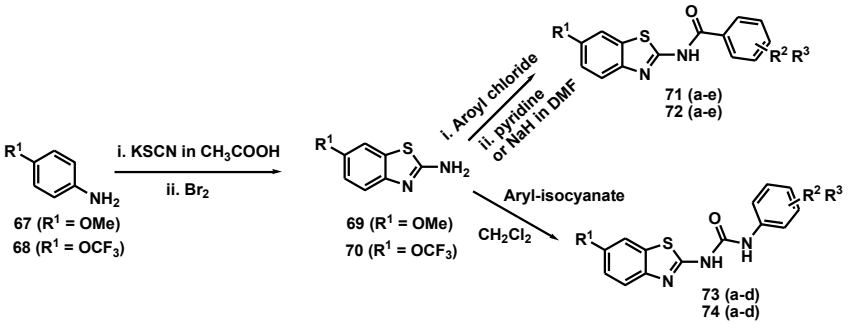
The present account focuses on the synthesis of benzothiazole scaffold in a one-pot or multistep process and used in Anticancer Drug Development.

10.2.2.1 Synthesis of urea-linked benzothiazole compounds

Caputo et al. [33] has described a one-pot, two-step synthesis towards biologically interesting benzothiazole derivatives. The reaction involves commercially available p-methoxy- or p-trifluoromethoxy aniline (**67** or **68**) with KSCN and Br_2 in presence of CH_3COOH to form 2-amino-6-substituted benzothiazole scaffold (**69** or **70**). The amide-linked compounds **71 (a–e)** and **72 (a–e)** were gained with 34–70% yield by reaction of the 2-amino-6-substituted benzothiazole scaffold (**71** or **72**) with different aroyl chlorides in basic medium. The urea-linked compounds **73(a–d)** alongwith **74 (a–d)** were found 35–70% yield with conjugating (**69** or **70**) and different aryl-isocyanates in dry CH_2Cl_2 at 25 °C temperature (Figure 10.21).

10.2.2.2 Preparation of (E)-2-benzothiazole hydrazones

A facile as well as efficient method to synthesize differently substituted (E)-2-Benzothiazole hydrazones (**77**) from reaction between an appropriate benzaldehyde



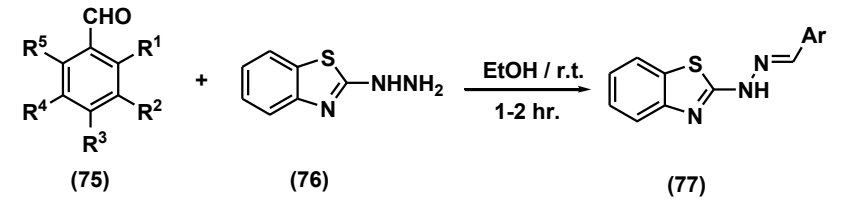
Comp.	R^1	R^2	R^3	Yield	Comp.	R^1	R^2	R^3	Yield
71a, 73a	-OMe	4-F	H	**(34%)/ (60%)**	**72a, 74a**	$-OCF_3$	4-F	H	**(35%)/ (91%)**
71b, 73b	-OMe	4-OMe	H	**(46%)/ (73%)**	**72b, 74b**	$-OCF_3$	4-OMe	H	**(51%)/ (84%)**
71c, 73c	-OMe	4-CN	H	**(70%)/ (85%)**	**72c, 74c**	$-OCF_3$	4-CN	H	**(58%)/ (93%)**
71d, 73d	-OMe	2 - F	6-F	**(43%)/ (78%)**	**72d, 74d**	$-OCF_3$	2 - F	6-F	**(43%)/ (81%)**
71e	-OMe	4 - NHCOMe	H	**(37%)**	**72d**	$-OCF_3$	4 - NHCOMe	H	**(41%)**

Figure 10.21: One-pot, two step synthesis of benzothiazole derivatives.

(**75**) with 2-hydrazinyl-1,3-benzothiazole (**76**) in ethanol at 25 °C temperature in good yields (73–78)% has been described by Lindgren et al. [34]. This reaction was carried out by applying only ethanol as a solvent (Figure 10.22). The Benzothiazole hydrazones have been selected for their *in vitro* antiproliferative potential in human cancer cell lines such as HL-60 (leukemia), HCT-8 (colon), and MDAMB-435 (breast).

10.2.2.3 Preparation of *s*-triazine-bearing benzothiazole compounds

Kumar et al. [35] has reported an efficient methodology for the preparation of new *s*-triazine-teeming benzothiazole compounds as an anticancer agents. The substitution reaction by nucleophiles was performed on mono-substituted triazine compounds (**78 & 79**) with employing benzothiazole derivatives (**80**) in the appearence of K_2CO_3 at 70–80 °C temperature under THF solvent medium to get the products (**81**) & (**82**) in excellent yields (85–91%) (Figure 10.23). These *s*-triazine-bearing benzothiazole derivatives have been evaluated for their *in vitro* inhibitory potential against the outgrowth of six type's cancer cell lines, *viz;* DU-145, MDAMB-231, PC-3, MCF-7, HGC-27, and HT-29.



Ar = 2-OH, 5-$NO_2C_6H_3$, 3-OMe, 4-OHC_6H_3, 3,4-$(OH)_2C_6H_3$, 4-ClC_6H_4, 4-$N(Me)_2C_6H_4$, 2-$COOHC_6H_4$, 2,4-$(Cl)_2C_6H_3$, 2,4-$(F)_2C_6H_3$, 2,4-$(Me)_2C_6H_3$, 2,4-$(OMe)_2C_6H_3$, 4-BrC_6H_4, 2,3-$(OH)_2C_6H_3$etc.

Figure 10.22: Synthetic method for formation of the (E)-2-benzothiazole hydrazones.

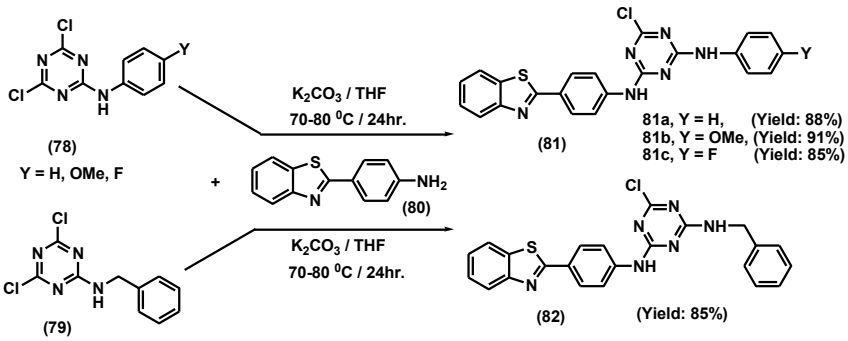


Figure 10.23: Synthesis of s-triazine containing benzothiazole derivatives.

10.2.2.4 Synthesis of ester bearing benzothiazole compounds

Bao-An and his co-workers [36] have developed a three step synthesis of 2,3,4-trimethoxyacetophenoxime ester based benzothiazole derivatives. It is accomplished from Et_3N as a base-catalyzed reaction of 2-mercaptobenzothiazole (**83**) and 2-bromo-1-(2,3,4-trimethoxyphenyl) ethanone (**84**) at 25 °C for 8 h to afford 2-(benzothiazol-2-ylthio)-1-(2,3,4-trimethoxyphenyl)ethanone (**85**). The oximation of compound (**85**) with hydroxylamine was performed in CH_3OH catalyzed by NaOAc to produce 2-(Benzothiazol-2-ylthio)-1-(2,3,4-trimethoxyphenyl)-ethanoxime (**86**).

The above reaction was completed into 8 h under refluxing temperature to yield (80–90)%. Finally, the esterification of compound (**86**) with acyl chloride (**87**) in $CHCl_3$ in presence of a pyridine base produced 2-(Benzothiazol-2-ylthio)-1-(2,3,4-trimethoxyphenyl)ethanoxime ester (**88**) in moderate yield (32–61%) (Figure 10.24).

10.2.2.5 Synthesis of benzothiazole-pyrazole hybrids

A synthetic route to novel anticancer agents benzothiazole-pyrazole hybrids (**91** and **92**) has been described by Belal et al. [37]. The reaction was performed on benzothiazole derivatives (**80**) in the presence of $HCl/NaNO_2$, ethyl cyanoacetate, CH_3COONa, and aqueous ethanol at room temperature to get the compound [(4-Benzothiazol-2-yl-phenyl)-hydrazono]-cyano-acetic acid ethyl ester (**89**) in good yields (90%). The synthesized compound (**89**) also reacts with hydrazine hydrate ($NH_2NH_2.H_2O$) in ethanol to give the starting materials 5- amino-4-[(-benzothiazol-2-yl-phenyl)-hydrazono]-2,4-dihydro-pyrazol-3-one (**90**). For substituting the C3 atom to get pyrazolinone, the compounds (**90**) have been subjected to further reactions with (i) acid chloride in solvent dioxane at rt. for 24 h to give *N*-{4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl) acetamide in good yields (83–87%) (**91**) and (ii)

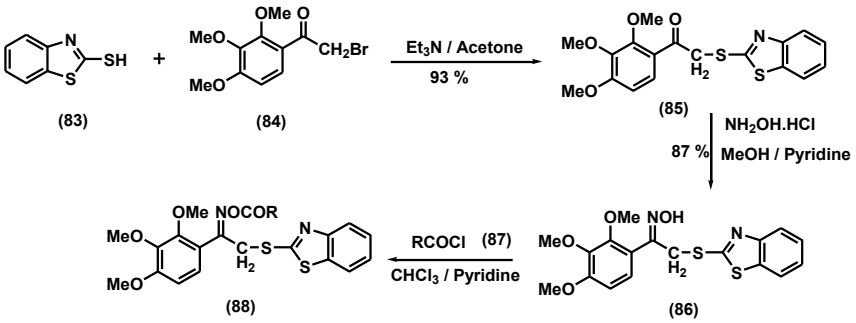


Figure 10.24: Synthesis of 2,3,4-trimethoxyacetophenoxime ester contain-ing benzothiazole.

aldehyde/glacial acetic (cat.) in absolute ethanol under reflux condition for 20 h to give 4-[(4-Benzothiazol-ylphenyl)hyrazono]-5-[(4-chlorobenzylidene) amino]-2,4-dihydro pyrazol-3-one (**92**) in good yields (78–82%) (Figure 10.25).

10.2.2.6 Synthesis of benzothiazole thiourea compounds

Eshkil et al. [38] has proposed a newly synthesized benzothiazole thiourea derivatives (**94**) by the reaction of 2-amino benzothiazole derivatives (**18**) with CS_2 in alkali medium to give an intermediate, which are methylated with dimethyl sulfate in very good yields (73–85%). In the presence of NH_3, compounds (**93**) undergoes ammnolysis reaction in ethanol under reflux conditions to produce substituted benzothiazole thiourea (**94**) in good yields (60–68%) is shown (Figure 10.26).

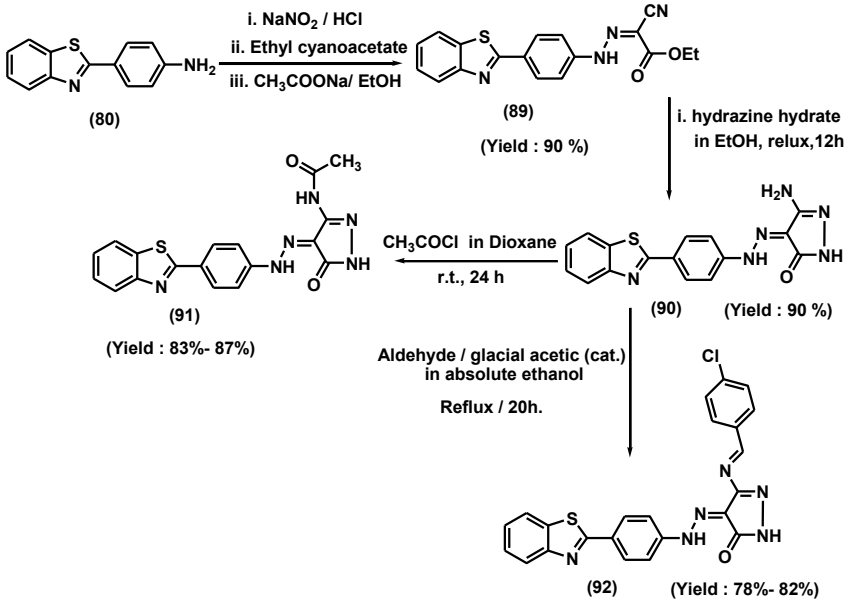
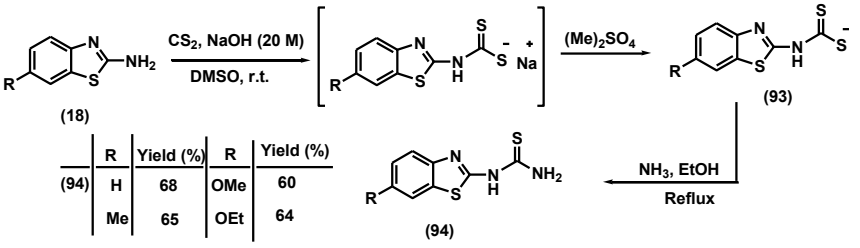


Figure 10.25: Synthesis of benzothizole–pyrazole hybrids.



(94)	R	Yield (%)	R	Yield (%)
	H	68	OMe	60
	Me	65	OEt	64

Figure 10.26: Synthesis of the benzothiazole thiourea derivatives.

10.2.2.7 Preparation of 5-arylidene-3- (benzothiazol-2-ylamino)–2-thioxo 4-thiazolidinones

Havrylyuk and co-workers [39] have disclosed the synthesis of 3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidone (**96**) from (benzothiazole-2-yl)hydrazine (**76**) and trithiocarbonyl diglycolic acid (**95**) under refluxing EtOH in good yield 78%. The freshly prepared compound (**96**) undergoes smooth reaction with aromatic aldehydes (**97**) to give 5-arylidene a derivative (**98**). Finally, the *N*-acetylation was performed with following addition of acetic anhydride to the reactive mixture to produce the target compounds (**99**) (Figure 10.27).

10.2.2.8 Synthesis of aminopyrazole-benzothiazole compounds

Hassan and Hussein group [40] has disclosed that the synthesis of novel benzothiazole analogs as shown (Figure 10.28). In the first step, 2-(Benzo[d]thiazol-2-yl)acetonitrile compound (**100**) are treated with carbon disulfide/dimethyl sulfate, phenyl isothiocyanate/ethyl iodide, or several 4-bromo benzaldehyde derivatives to give the desired acrylonitrile compounds (**101**), (**102**) and (**103**) respectively. Further, they were undergoes hydrazinolysis through hydrazine hydrate ($NH_2NH_2.H_2O$) 99% in EtOH solvent to produced amino-pyrazole compounds (**104**), (**105**), and (**106**) respectively.

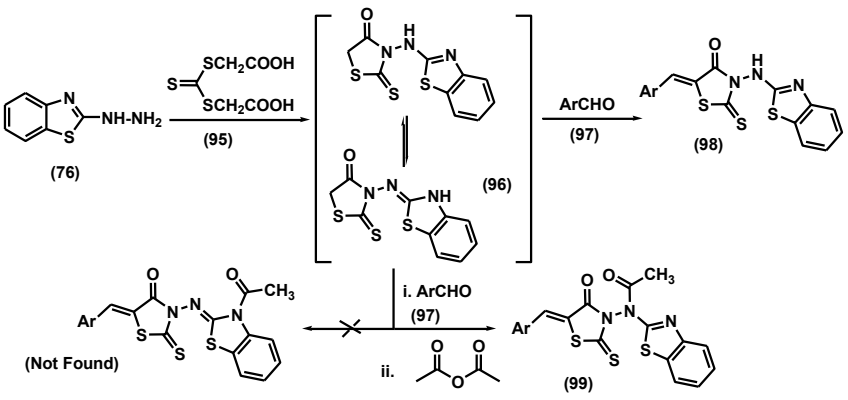


Figure 10.27: Preparation of 5-arylidene-3- (benzothiazol-2-ylamino)–2-thioxo 4-thiazolidinones and *N*-(benzothiazol-2-yl)-*N*-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-acetamides.

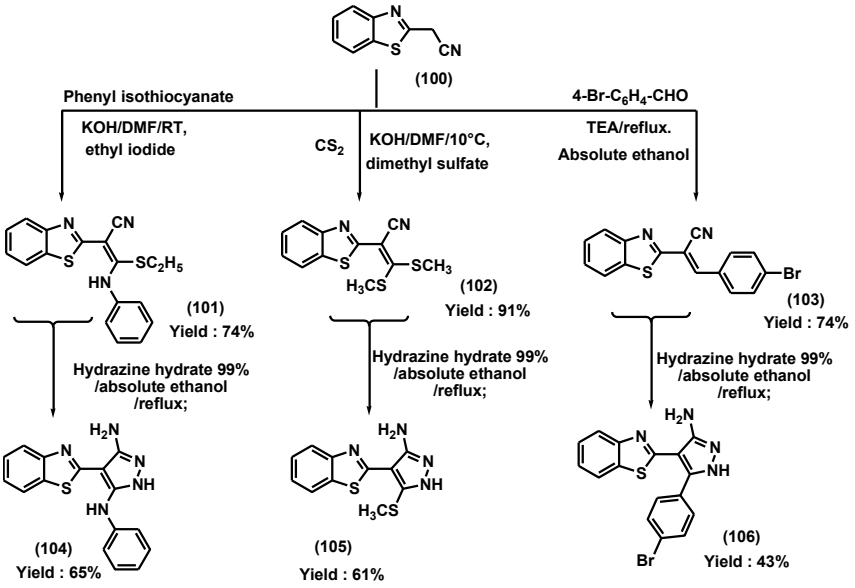


Figure 10.28: Synthesis of the novel benzothiazole analogues.

Benzothiazoles demonstrated the exciting pharmacological activities and widely studied for their anticancer potential [41]. 2-(4-aminophenyl)benzothiazoles [42] and their *N*-acetylated derivatives (**88** and **107**) [43] have shown amazingly notable *in vitro* anticancer potential against cell lines of cancer particularly ovarian, colon, and breast cell lines etc.

Whereas, 2-(3,4-dimethoxyphenyl) group and a fluoro substituent in the benzothiazole moiety, particularly at the 5-position showed potent anticancer potential (**108**). Newly synthesized 7-methoxy-8-{4-[4-(1,3-benzothiazol-2-yl)phenoxy]-butyl} oxy-(11aS)1,2,3,-11a-tetra-hydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one (**109**) have showed incredibly anticancer activity, molecular modelling, and DNA-binding ability etc. (Figure 10.29) [44].

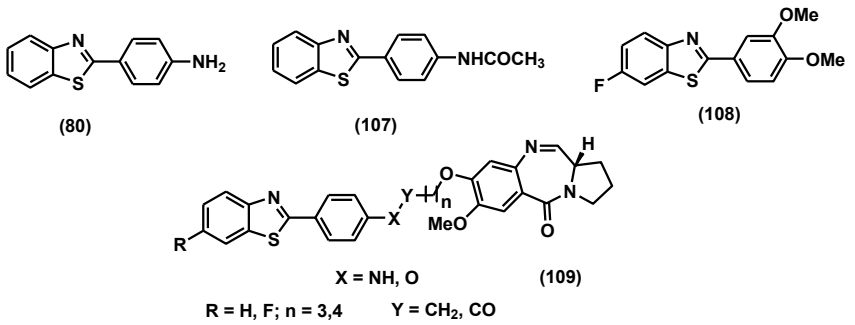


Figure 10.29: Structures of anticancer active molecules.

10.2.2.9 Synthesis of benzothiazole-pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates

A synthetic route to novel fused benzothiazole-pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates (**92**) has described by Kamal et al. [44a] as shown (Figure 10.30). In the first part, 2-(4-Aminophenyl)benzothiazoles derivatives (**88**) was treated with bromopentanoyl chloride (**110**) in presence of Et_3N in THF at room temperature resulting to produce *N*1-[4-(6-halo-1,3-benzothiazol-2-yl)phenyl]-5-bromopentanamide (**111**) in good yield. In the second part, 2-nitrobenzoic acid (**112**) reacts with L-proline methyl ester hydrochloride (**113**) in appearance of thionyl chloride and Et_3N to give the nitro ester derivative (**114**). The ester groups of compound (**114**) were reduced by reducing agent DIBAL-H to generate the corresponding aldehyde compound (**115**) and protected by EtSH/TMSCl to afford (**116**). The compound (**116**) undergoes debenzylation to give (2S)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxal-dehyde diethylthioacetal (**117**).

This nitrothioacetal intermediates (**117**) take part on reduction reaction in refluxing methanol in the presence of $SnCl_2.2H_2O$ to give aminothioacetal derivatives

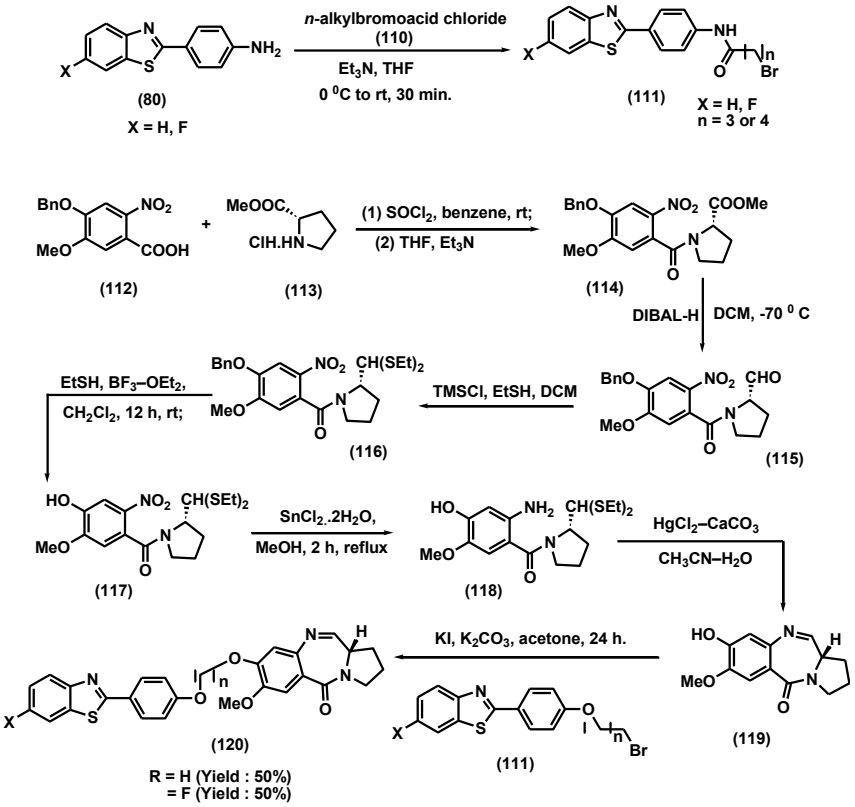


Figure 10.30: Preparation of novel fused benzothiazole-pyrrolo[2,1-*c*][1,4] benzodiazepine conjugates.

(**118**). In the next step, deprotection of thiol by $HgCl_2/CaCO_3$ in H_2O-CH_3CN at room temperature is to give DC-81 (**119**). In addition of 2-[4-(n-alkyloxy)-phenyl) benzothiazoles (**111**) to the intermediate (**119**) by using KI/K_2CO_3 in acetone at rt. to give the desired PBD conjugates 7-methoxy-8-{4-[4-(1,3-benzothiazol-2-yl)phenoxy]-butyl} oxy-(11aS)1,2,3,11a-tetra-hydro-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5-one (**120**).

Docking studies and mechanism of action:

The molecular modification is required for more perfect structure activity relationship (SAR) with increasing their anticancer potential in human tumour cell lines with study of its mechanism of action. The C8-linked benzothiazole–PBD conjugates 7-methoxy-8-{5-[4-(1,3-benzothiazol-2-yl)-2-methoxyphenoxy]pentyl}oxy(11aS)1,2,3, 11a-tetra-hydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepin-5-one (**120**) has shown distincted *in vitro* anticancer potential next to 60 types human tumour cell lines. The GOLD 3.2 program protocol [44b] was designed for the calculations of molecular docking of the cross-linked complex formed among the host DNA duplex and synthesized molecules. Studies of **molecular modelling** propose that the sulfur (S) atom in benzothiazole ring skeleton and raise of the linker chain length to a 5-carbon chain notably enhance the DNA-binding capability of the minor groove binders with the observation of DNA sequence in **GOLD docking**. The distortions of the DNA double helix on the binding site of ligand noticed on molecular dynamics simulation of the respective docked pose enlightening the local displacement the H_2O of the hydration spine and the binding effect of inhibitor on conformation of DNA viewing the uncoiling of the DNA double helix from the sites of flanking the bound inhibitor, in difference to the shrinking tendency displayed by the area of DNA strands bound by the inhibitor may be owing to non-covalent interaction [44a].

10.2.3 Synthesis of 2, 3-disubstituted imidazo-benzothiazoles compounds

In recent times, Singh et al. [45] has established an efficient method for the preparation of a new series of 2, 3-disubstituted imidazo [2,1-*b*] benzothiazole derivatives as shown (Figure 10.31). Singh groups have performed the reaction between 2-(4-Aminophenyl) benzothiazoles derivatives (**18**) and substituted 2-bromo-1-phenylethanone (**121**) in the presence of P_2O_5 under refluxing condition to produce 2-(4′-substituted) phenyl imidazo [2, 1-*b*] benzothiazole (**122**) in good yield. The compounds (**122**) were treated with different types of reagents to build up different substituted benzothiazole derivatives.

In the first case, compounds (**122**) were introduced to Vilsmeir Haack reagent (by adding phosphoryl chloride in dimethylformamide) at 0 °C to rt. with stirring to give 2-(4′-substituted) phenyl imidazo [2, 1-*b*] benzothiazole-3-carbaldehyde (**123**) in good yield (50–68%). In the second case, 3-nitroso-2-(4′-substituted) phenyl imidazo-benzthiazole (**124**) was synthesized by reaction between 5-(4-substituted) phenyl-imidazo benzothiazole (**122**) and sodium nitrite solution in the acetic acid medium under refluxing

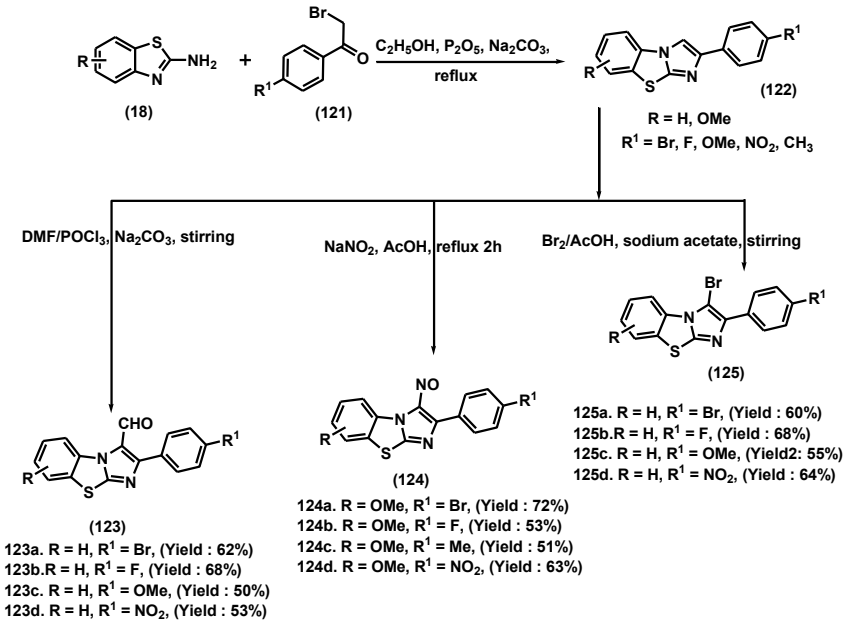


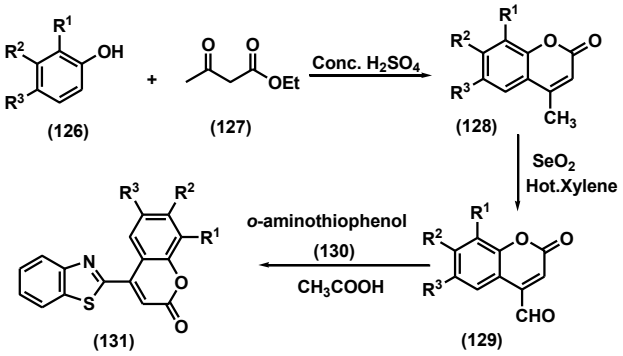
Figure 10.31: Preparation of 2,3-disubstituted imidazo [2, 1-*b*] benzthia-zole derivatives.

conditions for 30 min. In the third case, the synthesized compounds (**122**) readily undergo bromination with Br_2 in CH_3COOH stirring at room temperature to afford 3-bromo-2-(4-sustituted) phenyl imidazo-benzothiazole (**125**) in good yield (55–68%).

10.2.3.1 Synthesis of benzothiazolyl chromenone compounds

Kini et al. [46] has been interested in developing a series of coumarin substituted benzothiazole moiety for the sake of anticancer activities. For this purpose, this chemist group focused on the synthesis of coumarin substituted benzothiazole carrying different functionalities. The preparation of 4-(benzo[*d*]thiazol-2-yl)-7-substituted-2*H*-chromen-2-one (**131**) based on coupling and cyclization reaction as the key step has been achieved using conventional heating methods (Figure 10.32).

In the first step, substituted resorcinol (**126**) with concentrated sulphuric acid, followed by an addition reaction with ethyl acetoacetate (**127**) afforded the corresponding substituted coumarin (**128**) in good yields. In the second step, substituted coumarins (**128**) undergo a formylation reaction in the presence of selenium dioxide in hot xylene as a solvent medium to produced coumarin-4-carboxaldehyde derivatives (**129**).In the final step, the coumarin-4-carboxaldehyde (**129**) is being treated with



131a. R^1 = H, R^2 = OCH_3, R^3 = H , (Yield : 74%)
131b. R^1 = H, R^2 = H, R^3 = OCH_3, (Yield : 72%)
131c. R^1 = OCH_3, R^2 = OCH_3, R^3 = H, (Yield : 66%)
131d. R^1 = OAc, R^2 = OAc, R^3 = H ,(Yield : 62%)
131e. R^1 = H, R^2 = OEt, R^3 = OEt, (Yield 73%:)
131f . R^1 = NO_2, R^2 = OCH_3, R^3 = H, (Yield :68%)
131g. R^1 = H, R^2 = $OCH_2CH{=}CH_2$, R^3 = $OCH_2CH{=}CH_2$, (Yield :63%)

Figure 10.32: Synthesis of 4-(benzo[*d*]thiazol-2-yl)-7-substituted-2*H*-chromen-2-one.

o-aminothiophenol (**130**) under the refluxing condition in acetic acid for 5 h to give 4-(benzo[*d*]thiazol-2-yl)-7-substituted-2*H*-chromen-2-one (**131**) good yield (56–75%).

Docking studies and mechanism of action:

The molecular modification is required for more exact structure activity relationship with its anticancer potential on cancer cell lines of breast or various tyrosine kinase domain structure could be chosen from PDB to study its mechanism of action. The activation of receptor tyrosine kinase (RTK) signalling pathways is effectively linked with carcinogenesis. On this regard, researchers preferred RTK for docking study of newly synthesized compounds (**131**). The docking simulation was complete by GRIP batch docking method [46]. All the conformers of the compounds (**131**) were docked at the receptor cavity. The essential parameters were stilled for docking simulation like as exhaustive method, rotation angle: 30°, number of placements: 30, scoring function: dock score. The ligands having minimum dock score, which making most stable drug receptor complex. Then docking simulation, most excellent docked conformer of all receptor and ligand were merged. Their complexes were energetically optimized through defining the radius of (10 Å) calculated from the docked ligand. The optimized complexes were after that checked for several ligand interaction with receptor like hydrophobic bonding, hydrogen bonding, and vander Waal's interaction.

Selectively tyrosine kinase domain was the main objects for anticancer activity in docking studies and identify the compound (**131g**) have maximum negative dock score (**–64.67**). This negative dock score means that the compound can perfectly packed in the receptor cavity making most stable drug receptor complex.

10.2.3.2 Synthesis of benzothiazolyl arylpropenone compounds

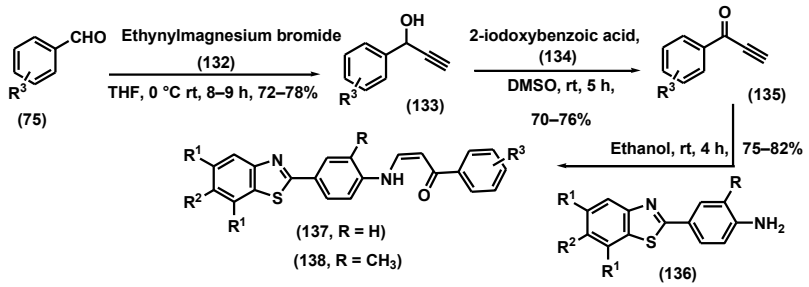
Subba Rao and Kamal research group [47] have been disclosed the preparation of desired compounds (Z)-3-((4-(benzo[*d*]thiazol-2-yl)aryl)amino)-1- arylprop-2-en-1-ones (**137**) and (**138**) are illustrated in (Figure 10.33). Substituted benzaldehydes (**75**) are reacts with ethynylmagnesium bromide (**132**) in THF at 0 °C temperature to afford 1-aryl-2-propyn-1-ol (**133**). In the second step, 2-iodoxybenzoic acid (IBX) (**134**) used as an oxidizing agent to oxidized the compound (**133**) in dimethyl sulfoxide (DMSO) solvent medium to give 1-aryl-2- propyn-1-one (**135**). Finally, the compound (**135**) take part in condensation reaction with aryl amines (**136**) in EtOH under room temperature to produced (Z)-3-((4-(benzo[*d*]thiazol-2-yl)aryl)amino)-1-arylprop-2-en-1-ones (**137**) and (**138**) in high yield.

10.2.3.3 Synthesis of benzothiazole-chromene compounds

A new series of 2-amino-3-benzothiazole-4-heterocycle-4*H*-chromene derivatives (**141**) are three component synthesized successfully by using a heterocyclic carbon nucleophile (**139**), substituted salicylaldehyde (**140**), and benzothiazole acetonitrile (**100**) promoted by DBU catalyst at room temperature have been described by Pazhanivel et al. [48]. (Figure 10.34) to give the required product (**141**) with 95% yield.

10.2.3.4 Preparation of 2-anilinopyridyl linked benzothiazole hydrazones

Sultana, et al. [49] has performed the synthesis of the 2-anilinopyridyl based benzothiazole hydrazone conjugates (**148**) in a sequential manner as shown (Figure 10.35). In



137a. R = H, R^1 = H, R^2 = OCH_3, R^3 = 3-OH,4-OMe, (Yield : 85%)

137b. R = H, R^1 = OCH_3, R^2 = H, R^3 = 3-OH,4-OMe (Yield : 86%)

137c. R = H, R^1 = OCH_3, R^2 = OMe, R^3 = 3-OH,4-OMe (Yield : 87%)

137d. R = H, R^1 = H, R^2 = OMe, R^3 = 3-F,4-OMe (Yield : 88 %)

138a. R = Me, R^1 = H, R^2 = OMe, R^3 = 3-OH,4-OMe (Yield: 83%)

138b . R = Me, R^1 = OMe, R^2 = H, R^3 = 3-OH,4-OMe (Yield : 85%)

138c. R = Me, R^1 = OMe, R^2 = OMe, R^3 = 3-OH,4-OMe (Yield : 83%)

Figure 10.33: Preparation of (Z)-3-((4-(benzo[*d*]thiazol-2-yl)aryl)-amino)-1-arylprop-2-en-1-ones.

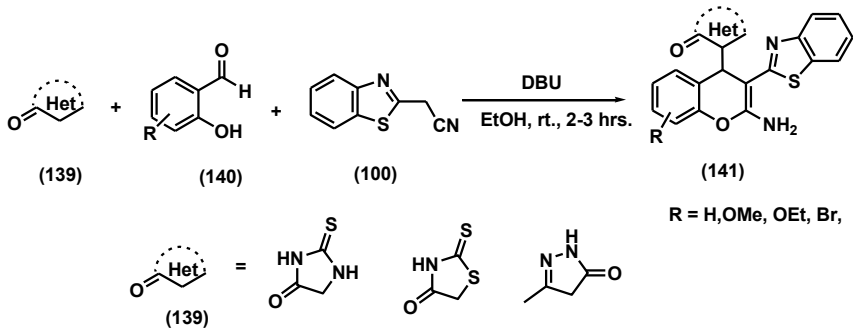
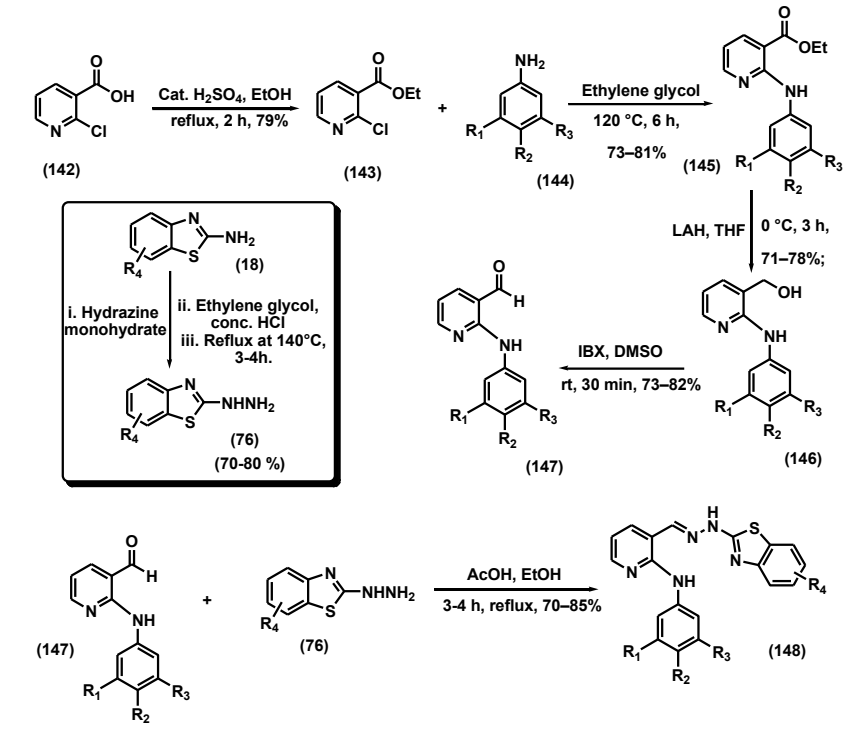


Figure 10.34: Synthesis of 2-amino-3-benzothiazole-4-heterocycle-4*H*-chromene derivatives.



148a. R^1 = H, R^2 = Cl, R^3 = H, R^2 = OCH_3, (Yield : 82%)

148b. R^1 = H, R^2 = CF_3, R^3 = H, R^2 = OCH_3, (Yield : 85%)

148c. R^1 = H, R^2 = F, R^3 = H, R^2 = OCH_3, (Yield :85%)

148d. R^1 = H, R^2 = H, R^3 = H, R^2 = OCH_3, (Yield : 92%)

148e. R^1 = H, R^2 = Cl, R^3 = H, R^2 = OC_2H_5, (Yield 85%:)

148f . R^1 = H, R^2 = CF_3, R^3 = H, R^2 = OC_2H_5, (Yield : 85%)

148g. R^1 = H, R^2 = F, R^3 = H, R^2 = OC_2H_5, (Yield : 87%)

Figure 10.35: Preparation of 2-anilinopyridyl based benzothiazole hydrazone conjugates.

the beginning step, 2-chloronicotinic acid (**142**) are being used as starting materials and transformed to ethyl-2-chloronicotinate (**143**) in presence of catalytic amount of conc. H_2SO_4 under refluxing in ethanol. In the next step, substituted anilines (**144**) heated with ester (**143**) in ethylene glycol for 6 h at 140 °C temperature to afford ethyl 2-(phenylamino)nicotinate (**145**).

The reducing agent LAH in dry THF are being used for reduction of ethyl 2-(phenylamino)nicotinate (**145**) to provide the compounds (**146**), which are selectively oxidized by using 2-iodoxy benzoic acid in DMSO to give 2-(phenylamino) nicotinaldehydes (**147**).

Conversely, synthesis of 2-hydrazinyl benzothiazoles (**76**) by the concentrated HCl catalyzed reaction between substituted 2-amino benzothiazoles (**18**) and hydrazine hydrate in ethylene glycol. To end by formation the desired compounds 2-anilinopyridyl based benzothiazole hydrazone conjugates (**148**) was achieved by condensation reaction between 2-(phenylamino)nicotinaldehydes (**147**) and 2-hydrazinyl benzothiazoles (**76**) under catalytic amount of glacial acetic acid in EtOH at room temperature in 82–92% yield.

10.2.3.5 Synthesis of benzothiazole-piperazine-1,2,3-triazoles

A new method allowing the preparation of novel benzothiazole-piperazine-1,2,3-triazole compounds has been established by Aouad et al. [50]. In this synthesis, base promoted acylation of 2-(piperazin-1-yl)benzo[*d*]thiazole (**149**) with bromo acetyl bromide (**150**) under triethylamine in dichloromethane to give the intermediate bromoacetyl piperazine (**151**). This intermediate take part in the reaction with NaN_3 in a mixture of water-acetone (1:4), furnished the desired azide compounds (**152**). Finally, using the $CuSO_4$ and sodium ascorbate as catalytic media and the mixed solvent DMSO/H_2O (1:1), 1,3-dipolar cycloaddition reaction was performed between the synthesized azide (**152**) and functionalized alkynes (**153**) at 80 °C to give the benzothiazole-piperazine-1,2,3-triazole compounds (**154**) in (80–90%) yield carried out several hydroxylated or ester based alkyl side chains as shown (Figure 10.36).

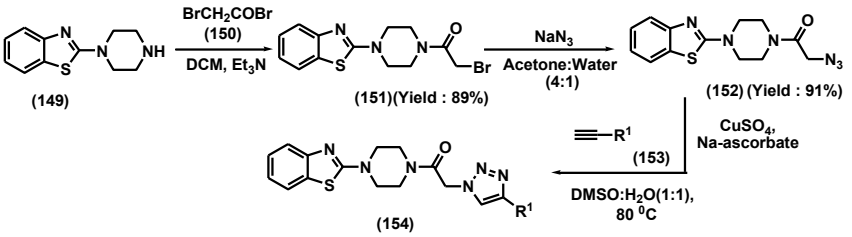


Figure 10.36: Synthesis of benzothiazole-piperazine-1,2,3-triazole compounds.

10.2.3.6 Synthesis of UK-1 analogues with benzothiazole

UK-1 shows the potent anticancer potential against epithelial carcinoma and human lung cell lines. UK-1 analogs based benzothiazole moiety has been synthesized by Huang et al. [51] and schematic described (Figure 10.37). In the beginning, compound (**155**) was introduce with *N*,*N*-dimethylformamide and oxalyl chloride to give the analogous acyl chloride, which was readily reacts with methyl 2-aminobenzoate (**156**) to give the amide (**157**) in 81% yield. The newly prepared amide (**157**) was transformed into thioamide (**158**) in 51% yields by the reaction with Lawesson's reagent. In the next step, thioamide (**158**) was readily cyclized to form benzothiazoles (**159**) followed by Jacobson synthesis using alkaline potassium ferricyanide. Compound (**160**) was synthesized by the debenzylation of newly prepared compound (**159**) using boron trifluoride diethyletherate and ethanethiol. The synthesized compound (**159**) underwent saponification step to give the corresponding acid (**161**) which was further reaction by oxalyl chloride in DMF to generate the analogous acyl chloride. Finally, the synthesized acyl chloride derivatives were coupled with methyl 2-aminobenzoate (**156**) to give the amide methyl 2-(2-(2-(benzyloxy)phenyl)benzo[d]thiazole-4-carboxamido)benzoate (**162**) in 54% yield.

10.2.3.7 Preparation of 2-(4-aminophenyl)benzothiazoles

Hutchinson et al. [52] has reported that the benzanilides (**163**) transformed to their thiobenzanilides (**164**) using Lawesson's reagent in HMPA. The thiobenzanilide

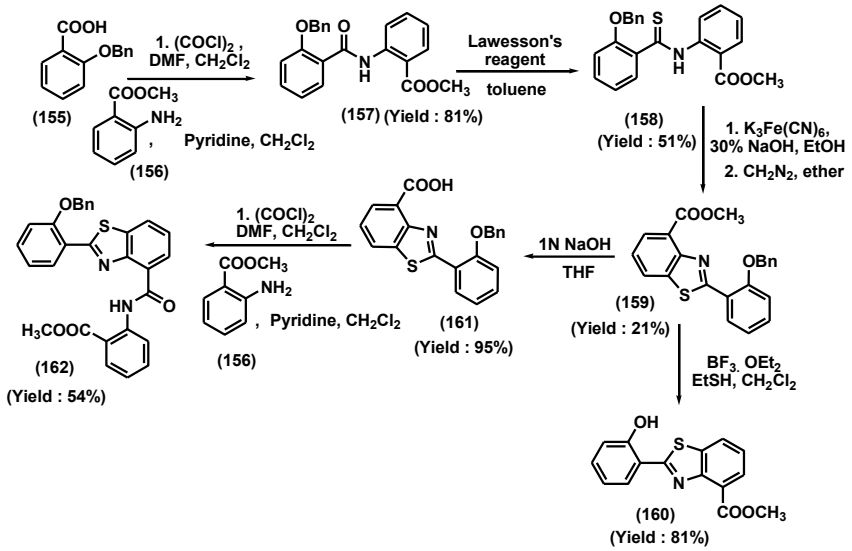


Figure 10.37: Preparation of UK-1 analogues based benzothiazole moiety.

derivatives (**164**) were readily undergoes to cyclization followed by the Jacobsen synthesis to provide the fluoro substituted nitrophenyl benzothiazoles (**165**) using alkaline potassium ferricyanide at 90 °C temperature. Finally, tin (II) chloride dihydrate reduced the nitrophenyl benzothiazoles (**165**) to give the corresponding arylamines (**166**) under refluxing in ethanol (Figure 10.38).

10.2.3.8 Synthesis of benzothiazole-thiazolidine compounds

Osmaniye et al. [53] has reported the multistep synthesis of 2-(benzothiazol-2-ylthio)-*N*-(3-substituted-4-(3,4-substitutedphenyl)thiazol -2(3*H*)-ylidene) acetohydrazide (**173**) as an anticancer agent (Figure 10.39). In the first step, ethyl 2-(benzothiazol-2-ylthio) acetate (**168**) have prepared through setup the reaction between 2-mercaptobenzothiazole (**83**) and ethyl 2-chloroacetate (**167**) in appearance of mild base K_2CO_3.

The compound ethyl-2-(benzothiazol-2-ylthio)acetate (**168**) readily take part the reaction with extra hydrazine hydrate to provided 2-(benzothiazol- 2-ylthio)acetohydrazide (**169**). Thiosemicarbazide derivatives (**171**) was isolated through the reaction of 2-(benzothiazol-2-ylthio)acetohydrazide (**169**) with phenyl isothiocyanate or cyclohexyl isothiocyanate (**170**). Finally, the desired compounds (**173**) was synthesized by using of thiosemicarbazide derivatives (**171**) and 2-bromoacetophenone (**172**) *via* ring closure reaction under reflux in ethanol at 150 °C.

10.2.3.9 Synthesis of chrysin–benzothiazole conjugates

A synthetic route to novel Chrysin–benzothiazole conjugates (**177**) has described by Mistry and Kim groups [54]. This synthetic process are proceed through the reaction of

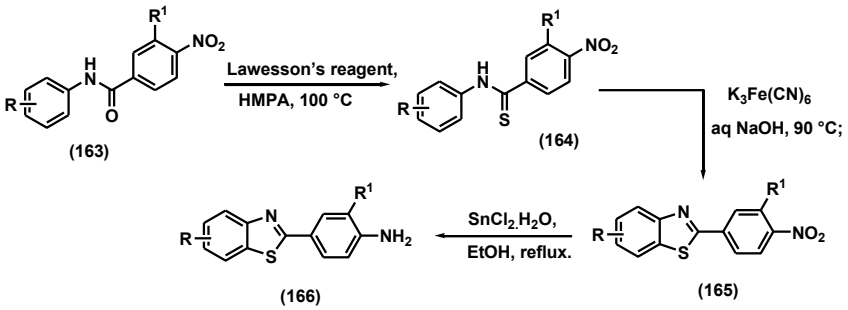


Figure 10.38: Preparation of fluorinated 2-(4-aminophenyl)benzothiazoles.

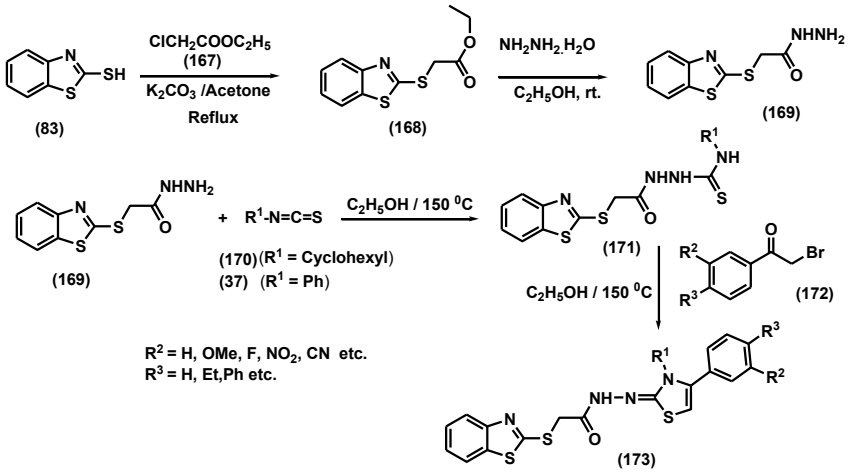


Figure 10.39: Synthesis of novel benzothiazole-thiazolidine derivatives.

chrysin (**174**) and 1,4-dibromobutane (**175**) under inert atmospheric condition using a mild base K_2CO_3 to produced an intermediate 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**176**) in 83% yield. After that, benzothiazoles (**18**) were coupled with compound (**176**) under refluxing in acetonitrile to give desired products (177) in 50–67% yield (Figure 10.40).

10.2.4 Synthesis of pyrazolo-benzothiazole hybrids

Reddy and Kamal group [55] has discloesd a remarkable approach to synthesis the *N*-(benzo[*d*]thiazol-2-yl)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide hybrids (**181**) as

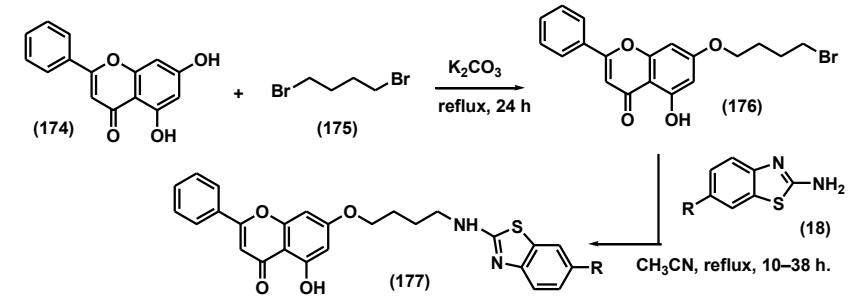


Figure 10.40: Synthesis of chrysin–benzothiazole conjugates.

shown (Figure 10.41). The precursors of pyrazole carboxylic acid (**180**) were synthesized by performed the reaction between phenylhydrazine (**178**) and (un) substituted acetophenones (**179**) followed by cyclocondensation as well as oxidation reaction. The compound (**180**) were readily reacts with 2-(4-Aminophenyl)benzothiazoles derivatives (**18**) under an appropriate catalytic combination such as *N*,*N*-diisopropylethylamine (DIPEA), *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI), along with 1-hydroxybenzotriazole (HOBt) in dry DMF solvent to construct the essential pyrazolo benzothiazole derivatives (**181**) in (75–90%) yields.

Docking studies and mechanism of action:

Docking studies give important structural information regarding drug-protein interactions which take part in a vital role in the development of drugs. Molecular docking tools were used for forecast the orientation of the recently designed hybrids of pyrazolo-benzothiazole (**181**) in the constraints of protein binding pockets. The docking studies was observed that the most potent pyrazolo-benzothiazole hybrids (**181i** and **181j**) perfectly packed in AXI binding pocket of VEGFR-2 (PDB ID – 4AGC) with comparison of the different binding pose of axitinib [55]. The carboxamide portion of axitinib nestles into the hydrophobic pocket even as the pyridin-2-yl terminal

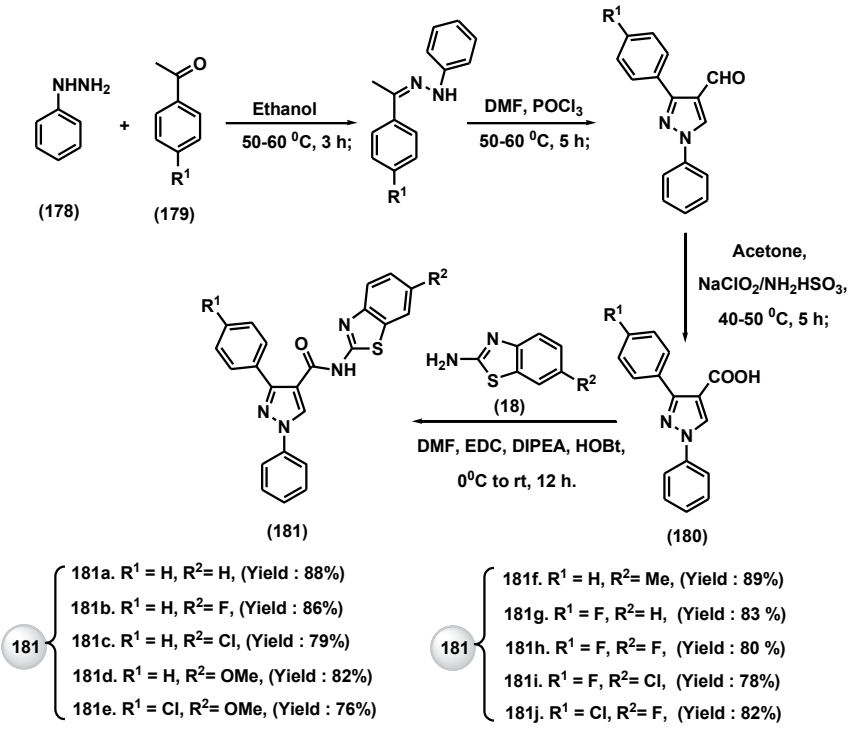


Figure 10.41: Synthesis of pyrazolo-benzothiazole hybrids.

protrudes. Conversely, in hybrids (**181i**) and (**181j**) jointly the pyrazole ring as well as aryl-substituent in the third position optimally occupy the hydrophobic core and demonstrate alike interactions with the protein molecules (ref. [55]). Studies of mechanism of action give an idea about the interaction between the both hybrids and active parts of the protein. The carbonyl oxygen part of both hybrids makes hydrogen bonding along the amide NeH side chain of Asn923 and peptide linkage. Additionally, the side chain NeH further interacts with N-atom of benzothiazole ring. The amide NeH part of both hybrids (**181i**) and (**181j**) was demonstrating an interaction with the carbonyl oxygen of Leu840 along with hydrogen bond lengths. The substituted benzo [d]thiazol-2-amine skeleton in hybrids (**181i**) and (**181j**) was shown π-interactions with Phe1047 and hydrophobic interactions with Arg1032, Asp1056, and Gly841 residues. Another substituted pyrazole ring moiety in hybrids (**181i**) and (**181j**) shows interactions with Val848, Leu840, Ala866, Glu850, Lys838, Lys920 Phe918, Phe921, Gly922, Leu1035, Asn923 and Cys919 amino acid residues.

10.2.4.1 Synthesis of bis(benzo[d]thiazolyl)quinoxaline analogues

Babu et al. [56] has reported an efficient methodology for the preparation of new bis(benzo[*d*]thiazolyl)quinoxaline (**186**) as cancer theranostic agents as summarized (Figure 10.42). Initially, benzothiazolylphenol derivatives (**182**) were afforded

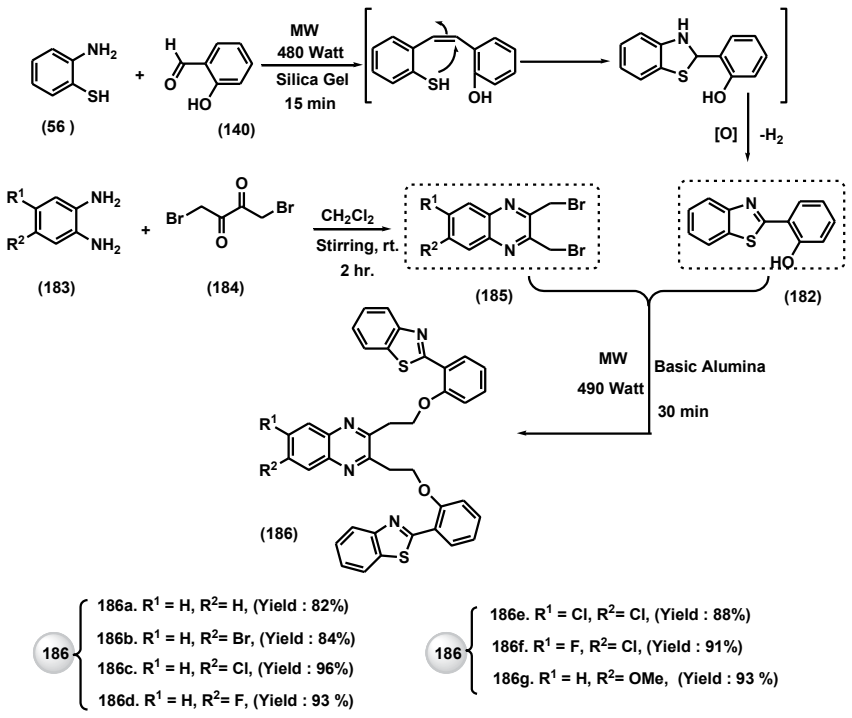


Figure 10.42: Synthesis of bis(benzo[d]thiazolyl)quinoxaline analogues.

by the microwave irradiation reaction of appropriate 2-aminothiophenol (**56**) and 2-hydroxybenzaldehyde (**140**) in front of silica gel.

Secondly, another precursor quinoxaline dibromides (**185**) were synthesized by liquefying the equivalent quantity of phenylene-1,2-diamines (**183**) and 1,4-dibromobutane-2,3-dione (**184**) in dichloromethane (DCM) stimulating for 2 h at ambient temperature.

Finally, quinoxaline dibromides (**185**) were treated with benzothiazolylphenol derivatives (**182**) in the presence of K_2CO_3 using basic alumina under microwave irradiation (MW) to give the desired compound bis(benzo[*d*]thiazolyl)quinoxaline derivatives (**186**) with high yield (82–96%).

10.2.4.2 Synthesis of benzothiazole derivatives as an "allosteric Hsp70 inhibitors"

Whadwa and his coworkers recognized the benzothiazole, MKT-077 (**187**), as a potential inhibitor of Hsp70s. These Inhibitors containing a pyridinium-modified benzothiazole was presenting effective anti-proliferative activity with minimal toxicity in multiple cancer cells [57]. The medicinal chemistry campaigns was planned to improve MKT-077, giving analogs for example JG-231 (**188**) and JG-98 (**189**) with enhanced the longer lifetimes in rodents and anti-proliferative activity [58] (Figure 10.43).

On the other hand, MKT-077 has no penetration effect on the blood-brain barrier (BBB), and it has limitation on probe for analyzing Hsp70 as a targeted drug in the central nervous system. Scientists imagined that changing the cationic pyridinium skeleton in MKT-077 by neutral pyridine moiety enhance its blood-brain barrier (BBB) penetrance.

The researchers successfully planned and synthesized YM-08 (**190**) a neutral analogue of MKT-077. The newly synthesized YM-08 *in vitro* bound to Hsp70 and easily waned phosphorylated tau levels in cultured brain slices [59] (Figure 10.44).

Shao and Gestwicki group [60] has discloesd a remarkable approach to synthesis of the (2Z,5E)-3-ethyl-5-(3-methylbenzo[d]thiazol-2(3H)-ylidene)-2-((pyridin-2-yl)methylene)thiazolidin-4-one (**199**) as depicted (Figure 10.45). Initial step, the substituted anilines (**191**) was treated with potassium ethylxanthate in DMF for 4 h,

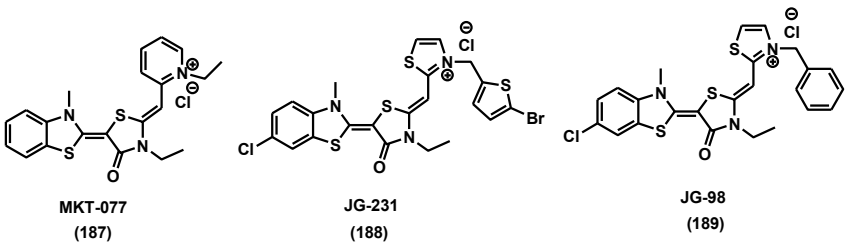
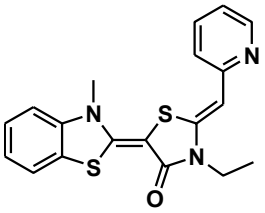


Figure 10.43: Some benzothiazole rhodacyanine probes.



YM-08
(190)

Figure 10.44: Pyridine ring based benzothiazole derivative (YM-08).

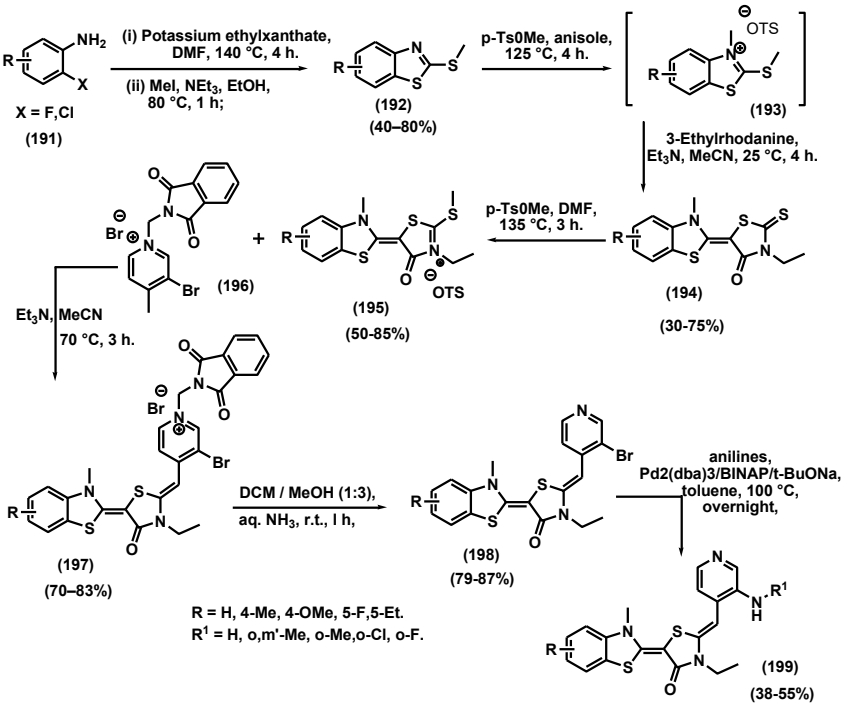


Figure 10.45: Synthesis of (2Z,5E)-3-ethyl-5-(3-methylbenzo[d]thiazol-2(3H)-ylidene)-2-((pyridin-2-yl)methylene)thiazolidin-4-one.

pursued by methylation with methyl iodide (MeI) under basic medium. The resulting benzothiazoles (**192**) was first reacted with methyl p-toluenesulfonate (p-TsOMe) to generate an intermediate (**193**) and then coupled with 3-ethylrhodanine to give the intermediate (**194**). Under boiling condition the Intermediate (**194**) was further treated by methyl *p*-toluenesulfonate (p-TsOMe) to give the compound (**195**),

pursued by condensation with 1-((1,3-dioxoisoindolin-2-yl)methyl)-2-methylpyridin-1-ium bromide (**196**) to give compound (**197**). Using aqueous ammonium hydroxide for the deprotection of (**197**) to obtained (**198**). Finally, the catalyst [$Pd_2(dba)_3$/BINAP/*t*-BuONa] was catalyzed the coupling reaction between compound (**198**) and anilines to give the desired compounds (**199**).

Docking studies and mechanism of action:

In molecular docking studies, Shao and Gestwicki group [60] used the structure of human Hsc70 (PDB code 3HSC) truncated form. In this docking model, the compound **199** (R = 4-Me, R^1 = *O*-F) was attached in a core pocket formed by residues R72, F150, T13, K71, P147 and T204. On the other hand, the pyridine ring of **199** (R = 4-Me, R^1 = *O*-F) was mostly solvent exposed, and it situated the benzyl group into a neighboring hydrophobic pocket. This binding pose was explained the bias for ortho-substitutions, because nestled this group against Y149 along with β-sheet composed of residues T222–T226. Further study of mechanism of action indicated that the compound pyridine-modified benzothiazoles, **199** (R = 4-Me, R^1 = *O*-F) was retain promising anti-proliferative potentiality in breast and cell lines of prostate cancer.

10.2.4.3 Synthesis of benzothiazole derivatives as "tubulin" inhibitors

Song and his coworkers [61] has demonstrated that substituted benzaldehyde derivatives (**75**) was treated with 3,4,5-trimethoxyaniline (**200**) under refluxing condition to afford the compounds (**201**). The Compounds (**201**) then treated with reducing agent (NaBH4) in appearance of benzoic acid in dichloromethane to produced compounds (**202**). In the next step, the compounds (**202**) coupled with chloroacetyl chloride to give the compounds (**203**) in DMF. Finally, in the presence of K_2CO_3, the compounds (**203**) were interacted with substituted benzothiazoles (**83**) to give the desired compounds (**204**) in acetonitrile (Figure 10.46).

Docking studies and mechanism of action:

Tools of molecular docking were used to forecast the orientation of the recently planned compound (**204h**) in the constraint of colchicines binding site of β-tubulin (PDB ID: 6O61) [61]. Demonstrated the docking result was that compound (**204h**) perfect bind to the colchicine binding pocket of β-tubulin. In compound (**204h**), the 3, 4, 5- trimethoxyphenyl group was formed H-bonding with Cys239 side chain in 1.9 Å distance. The 3-hydroxyl-4- methoxyphenyl was formed two H-bonds with Leu246 main chain and Asn101 side chain. The H-bonds distances was observed 1.6 and 2.0 Å, respectively. These two groups (3,4,5-trimethoxyphenyl group and 3-hydroxyl-4-methoxyphenyl) were also participate in hydrophobic interaction with Leu246, Lys252, and Leu240 etc. Benzothiazole group was situated in hydrophobic pocket form of Leu250, Val236, Tyr200, Leu240, Leu253, and Ile316. Hitherward, the carbonyl (C=O) group of the compound (204 h) was formed H-bond with Asn256 side chain along with 1.9 Å distance. These interactions helps to strong bind the compound (**204h**) with colchicine binding pocket and show a rigid polymerization inhibiting potentiality

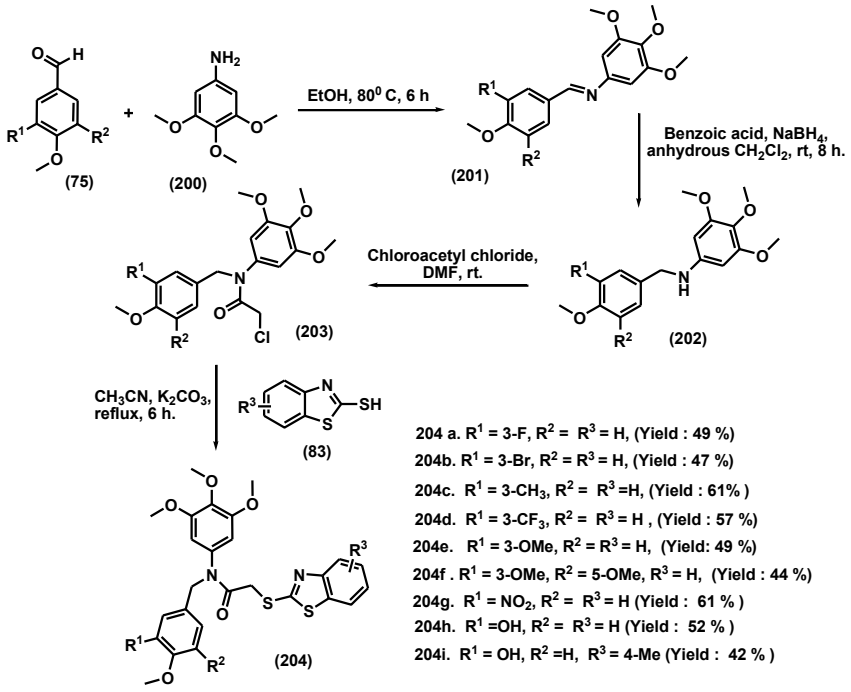


Figure 10.46: Synthesis of 2-(benzo[d]thiazol-2-ylthio)-N-(3,4-substituted-methoxybenzyl)-N-(3,4,5-trimethoxyphenyl)acetamide.

against β-tubulin. Further study of mechanism of action indicated that the compound (**204h**) was induced intrinsic and extrinsic apoptosis of two gastric cancer cells [61]. As a result, the compound (**204h**) activates Hippo signaling way to selectively inhibited gastric cancer cells.

Mokesch et al. [62] has reported an efficient methodology for the preparation of new benzothiazole-based metallacycles as an anticancer agents. In the first step, an acid catalysed nucleophilic addition reaction was carried out 2- aminothiophenol (**56**) with employing appropriate aldehydes (**75**) in the addition of hydrogen peroxide (H_2O_2) and HCl at 25 °C in EtOH to get the products (**205**) in good yields. In the final step, the complexation reaction was carried out in the presence of bis [dichlorido(η^6-*p*cymene)ruthenium(II)] (**206**), sodium acetate and 2- phenyl-benzothiazole (**205**). The reaction mixture was swunged at 40 °C for 24 h and red crystal products substituted [(Chlorido)(2-phenylbenzothiazolato-κN,C2′))(η^6-*p*-cymene)ruthenium(II)](**207**) was obtained in good yields (10–82%). The compound (**207**) was further treatment with water (H20) to give the compound (**208**) (Figure 10.47).

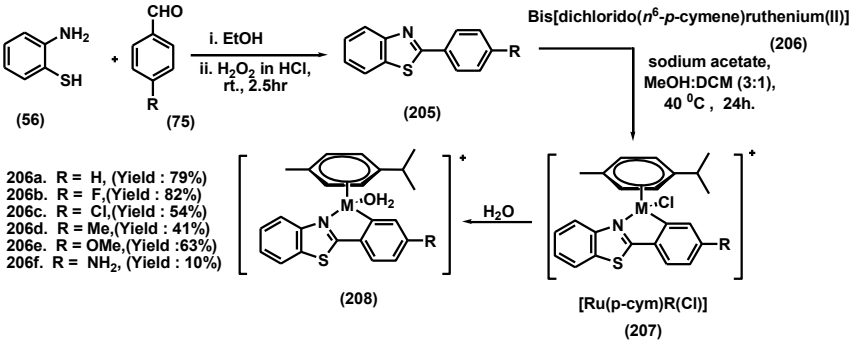


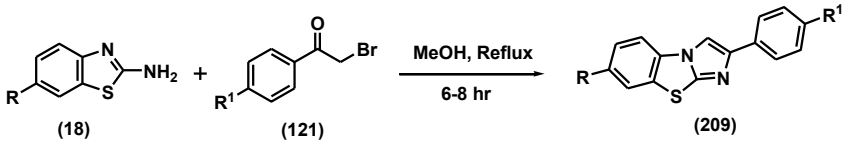
Figure 10.47: Synthesis of substituted [(Chlorido)(2-phenylbenzothiazolato-κN,C2′)(η^6-*p*-cymene) ruthenium(II)].

10.2.4.4 Synthesis of imidazo benzothiazole derivatives as radiosensitizers

A facile as well as efficient method to synthesize a family of imidazo-benzothiazole derivatives (**209**) as an radiosensitizers from reaction between an appropriate 6-substituted-1,3-benzothiazol-2-amine (**18**) with 4-substituted phenacyl bromides (**121**) in methanol and subjected to reflux for 6–8 h in good yields (71–77)% has been described by Majalakere et al. [63] (Figure 10.48). The anticancer potential screening of synthesized imidazo benzothiazole derivatives (**209**) was performed in twice parental melanoma cell line (mouse melanoma cell line, A375C and human melanoma cell line, A375C), HepG2 cell line, along with two drug resistant sublines (A375R and B16F10R) [64].

10.2.4.5 Synthesis of benzothiazole substituted 4-thiazolidinone derivatives

In recent times, Shetty et al. [65] has established a method of preparation of benzothiazole substituted 4-thiazolidinone derivatives (**212**) as shown (Figure 10.49). Shetty groups



209a. R = 7-Br, R^1 = 4-OMe(Yield : 75%)
209b. R = 7-Br, R^1 = 4-Me(Yield : 77%)
209c. R = 7-Br, R^1 = 4-F (Yield : 75%)
209d. R = 7-Br, R^1 = 4-NO_2 (Yield : 74%)
209e. R = 7-SO_2NH_2, R^1 = 4-NO_2, (Yield :72%)
209f. R = 7-SO_2NH_2, R^1 = 4-F, (Yield : 74%)

Figure 10.48: Synthesis of substituted imidazo[2,1-*b*][1,3]benzothiazole de-rivatives.

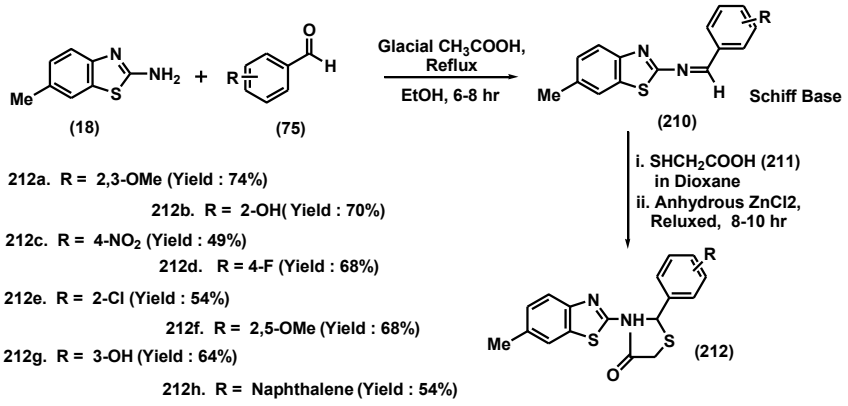


Figure 10.49: Synthesis of novel benzothiazole substituted 4-thiazolidinones derivatives.

have performed the reaction between 2-amino-6-methyl benzothiazole derivatives (**18**) and different substituted aromatic aldehydes (**75**) with glacial acetic acid in ethanol under refluxing condition for 6–8 h to produce various schiff bases (**210**) in good yield. The schiff base compounds (**210**) were treated with thioglycolic acid (**211**) undergoes cyclization reaction in dioxane containing a little bit of anhydrous ZnCl2 and subjected to refluxed for 8–10 h to build up benzothiazole substituted 4-thiazolidinone derivatives (**212**) in good yield (49–74%).

Docking studies and mechanism of action:

Molecular docking was performed by utilizing two unlike proteins which associate to VEGFR2 family PDB ID such as 2QU5 and 5HS3. VEGFR-2 acts as a significant role as signal transducer chiefly for angiogenesis. Benzothiazole substituted 4-thiazolidinone derivatives were look into whether these compounds mode of binding is associated to VEGFR-2 inhibitors or not, with docking against VEGFR-2. Docking of "Pazopanib" well-known anticancer agent is used as standard drugs expressed that it has maximum binding affinity towards simultaneously both 5HS3 and 2QU5 with binding free energies of (–8.32) and (–7.137) respectively. The compound 2-(3-hydroxyphenyl)-3-(6-methylbenzo[*d*]thiazol-2-yl)thiazolidin-4-one (**212g**) interact with 2QU5, H-bonding interactions of keto (C=O) group of thiazolidinone ring and hydroxyl positioned on the phenyl ring with ASN 923 and GLU 850 respectively [65]. In 3-(6-methyl- benzo[d] thiazol-2-yl)-2-(4-(naphthalen-1-yl)phenyl)thiazolidin-4-one (**212h**) interact with 2QU5, π–π stacking observed between naphthalene moiety PHE 368. Naphthalene ring was found in the hydrophobic pocket formed with PHE 368, ALA 371, and PHE 370. Based on the docking score compound 2-(4-fluorophenyl)-3-(6-methylbenzo[d]thiazol-2-yl) thiazolidin -4-one (**212d**) was found to demonstrate superb cytotoxic effect. It was selected for *in vivo* anticancer potential and which raises life span of EAC bearing mice with concentration of 10 mg/kg [65].

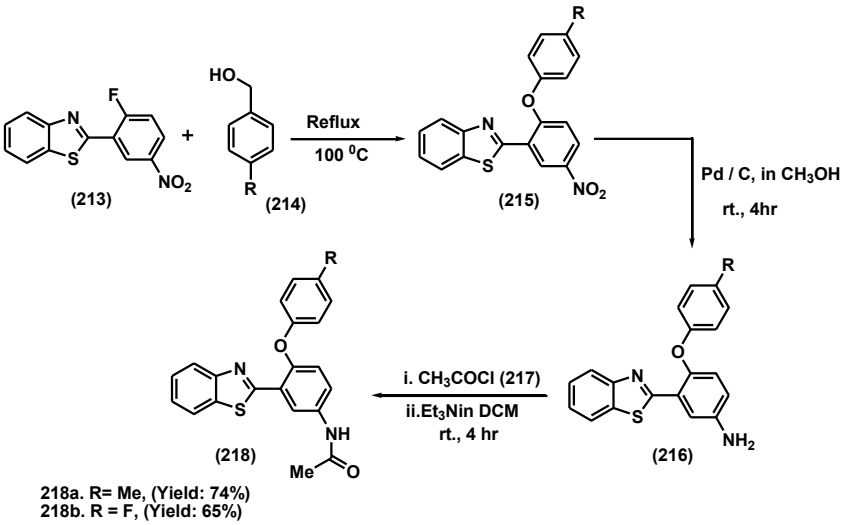


Figure 10.50: Synthesis of novel benzothiazole acetamide derivatives.

10.2.4.6 Preparation of benzothiazole acetamide derivatives as a EGFR-inhibitors

A new method allowing the synthesis of benzothiazole derivatives as promising epidermal growth factor receptor (EGFR) inhibitors has been established by Shahare et al. [66]. In this synthesis, aromatic nucleophilic sustitution of 2-(2-fluoro-5-nitrophenyl)benzo[d]thiazole (**213**) with 4-substituted benzyl alcohol (**214**) under refluxing condition at 100 °C temperature for 5 h to give the substituted 2-(5-nitro-2-phenoxyphenyl)benzo[d]thiazole (**215**). The compounds (**215**) take part in the reduction reaction with **Pd/C** at room temperature in methanol furnished the desired substituted 3-(benzo[d]thiazol-2-yl)-4-phenoxybenzenamine compounds (**216**). Finally, using the acetyl chloride (**217**) in the presence of Et_3N, acetylation reaction was performed with compounds (**216**) at room temperature to give the N-(3-(benzo[d]thiazol-2-yl)-4-phenoxyphenyl)acetamide compounds (**218**) in (65–74%) yield (Figure 10.50).

10.3 Concluding remarks

This book chapter has attempted to represent the different part of the benzothiazole scaffold along with anticancer profile, drug development and synthesis. Several stupendous achievements exposed that benzothiazole-based compounds occupy extensively potential application as diagnostic agents and medicinal drugs. Benzothiazole scaffold is a multipurpose and multifunctional molecule that possesses a therapeutic

effect in several types of cancers for example central nervous system, prostate, ovarian, pancreatic, breast, gastric, colon, renal, and liver cancers. SAR studies revealed that the anticancer and antioxidant activities of benzothiazole scaffolds depend upon the behavior of substituents exist in these molecules. Here, we have strived to accumulate a good number of methods have recently been disclosed to supporting the development of the variety of synthetic benzothiazole compounds involving anti-cancer activities. This kind of information would help us to design and construction of better molecules with enhanced higher specificity and biological properties and together with the advancement of new synthetic strategies. Future research of this benzothiazole scaffolds could provide several heartening results in the medicinal field. We sincerely believe that this chapter would be found useful by researchers engrossed in respective field of work.

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11 Triazine based chemical entities for anticancer activity

Abstract: Triazine is a six-membered aromatic nitrogen heterocyclic moiety that was extensively investigated because of its biological properties and, in particular anticancer potentials. Kinases play a crucial role in cancer cell proliferation and metabolism. Triazine derivatives show anticancer activity by inhibiting the lipid kinases like phosphoinositide 3-kinases, mammalian target of rapamycin, receptor tyrosine kinases, like focal adhesion kinase, cyclin-dependent kinases, Rho-associated protein kinases, p21-activated kinases, carbonic anhydrases, enolase inhibitors, microtubules inhibitors, and histone deacetylases. The present chapter highlights the synthesis of triazine-based derivatives, their characterization, evaluation of anticancer properties, and their journey towards possible medicine for cancer.

Keywords: anticancer activity; kinase inhibitors; several kinases; therapeutic value; triazine synthesis.

11.1 Introduction

Despite enormous investments, the persistent fatal characteristic of cancer continues, with very modest overall gains in therapeutic results. Research for new anticancer leads is a never-ending process and the development of anticancer medication is a complicated task that continues to be arduous. Abnormal kinase activity has been regarded as a prominent part of cancer research for identifying a lead molecule. It is one of the critical processes by which cancer cells circumvent normal growth and proliferation constraints. The human kinome contains around 518 kinases, of which only a couple have been effectively targeted for ailments including cancer and inflammation. Amongst the most, frequent reasons for treatment failure and mortality in cancer is resistance to presently available chemotherapeutics. As a result, we must seek out novel alternative anticancer drugs.

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Many researchers utilized different chemical scaffolds including pyrazolopyrimidines (as mTOR inhibitors), imidazolopyrimidines, and pyrrolopyrimidines, (as PI3K/mTOR inhibitors), and designed various compounds. Similarly, triazine, a six-membered aromatic heterocyclic ring with three N atoms. There are three isomers based on the location of the nitrogen atoms in the ring. All three isomers of the triazine, 1,2,3-triazines (**1**), 1,2,4- triazines (**2**), and 1,3,5-triazines (**3**) (Figure 11.1) are often the emphasis of attention in medicinal chemistry owing to their diverse range of pharmacological actions: anticancer [1], analgesic, anti-inflammatory [2], antimicrobial [3], antiviral [4], antihistaminic [5], antiangiogenic [6] activities, etc.

This chapter discusses the recent development of antiproliferative triazine derivatives. The synthesis, anticancer activity, and potential mechanism of action of the most potent molecules in this class are described.

11.1.1 1,2,3-triazines synthesis and anticancer activity

Vic-triazines or *v*-triazines, the alternative terms for 1,2,3-triazines, are among the less exploited triazines in the research, owing to the lack of ring stability among the three isoforms. Substituted monocyclic 1,2,3-triazines have yet to be found to have any biological action. Instead, benzo- and hetero-fused fused 1,2,3 triazines derivatives exhibited a diverse set of biological activities like antimicrobial [7], antiviral [8] etc., However, in this chapter, we focus on anticancer activity. 1,2,3-triazine derivatives **10a–e** were synthesized as described in Figure 11.2. The crucial intermediate **6** was synthesized by reacting 2-fluorobenzonitrile **4**, ethyl thioglycolate **5**, and NaH in the presence of dry dimethyl sulfoxide (DMSO). Further, diazotization of compound **6** in acetic acid and subsequent coupling of the resultant diazonium salt **7** with primary amines **8a–e** gives compounds **9a–e**. Finally, targeted compounds **10a–e** were attained by intramolecular cyclization of compounds **9a–e**.

The antiproliferative activity of the synthesized benzothiophene fused 1,2,3-triazine compounds **10a–e** was investigated, and compound **10d** exhibited the most potent antiproliferative activity on HeLa cells. Examination of the hit molecule's mechanism of action revealed that it covalently binds in the DNA minor groove, as evidenced by spectroscopic and *in silico* analyses. Further, the cell cycle analysis of the potent derivative **10d**, unveiled that the G2/M phase was suppressed, with a larger concentration of cells in the G0/G1. The specific growth inhibition of cells in the G1 phase implies that cell exposure to **10d** can have severe repercussions such as DNA damage and disruption of DNA synthesis. Furthermore, there was a substantial rise in the population of cells in

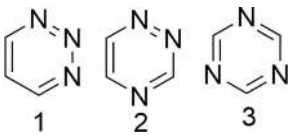


Figure 11.1: Triazine isomers.

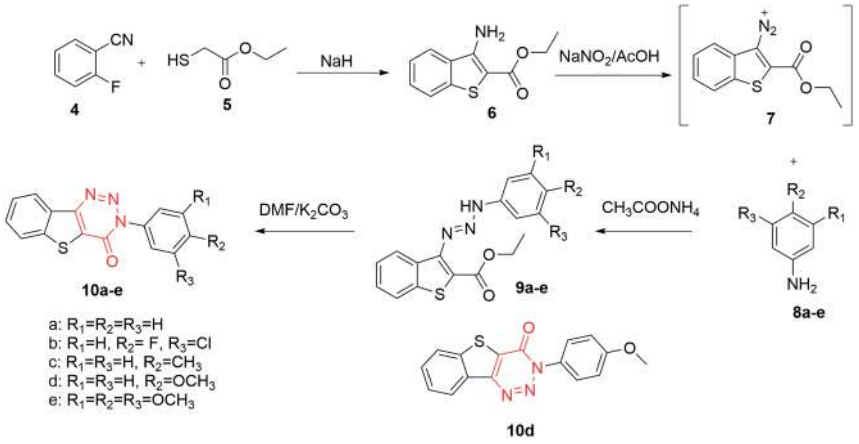


Figure 11.2: Benzothiophene fused 1,2,3-triazines.

the sub-G1 phase, which is suggestive of apoptotic cells. G1 arrest is also consistent with DNA-topoisomerase-I inhibition and possible disruptions of replication or DNA damage [9].

4-Anilino side chain associated with quinazoline/quinoline is the pharmacophore in the majority of approved and developed first-generation epidermal growth factor receptor (EGFR) inhibitors. This side chain gets accommodated into a vital lipophilic selectivity pocket in the EGFR-binding domain. According to these pieces of evidence, utilizing pharmacophore modelling, three-dimensional quantitative structure-activity relationship (3DQSAR), and molecular docking, two sets of thieno[2,3 *d*][1,2,3]triazine derivatives were designed. Figure 11.3 Represents the synthesis of the *in silico* designed molecules. Under Gewald reaction conditions, the intermediate **12a, b** was obtained from cyclic ketone **11a–b**, and it was further reacted with sodium nitrite and HCl in the presence of glacial acetic acid to yield compound **13a–b**. Then the resultant compounds **13a, b** reacted with *m*-phenylenediamine, heteroaryl amines, and benzylamine to produce different derivatives (**14a–d, 15a–d, 16a–b, and 17a–b**).

The obtained compounds were investigated on H1299, a nonsmall-cell lung cancer cell line as a potential EGFR inhibitor. The synthesized derivatives displayed potent activity with IC50 values ranging from 25 to 58 nM, superior to the marketed drug gefitinib with an IC50 value of 40 μM. Among the synthesized compounds, compound **16b** displayed potent anticancer activity against the H1299 cell line with an IC_{50} value of 25 nM and it could suppress the cellular concentration of EGFR from 7.22 to 2.67 pg/mL. *In vitro* assessment of EGFR inhibition revealed that compound **16b** could effectively inhibit the expression of the EGFR with an IC50 value of 0.33 nM, which is higher than the values displayed by gefitinib and erlotinib (IC50 values of 1.9 and 4 nM). The proposed compound **16b** might be a viable lead molecule in the search for effective anti-lung cancer agents that target EGFR [10].

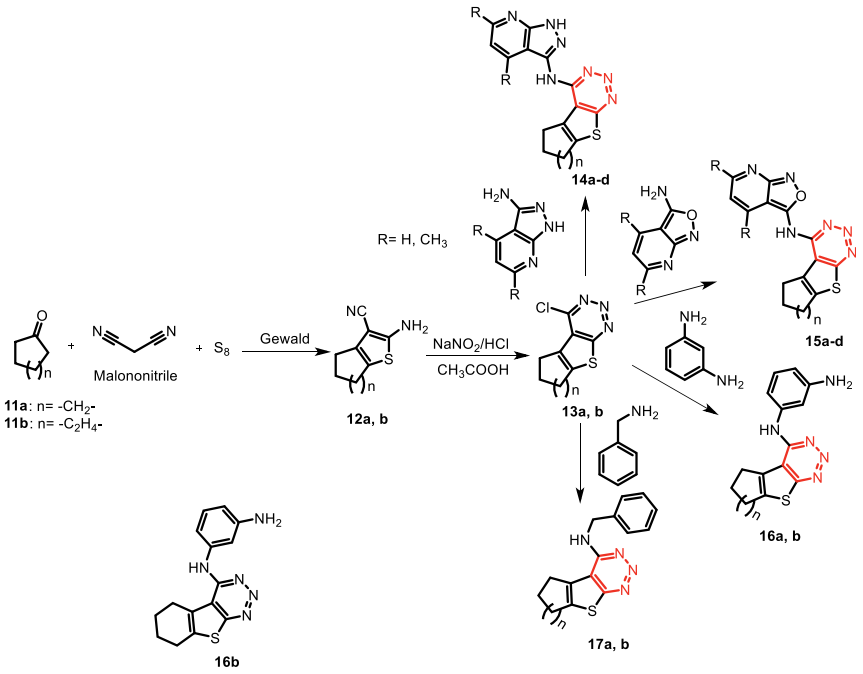


Figure 11.3: Thieno[2,3 *d*][1,2,3]triazine derivatives.

Diazolo fused 1,2,3-triazine compounds **25a–e** were synthesized according to Figure 11.4. The intermediate **20** was synthesized by cyanoacetylation of compound **18** with compound **19** in dioxane under reflux. The non-isolable potassium salt of ketene N,S-acetals was then obtained by reacting it with substituted isothiocyanates **21** in DMF containing KOH, yielding compounds **22a–e** *in situ* after treatment with methyl iodide. Treating compounds **22a–e** with hydrazine hydrate in hot ethanol gave **23a–e**. Furthermore, diazotization of molecules **23a–e** with aqueous sodium nitrite in the presence of HCl/AcOH (3:1 mixture) generated products **25a–e** via the formation of pyrazole diazo carboxamides **24a–e**, that cyclize to a triazine ring system through intramolecular nucleophilic addition of the carboxamide NH group to a diazonium salt.

The anticancer effects of the newly discovered compounds were tested on several of cancer cell lines, including Huh-7, Panc-1, and CCRF. Compounds **22a, 22c, 25a,** and **25c** displayed IC50 values (4.93–8.84 μM) similar to the doxorubicin (5.43 μM) and substantial anticancer activity against Huh-7 cell line. On the other hand, in Panc-1 cancer cell line was efficiently suppressed by compounds **25a** (IC50 = 9.91 μM) and **25d** (IC50 = 4.93 μM) compared to doxorubicin (IC50 = 6.90 μM). To elucidate the mechanistic approach of these new derivatives, the compounds were assessed by Caspase Glo 3/7 assay7 assay. Results implied that compounds could provoke caspase 3/7 and display pro-apoptotic activity. To examine for further molecular alterations, a microarray

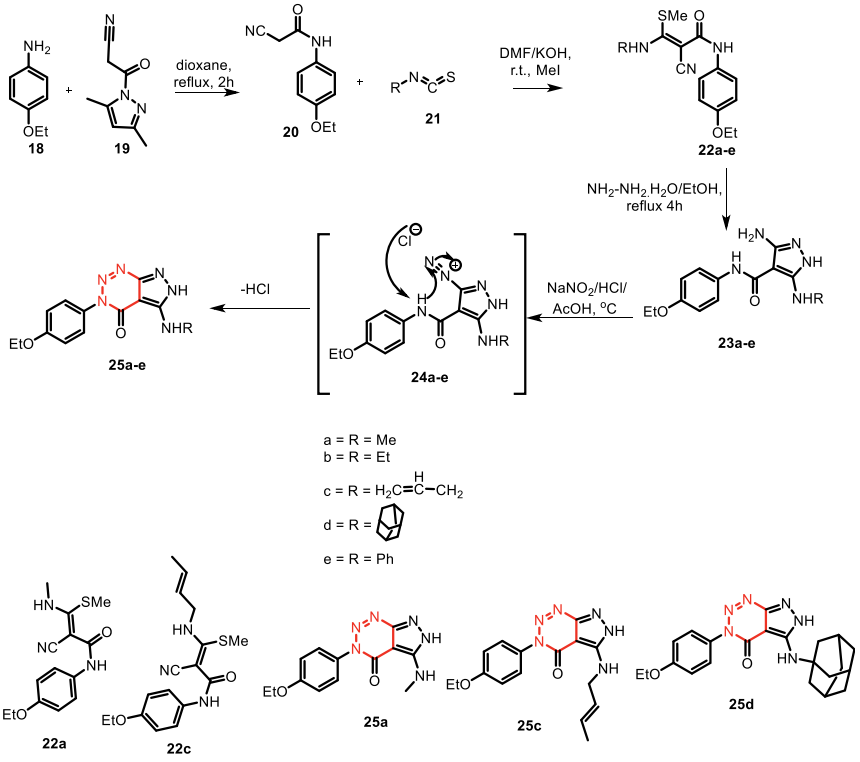
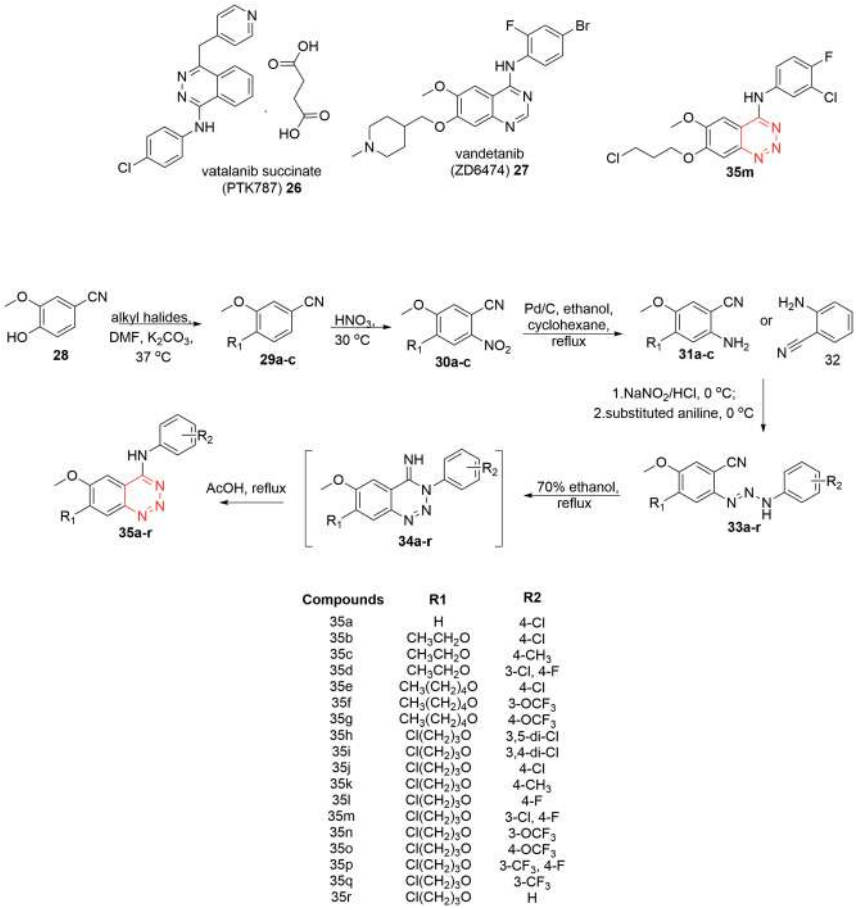


Figure 11.4: Diazolo fused 1,2,3-triazine compounds.

experiment was conducted for **25c** treated Huh-7 cells and specific genes related to apoptosis, metabolism, cell cycle, tumor growth, and suppression like MUCDH1, UGT1A1, SLC26A3, DNASE1, UGT2B15, UGT2B17, and UGT2B7 were upregulated and, FSCN1, IF127, TAGL, FSCN1, THBS1, and SOCS2 were the most substantially diminished [11].

Structural features of kinase inhibitors ZD6474 (vandetanib) **27** and PTK787 (vatalanib succinate) **26** were the basis for designing substituted benzo-1,2,3-triazine compounds **35a–r** and Figure 11.5 represents the outline of synthesis. The intermediates **29a–c** were obtained by reacting compound **28** with different alkyl halides in the presence of DMF and base potassium carbonate. Then compounds **29a–c** were selectively nitrated with nitric acid at 30 °C to give nitro compounds **30a–c**. Further, the nitro group of compounds **30a–c** was reduced to the corresponding amines **31a–c** by Pd/C. Compounds **31a–c** & **32** were initially diazotized and then coupled with substituted anilines at 0 °C, to get triazenes **33a–r**. Further, the targeted compounds **35a–r** were synthesized by cyclization of compounds **33a–r** in presence of ethanol (70%) via unstable intermediates **34a–r**. The synthesized compounds were screened for cytotoxicity against MVECs (microvascular endothelial cells) by MTT assay and found **35m** was the hit compound. Compared to vatalanib succinate derivative **35m** could inhibit



Compounds	R1	R2
35a	H	4-Cl
35b	CH_3CH_2O	4-Cl
35c	CH_3CH_2O	4-CH_3
35d	CH_3CH_2O	3-Cl, 4-F
35e	$CH_3(CH_2)_4O$	4-Cl
35f	$CH_3(CH_2)_4O$	3-OCF_3
35g	$CH_3(CH_2)_4O$	4-OCF_3
35h	$Cl(CH_2)_3O$	3,5-di-Cl
35i	$Cl(CH_2)_3O$	3,4-di-Cl
35j	$Cl(CH_2)_3O$	4-Cl
35k	$Cl(CH_2)_3O$	4-CH_3
35l	$Cl(CH_2)_3O$	4-F
35m	$Cl(CH_2)_3O$	3-Cl, 4-F
35n	$Cl(CH_2)_3O$	3-OCF_3
35o	$Cl(CH_2)_3O$	4-OCF_3
35p	$Cl(CH_2)_3O$	3-CF_3, 4-F
35q	$Cl(CH_2)_3O$	3-CF_3
35r	$Cl(CH_2)_3O$	H

Figure 11.5: Substituted benzo-1,2,3-triazine compounds.

the growth of cell lines, like DU145 & PC-3, T47D, B16F0, and LL/2 murine Lewis, 4–10 times effectively [12].

Further, structural modifications were introduced on the hit compound CTZ12 (**35m**), substituted pyrido-1,2,3-triazine compounds **41a–j** were designed and synthesized as portrayed in Figure 11.6. The intermediate compound **37** was produced by reacting 2,6-dichloro-3-nitropyridine **36** with CuCN in NMP. Utilizing phase-transfer catalyst TBAB, intermediate **37** was reduced with $Na_2S_2O_5$ in dichloromethane/aqua solution to obtain compound **38**. Consequently, compound **38** was diazotised and coupled with substituted anilines **39a–j** to obtain compounds **40a–j**. In the final step compounds **40a–j** were directly rearranged to the targeted compounds **41a–j**. The synthesized analogues were examined for their VEGFR-2 kinase inhibitory activity and cytotoxicity against MVECs and it was discovered that VEGFR-2 kinase inhibitory

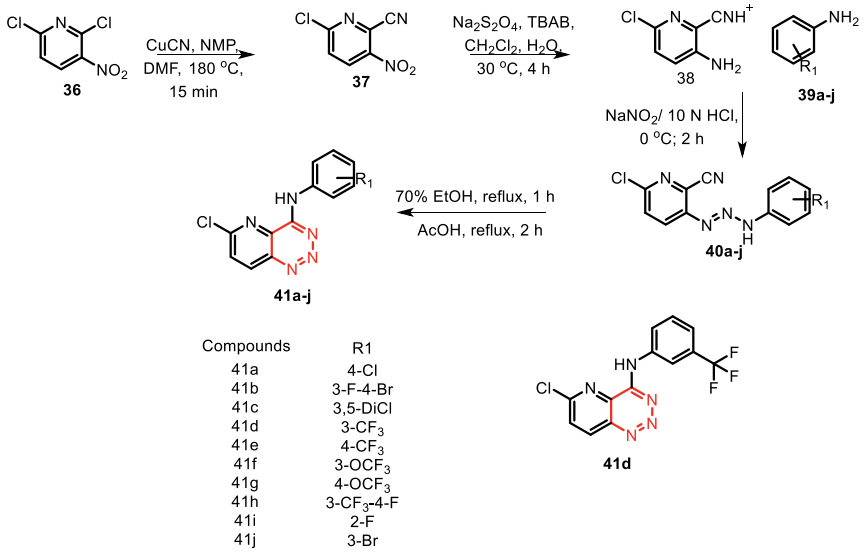


Figure 11.6: Chloropyrido substituted 1,2,3-triazine compounds.

activity by the compound **41d** is less than PTK787 albeit it had more MVECs growth inhibition than PTK787 [13].

As shown in Figure 11.7, chloropyrido fused 1,2,3-triazine substituted compounds **48 o–q** were synthesized. In the first step, upon reaction with cuprous cyanide in NMP with reacting 2,6-dichloro-3-nitropyridine **42** at reflux condition, yielded the desired intermediate **43.** Further, the nitro group of compound **43** was reduced to the amine group by sodium thiosulphate producing 3-amino-6-chloro-2-cyanpyridine **44**. Compound **46** was prepared by diazotization of **44** and coupling with 3-trifluoromethoxyaniline **45.** Cyclization followed by Dimroth rearrangement of compound **46** resulted in intermediate **47**, which was further reacted with aliphatic N-heterocycles to get compounds **48a–1**, de-protection Boc group of the compound **48l** with HCl in EtOAc gives compound **48m**. Moreover, compounds **48n** and **48o–q** were synthesized by reacting compound **47** with monoethanolamine in neutral ethanol under reflux, and sodium alkoxide in corresponding alcohol to get alkyl ethers **48o-q,** respectively. The synthesized compounds were evaluated for anti-proliferative and Pim-1 inhibitory activity against prostate cancer cells. The majority of derivatives presented potent *in vitro* Pim-1 inhibition and anti-proliferative effects. Among them, **48b, 48h,** and **48m** exhibited potent inhibition of Pim-1 activity possessing IC_{50} values of 0.69, 0.60, and 0.80 μM, correspondingly. Additionally, compounds **48b, 48i, 48j, and 48m** displayed cytotoxic activity at a low micromolar level in LNcap and PC-3 (human prostate cancer) cell lines. Furthermore, the western blot experiment revealed that **48j** could reduce the p-BAD and p-4EBP1 expression levels in PC-3 cells in a dose-dependent manner [14].

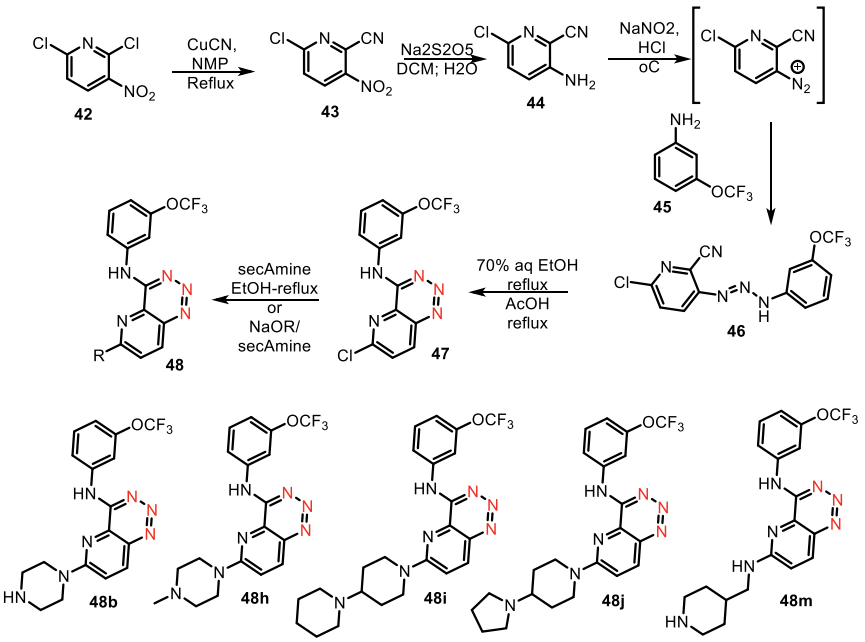
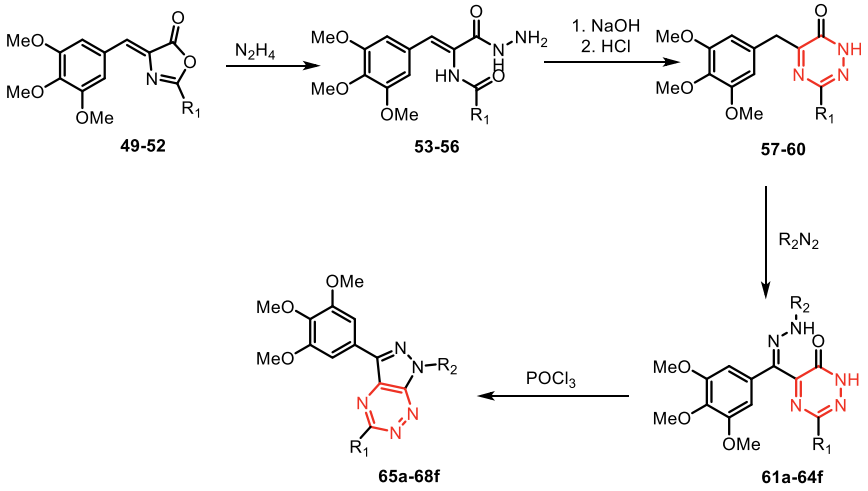


Figure 11.7: Pyrido fused 1,2,3-triazine substituted compounds.

11.1.2 1,2,4-triazines synthesis and anticancer activity

1,2,4-Triazine is one of the three isomers of triazine (Figure 11.1) called *as*-triazines (asymmetric triazines). The 1,2,4-triazine ring is frequently characterized as a scaffold for a wide range of biologically active molecules, both natural and synthetic, with a wide range of pharmacological actions, including anti-HIV [15], CRF receptor antagonists [16], antimicrobial [17], anti-anxiety, and antinflammatory agents [18]. The NCNN sequence of atoms in the 1,2,4-triazine ring was thought to be crucial for a variety of pharmacological actions. The anticancer tirapazamine (TPZ) is an example of medicine that includes the 1,2,4-triazine nucleus. In the present chapter, synthetic approaches for 1,2,4-triazines, related benzo- and heterofused compounds having antitumor activity, and the possible mechanism of action, of the most promising derivatives are discussed.

Synthesis of diazo fused 1,2,4-triazine compounds **65a–70f** has been carried out in five steps as detailed in Figure 11.8. The intermediates **49–52** were prepared by the modified *Erlenmeyer´s* reaction. Then oxazolone ring of the compounds **49–52** undergo hydrazinolysis under mild conditions to get the compounds **53–56** without changing the stereochemistry on the exocyclic double bond. The hydrazides **53–56** undergo cyclization in boiling sodium hydroxide solution to produce compounds **57–60**.



For compounds		For compounds **61 - 68**
49, 53, 57, 61, 65	R_1 = phenyl	a = R_2 = phenyl
50, 54, 58, 62, 66	R_1 = 4-methoxyphenyl	b = R_2 = 2-methoxyphenyl
51, 55, 59, 63, 67	R_1 = 3,4,5-trimethoxyphenyl	c = R_2 = 3-methoxyphenyl
52, 56, 60, 64, 68	R_1 = 4-chlorophenyl	d = R_2 = 4-methoxyphenyl
		e = R_2 = 3,4,5-trimethoxyphenyl
		f = R_2 = 4-chlorophenyl

Figure 11.8: Diazolo fused 1,2,4-triazines.

Further, compounds **57–60** were used to synthesize of aryl hydrazones **61a–64f** by reacting with aryldiazonium salts in pyridine. The $POCl_3$ and catalytic amount of pyridine and aryl hydrazones **61a–64f** were cyclized to the target compounds **65a–68f.** The produced derivatives were assessed for anti-proliferative property against five cancer cell lines and compound **53–56** exhibited very slight cytotoxic activity against all of the tested cell lines. Interestingly the compound **57–60** displayed cytotoxicity and among them, **57** showed an impressive activity towards all the tested cell lines [19].

Figure 11.9 depicts an effective technique for synthesizing substituted pyrazolo [4,3-e][1,2,4]triazine compounds **74a–l**. Methylhydrazone **69**, was cyclized to produce pyrazolo[4,3-e][1,2,4]triazine **70**, then compound **70** was treated with potassium permanganate for 1 h at room temperature under phase transfer catalytic conditions to produce sulfone **71.** After that, compound **71** was reacted for 9 days at 150 °C in a sealed tube with anhydrous aniline to attain aniline substituted pyrazolotriazine **72** and it is selectively chlorosulfonylated in chlorosulfonic acid at 0 °C to produce the desired product **73.** As demonstrated in Figure 11.9, the chlorosulfonyl derivative **73** was linked to amines with ease to create the target sulfonamides **74a–1.** These sulfonamide derivatives were evaluated for anticancer potency and the results indicated that approximately half of the compounds showed moderate activity and compounds

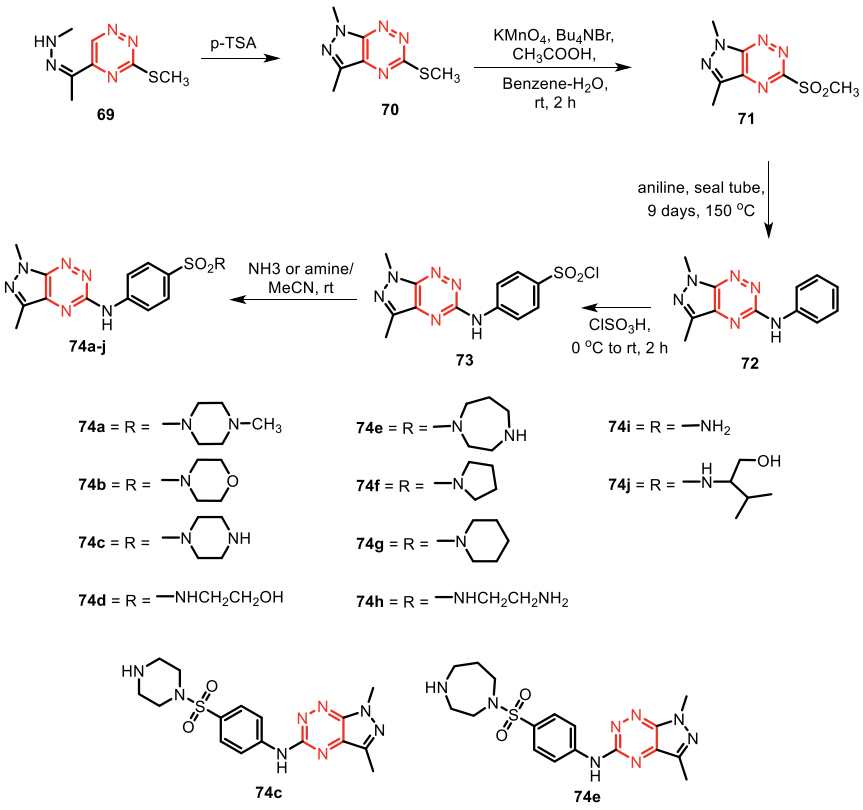


Figure 11.9: Substituted pyrazolo[4,3-e][1,2,4]triazines.

74c and **74e** were identified as hits. The hit compounds, **74c** and **74e** could display Abl protein kinase inhibition at micromolar concentration and selective cytotoxicity towards leukaemia cell lines with overexpression of Bcr-Abl oncogenic kinase [20].

A family of new aza-analogues sildenafil **78a–j** and pyrazolo[4,3-e][1,2,4]triazine sulfonamides with aniline **78a–j** were synthesized as represented in Figure 11.10. The intermediate **76** was synthesized by reacting compound **75** with 2-ethoxyphenylboronic acid in the prescence of copper(I) 3-methylsalicylate. Then compound **76** was treated with chlorosulfonic acid at 0 °C to get chlorosulfonation selectively at the 5′-position of the phenyl ring to get compound **77**. Further, it was coupled with the suitable amine to get sulfonamides **78a–j**. Sulfone **79** was produced by catalytic reaction of compound **75** with potassium permanganate under phase transfer catalytic conditions. Further, in a closed tube, anhydrous aniline was reacted with compound **79** at 150 °C for 9 days to get compound **80**. Chlorosulfonylation of the compound **80** with chlorosulfonic acid at 0 °C proceeded selectively at the 4′-position of the phenyl ring and gave compound **81**.

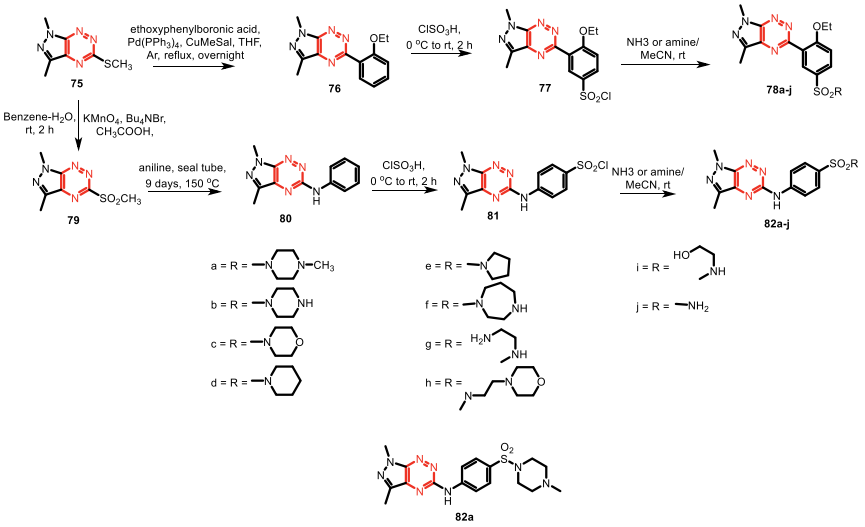


Figure 11.10: Anilino substituted pyrazolo[4,3-e][1,2,4]triazine sulfonamides.

As indicated in Figure 11.9, the chemical **81** was subsequently linked with amines to provide the intended sulfonamides **82a–j**.

Carbonic anhydrase (CA, EC 4.2.1.1) inhibitory potency and antiproliferative property of the synthesized compounds was assessed against MDA-MB-231 and MCF-7 human breast cancer cell lines and found that they were effective against tumor-related isoforms CA-IX and CA-XII, poorly inhibited CA-I and CA-II. Compound **82a** has higher cytotoxicity as per MTT assay, inhibits [3H] thymidine incorporation into DNA, and inhibits collagen formation in breast cancer cell lines [21].

Sildenafil analogues having diazolo fused 1,2,4-triazine **90a–o** and **94a–e** were synthesized as per the synthetic procedure Figure 11.11 and characterized. The synthesized derivatives were examined for their cytotoxicity potential against MCF-7 and K562 cell lines and carbonic anhydrase inhibitory activity and found that the cancer-linked isoform hCA IX is inhibited at nanomolar concentration with KIs in the range of 15.4–42.4 nM. Against the transmembrane isoform hCA XII; compounds, **90b, 90f, and 90o**, displayed inhibition in the range of 3.8–7.3 nM [22]. Carbonic anhydrase (CA, EC 4.2.1.1) inhibitory potency and antiproliferative property of the synthesized sulphonamide derivatives **94a–e** were assessed against MDA-MB-231 and MCF-7 human breast cancer cell lines. Compounds **94a–e** were ineffective against CA I and CA II but active against tumor-associated enzyme CA IX. Compound **94e** was the most efficient inhibitor of CA IX, with an inhibition constant KI of 13.8 nM, whereas derivatives **94c–94d** had intermediate activity (**94c**: KI = 25.4 nM; **94d**: KI = 24.5 nM). The activity of compounds **94g–94h** was satisfactory (**94g**: KI = 27.7 nM; **94d**: KI = 26.6 nM) [23].

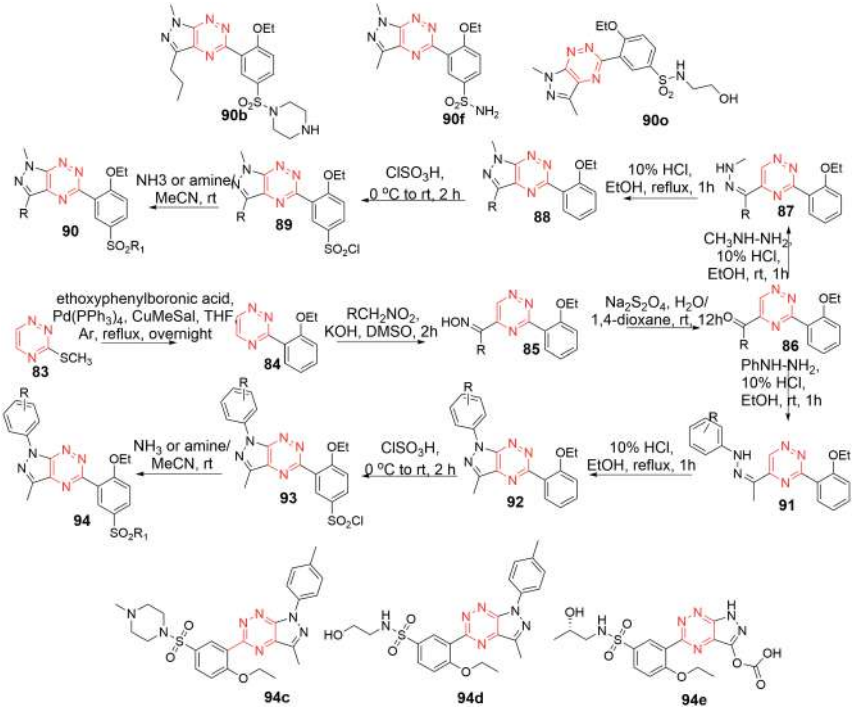


Figure 11.11: Sildenafil analogues.

As mentioned in Figure 11.12, compounds **96a–m** were synthesized by oxidation of radical **95**, with MnO_2. The synthesized compounds were evaluated for *in vitro* cytotoxicity and found that substituents at the 6th position of 1,3-diphenylbenzo[1,2,4] triazine-7-ones have a significant effect on toxicity. Compound **96a** is more cytotoxic to cancer cell lines, and compound **96b** displays greater specificity against solid tumor cells than the normal fibroblast cells. The CF_3-substituted compound **96b** is easily reducible and displays five-fold superior inhibition of TrxR [24].

11.1.3 1,3,5-triazines synthesis and anticancer activity

1,3,5-Triazine derivatives are also known as symmetrical triazines or *s*-triazines. Hexamethylmelamine **97** (HMM), irsogladine **98**, compounds **99**, **100, 101,** and **102** are the 1,3,5-triazine derivatives having the anticancer activity.

Compound **97** (Figure 11.13) is cytotoxic against breast, ovarian, and lung cancer, but it also causes side effects such as vomiting, nausea, abdominal pains, and anorexia [25]. Compound **98** (Figure 11.13) has been shown to have an antitumor effect in epidermoid carcinoma and glioma xenograft models in mice [26]. Compound **99** (Figure 11.13) is

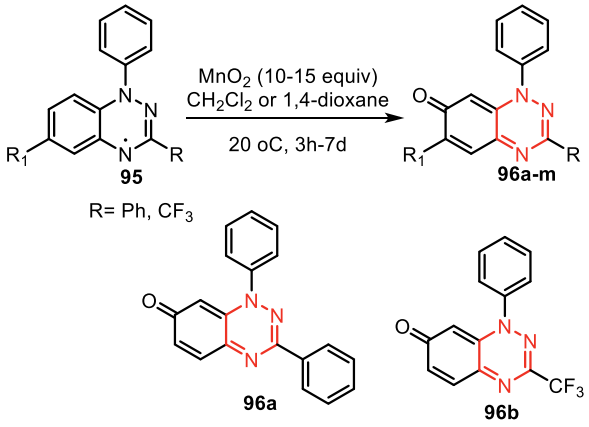


Figure 11.12: Fused 1,2,4-triazine compounds.

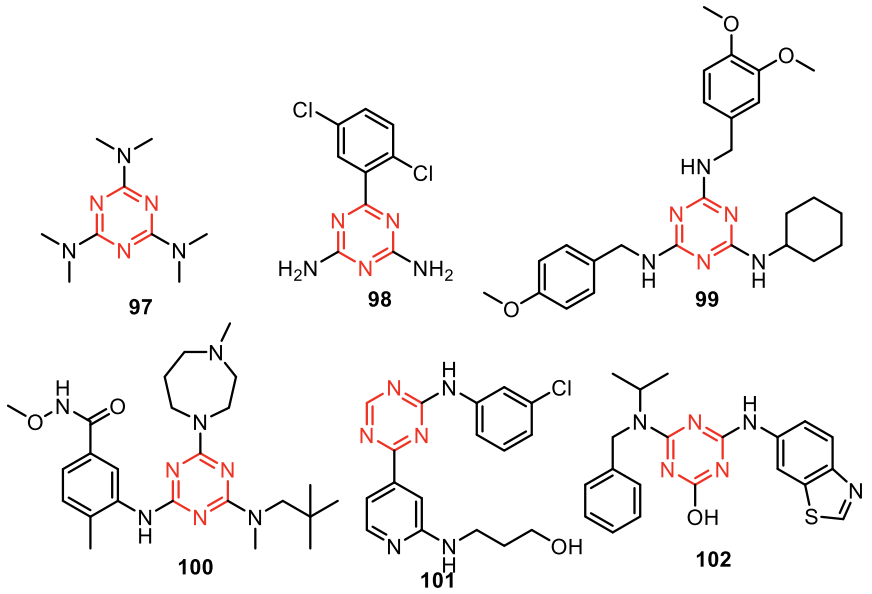


Figure 11.13: Selected 1,3,5-triazines.

a microtubule destabilizing compound that inhibits the development of U936 cells [27]. Compounds **100**, **101**, and **102** (Figure 11.13) block cyclin-dependent kinases [28], p38 MAP kinases [29], and VEGF-R2 (KDR) tyrosine kinase [30], respectively. The strategy followed for the synthesis of 1,3,5-triazines with β-carboline (indolo fused piperidine) compounds **106a–z & a^{I}–q^{I}** is depicted in Figure 11.14. Initially intermediate compounds, **105a–t** were attained via Pictet–Spengler cyclization of the tryptophan methyl ester

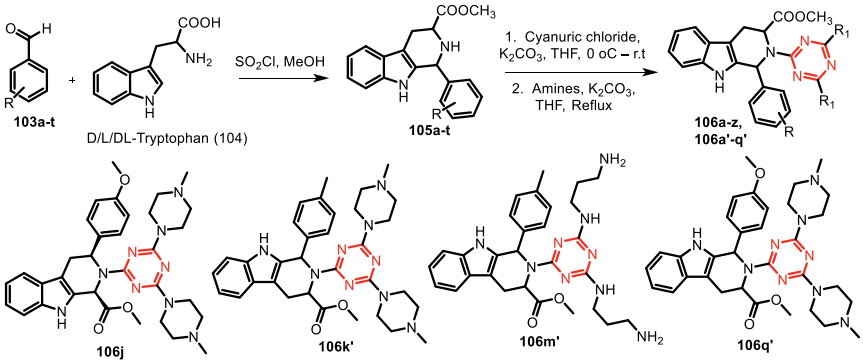


Figure 11.14: 1,3,5-Triazines with indolo fused piperidine.

with benzaldehydes (**103a–t**). Subsequently, compounds **106a–z and a^{I}–q^{I}** were synthesized in a two-step sequence. In the first step, cyanuric chloride was reacted with different tetrahydro-β-carbolines (**105a–t**) by nucleophilic substitution, then in the second step, it was further reacted with various amines. The racemic compounds **106k'** (IC50 = 105.8 nM), **106m** (IC50 = 664.7 nM), and **106q'** (IC50 = 122.2 nM), which are preferentially cytotoxic against KB (oral cancer) cell line, were evaluated for their cytotoxicity against a panel of eight human cancer cell lines and normal human fibroblasts. Enantiopure derivatives **106j** showed 2.5 times greater selectivity towards MCF-7 cells than normal fibroblast NIH3T3 cells, as well as arresting cell cycle in G1 phase and inducing apoptosis in MDA MB-231 and MCF-7 cell lines [31].

1,3,5-Triazine-ethanolamine compounds **113a–g, 114a–g, 115a–g, 116a–g,** and **117a–g** were synthesized as portrayed in synthetic procedure Figure 11.15 [27]. Primarily intermediate **109**, was synthesized by reacting 2,4,6-trichloro-1,3,5-triazine (**107**) with 4-aminoacetophenone **108**. The compound **111** was obtained by the reaction of compound **109** with ethanolamine **110** under microwave irradiation in the presence of dioxane for 5 min. Later with aldehydes **112a–g**, compound **111** was subjected to Claisen–Schmidt condensation to yield 1,3,5-triazine chalcones **113a–g**. Compounds **113a–g** were also reacted with hydrazine monohydrate before being functionalized with acetic anhydride and formic acid, yielding compounds **114a–g and 115a–g**. Under reflux in ethanol, chalcones **113a–g** were treated with 3,5-dichlorophenyl hydrazine and 4-chlorophenyl hydrazine, yielding **116a–g** and **117a–g**, respectively. Among the synthesized compounds 17 molecules were selected and screened against 58 different human tumor cell lines by the US NCI for their anticancer activity and found that compounds **113g and 116d, e, g** showed GI50 values 0.569–16.6 μM and LC50 values 5.15 to >100 μM [32].

1,3,5-Triazine attached with phenols and amine/aniline compounds **117–123** were synthesized as described in the synthetic procedure Figure 11.16. The intermediates **117** and **120** were synthesized by reacting cyanuric chloride with 4-hydroxybenzaldehyde and

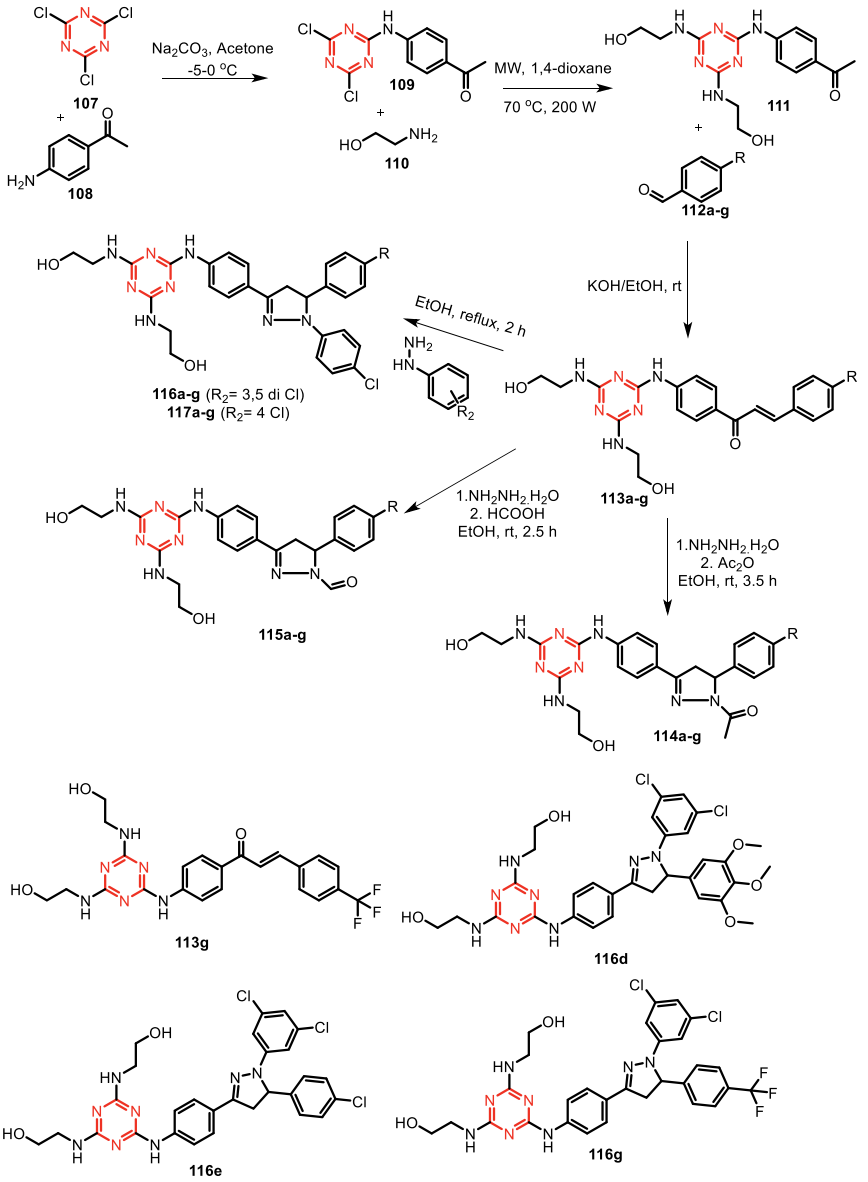


Figure 11.15: 1,3,5-Triazine-ethanolamine derivatives.

1-(4-aminophenyl)ethanone in acetone at 0–5 °C, respectively. Then compounds **118** and **119** were prepared by reacting compound **117** with 2 mol of piperidine or morpholine, respectively. Further intermediate **120** was reacted with and 2,6-disubstitutedphenol to get compounds **121** and **122.** In the last step pipecolic acid was reacted with cyanuric

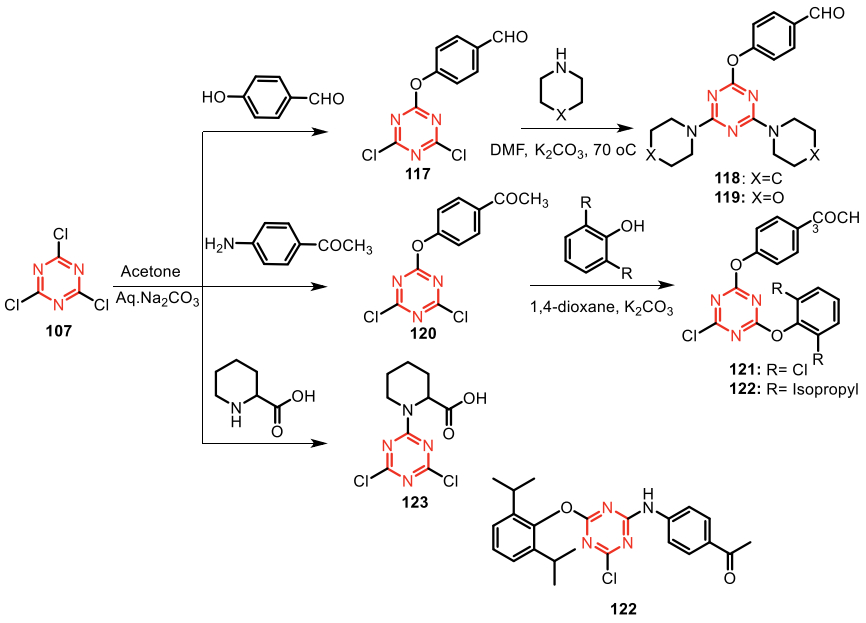


Figure 11.16: 1,3,5-Triazine attached with phenol and amine/aniline compounds.

chloride **107** in acetone at 0-5 °C to obtain compound **123**. The US National Cancer Institute (NCI) tested the produced compounds *in vitro* for their potential to inhibit 60 distinct human tumor cell lines. From the results of compound **122**, it was identified as a hit compound. It showed 60.13% inhibition in renal cancer (CAKI-1) cell line and in the cell-free assay, it also inhibits PI3Kγ at IC50 = 6.90 μM where standard wortmannin inhibits at IC50 = 3.19 μM [33].

Substituted 1,3,5-triazine compounds **132a–d, 133a–d, and 134a–d** were synthesized as shown in Figure 11.17. Initially, the intermediates **125a–c** were synthesized from the condensation of aromatic amines **123a–c** with *p*-aminobenzoic acid **124** in the presence of polyphosphoric acid at 200 °C. Then in the next step, the cyanuric acid **107** was reacted with substituted anilines **126a–c & 127** in the presence of DIPEA, generating monosubstituted triazine derivatives **128a–d**. In the subsequent step compounds **128a–d** reacted with one equivalent of compound **125a–c** in the presence of K_2CO_3 to get disubstituted derivatives **129a–d, 130a–d, and 131a–d**. In the final step, compounds **132a–d, 133a–d, and 134a–d** were synthesized by reacting compounds **129a–d, 130a–d, and 131a–d** with morpholine in the presence of base K_2CO_3 base and solvent DMF. The prepared s-triazine derivatives were assessed for their *in vitro* anticancer potential against the DU-145, A549, PA-1, PC 3, MDA-MB-231, HGC-27, MCF-7, HT-29 and found that tri-substituted s-triazine derivatives **132a–d, 133a–d, and 134a–d**

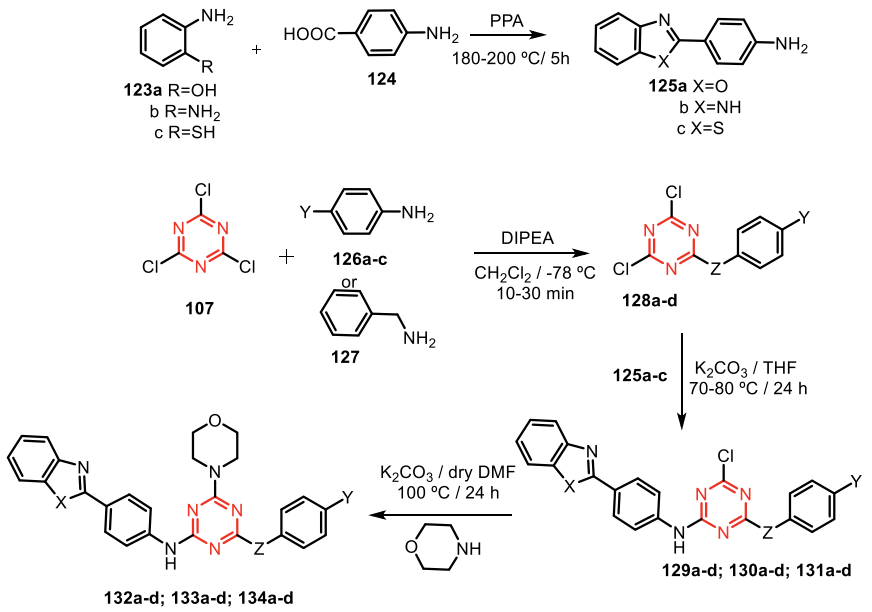


Figure 11.17: Substituted 1,3,5-triazine compounds.

with morpholino group exhibited potent anticancer activity compared to di-substituted s-triazine derivatives **129a–d, 130a–d, and 131a–d** [34, 35].

4-Quinolinol attached 1,3,5-triazine compounds **139a–u** were synthesized as illustrated in Figure 11.18. The first step, mono-substituted s-triazine intermediate **136** was prepared by reacting the 4-amino-2-trifluoromethyl-benzonitrile **135** with cyanuric chloride **107**. In the next step, disubstituted s-triazine intermediate **138** was achieved by reacting compound **136** with 4-hydroxyquinoline **137**. In the subsequent step, compound **138** is coupled with the desired piperazines and piperidines in the 1,4-dioxane solvent at 70–80 °C to get compounds **139a–u**. The synthesized compounds were screened in the DU-145 cell line (prostate cancer) and compounds **139n and 139s** were found as hit molecules [36].

As shown in Figure 11.19, porphyrin intermediate **143** was synthesized by reacting the *p*-hydroxy benzaldehyde **140** with benzaldehyde **141**, pyrrole **142**, and propanoic acid. Further compound **143** was treated with cyanuric trichloride **107** in the presence of $NaHCO_3$ and $CHCl_3$ at 35 °C for 5 h to generate compound **144** without isolation. It is further converted to the target compounds **145a–j**, by reacting with the alcohols or amines in $NaOH/CHCl_3$. The produced derivatives were examined for *in vitro* anticancer activity against MCF-7 cells. All the compounds exhibited similar activity in the absence of light compared to 5-fluorouracil and hematoporphyrin [37].

Initially, monosubstituted s-triazine **146** was synthesized by reacting cyanuric chloride **107** with morpholine at 0–5 °C in the presence of base TEA and solvent

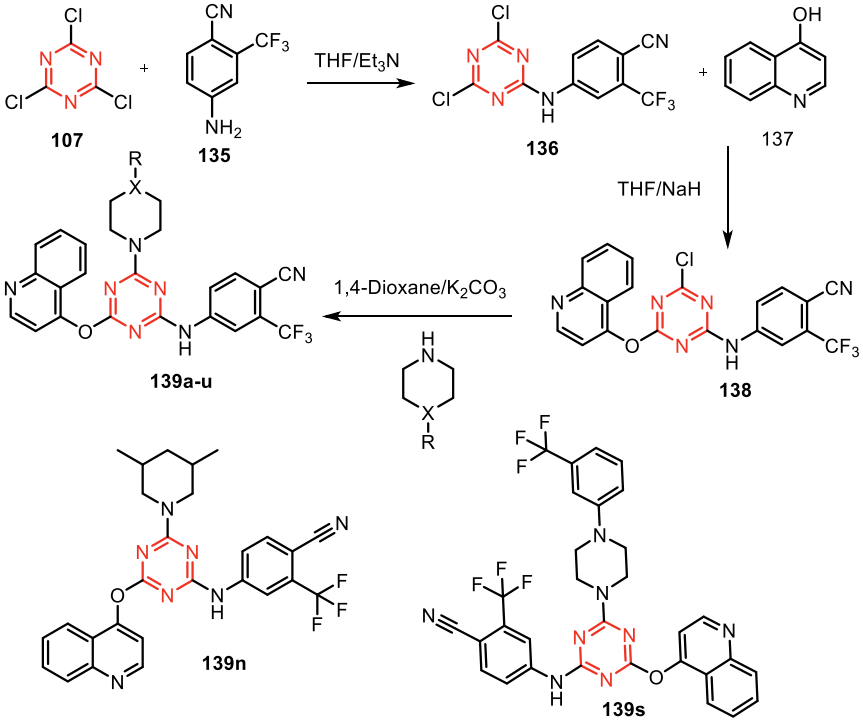


Figure 11.18: 4-Quinolinol attached 1,3,5-triazine compounds.

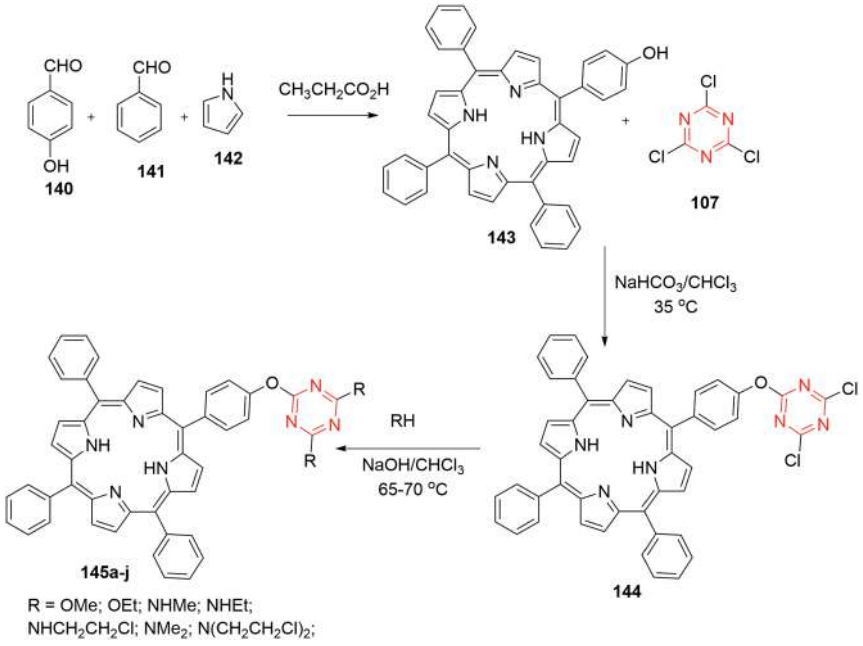


Figure 11.19: 1,3,5-Triazines attached with porphyrin.

acetone, as shown in synthetic Figure 11.20. Then the intermediates **147a–d, 150a–c** were synthesized from equimolar quantities of 4-hydroxy benzaldehyde and 3-fluoro-salicylaldehyde, followed by the addition of various amines, were dissolved in methanol (10 ml) in the presence of ionic liquid (1-butyl-3-methylimidazolium bis(trifluoro-methylsulfonyl) imide). In the next step, compound **146** was reacted with ionic liquid BMIM [Tf_2N], Schiff base **147a–d, and 150a–c** in the presence of base KOH and solvent THF/Acetone at a temperature of 50 °C to get disubstituted triazine derivatives **148a–d, 151a–c**. The trisubstituted s-triazine derivatives **149a–d, 152a–d** were synthesized by reacting the compounds **148a–d, 151a–c** with ionic liquid BMIM [Tf_2N], and morpholine in presence of base KOH and solvent dry THF/acetone at 50 °C for 2–3 h. The intermediate **154** was synthesized by reacting the cyanuric chloride **107** with amine **153**, ionic liquid in presence of base KOH and solvent THF. Further, it was utilized to synthesis of disubstituted derivatives **155a–c** by reacting with the intermediate **147a–c**. Tri substituted derivatives **156a–c** were synthesized from **155a–c** by reacting with morpholine (Figure 11.20). The synthesized 1,3,5-Triazine-Morpholine coupled compounds were tested *in vitro* against HT-1080 and HeLa, for their cytotoxicity, and compounds **148c, 148d, and 149c** have intermediate inhibitory activity towards the tested cell lines compared to the standard drug [38].

The synthesis of the compounds **160a**–**k** was accomplished as shown in Figure 11.21. In the first step cyanuric chloride **107** was reacted with different amines to get intermediates **157a**–**g**, then compounds the disubstituted s-triazine derivatives. The compounds **157a**–**g** were then reacted with hydrazine hydrate to obtain the derivatives **158a**–**g**. Further, compounds **158a**–**g** were treated with **159a–c**, in ethanol and a few drops of glacial acetic acid to get the products **160a**–**k**. The synthesized 1,3,5-triazine – thiobarbituric acid compounds **160a**–**k** were tested *in vitro* in four cancer cell lines: HCT-116, HepG2, A549, MCF-7, and found that thiobarbiturate derivatives showed more potency than the barbiturate derivatives. With IC50 values of 1.2 ± 0.5, 1.6 ± 0.6,

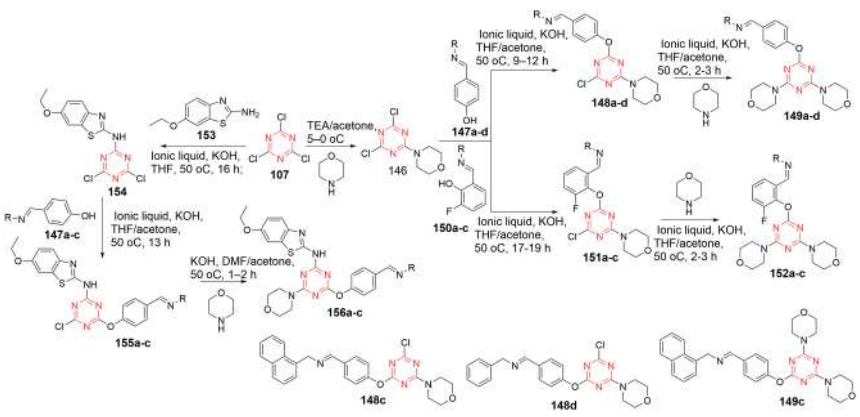


Figure 11.20: 1,3,5-Triazine-morpholine coupled compounds.

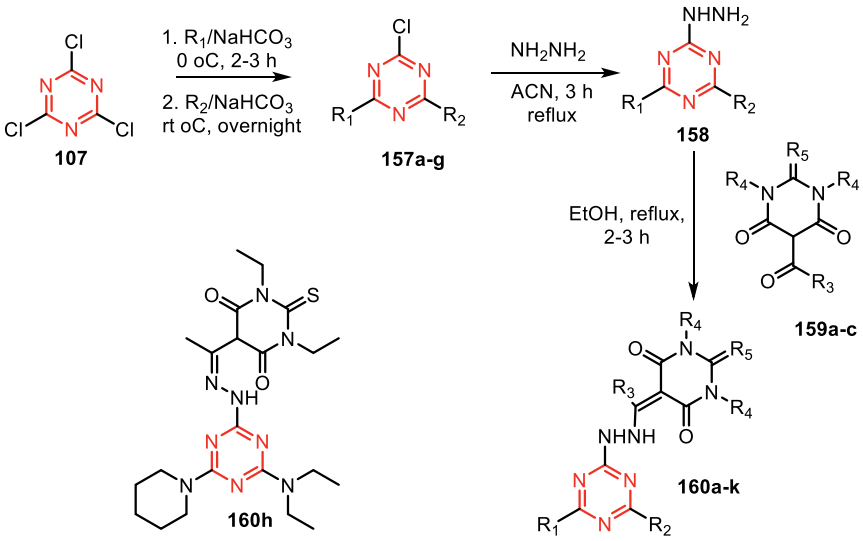


Figure 11.21: 1,3,5-Triazine – thiobarbituric acid coupled compounds.

1.9 ± 0.4, and 3.8 ± 0.3 g/mL for the examined cell lines MCF-7, A549, HCT-116, and HepG2, respectively, the hit molecule **160h** had the greatest activity. These findings reveal that thiobarbiturates-s-triazine hydrazone derivatives might be an excellent scaffold for developing an anticancer therapeutic candidate [39].

As depicted in Figure 11.22, compounds **161a–o** were produced by reacting cyanuric chloride **107** with 2 eq. of substituted amines. Then compounds **161 (a–o)** were further reacted with thiourea. In the final step, targeted compounds **163a–o** were synthesized by Biginelli's one-pot condensation, using bismuth nitrate, benzaldehyde, ethyl acetoacetate, and intermediate compounds **162a–o**. The compounds were screened for cytotoxic activity against cancer cells MCF-7, HeLa, HepG2, HL-60, and normal MCF 12A cells using the MTT assay. The results indicated that the maximum inhibitory effect of compounds was against the MCF-7 cell line and was non-toxic to MCF 12A cells. In the *in-vitro* experiment, the synthesized compounds also displayed epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibition substantially. The most active compound **1631** was further tested in a DMBA induced mammary tumor model in female Sprague-Dawley rats to assess its *in vivo* anticancer efficiency, as well as its influence on biochemical markers such as antioxidant status (SOD, CAT, GPX, and GSH) and lipid peroxidation. From the western blot analysis compound **163l** inhibiting EGFR downstream signaling [40].

Novel 4-aminoquinoline 1,3,5-triazine hybrids **168a–j** were synthesized as per the synthetic procedure given in Figure 11.23. Initially, in ethanol reflux conditions in the presence of ethanol, compound **164** (4,7-dichloroquinoline) was reacted with hydrazine hydrate to yield the intermediate compound **165**. Later in the second step,

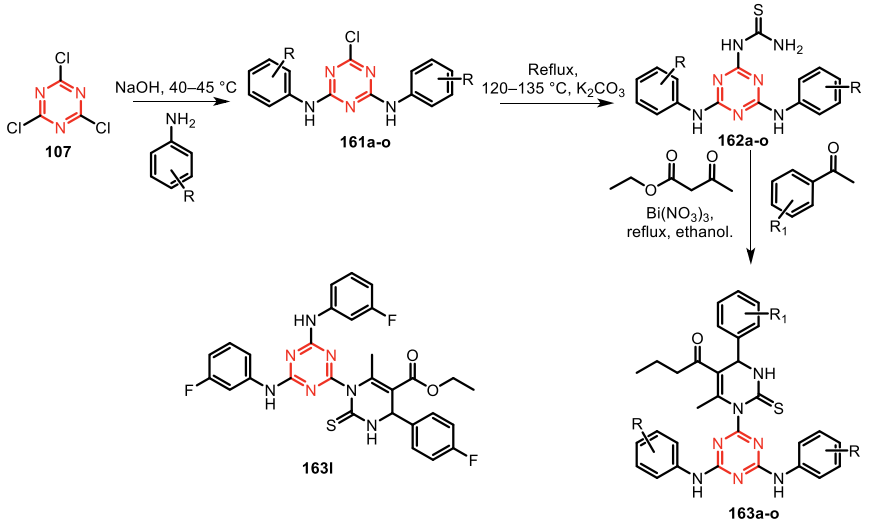


Figure 11.22: 1,3,5-Triazine – aniline – thiobarbituric acid coupled compounds.

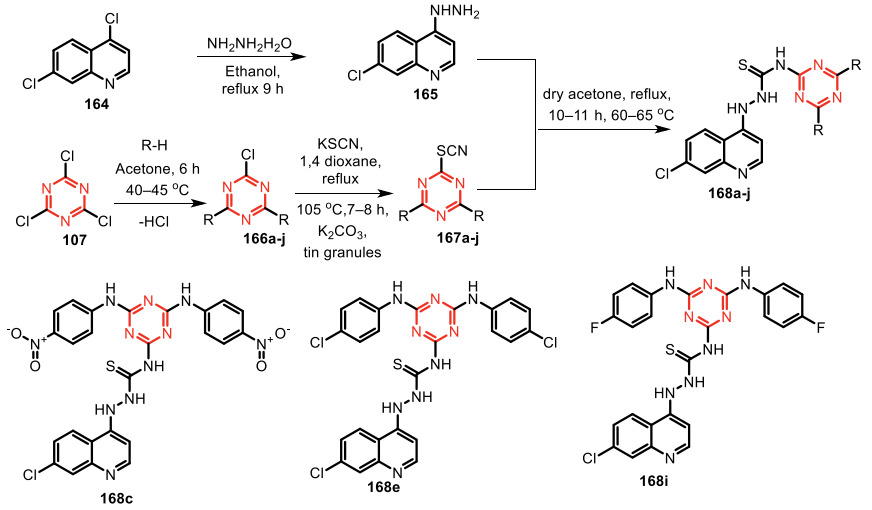


Figure 11.23: 4-Aminoquinoline - 1,3,5-triazine hybrids.

di-substituted 1,3,5-triazines **166(a–j)** were produced via nucleophilic substitution of various aliphatic and aromatic amines with Cl of the cyanuric chloride **107**. Further compounds **166(a–j)** were reacted with potassium thiocyanate by the nucleophilic substitution of Cl to afford 1,3,5-triazine derivatives with tri-substitutions **167(a–j)**. In the final step, the compounds **168(a–j)** were synthesized upon reacting compound **165** with **167(a–j)** in the presence of dry acetone. The anticancer activity of hybrid

4-aminoquinoline 1,3,5-triazine derivatives was tested against cancer cell lines HeLa, MCF-7, HL-60, and HepG2, with derivatives **168c, 168e**, and **168i** exhibiting considerable cytotoxicity and being nontoxic against MCF-12A. In the enzyme-based test, the compounds inhibited EGFR-TK more effectively than erlotinib [41].

The synthesis of the compounds **172a–e and 173a–g** were carried out as per the synthetic procedure shown in Figure 11.24. The first and second chlorine atom of cyanuric chloride **107** was replaced by nucleophilic substitution with amine (morpholine, piperidine, benzylamine, *N*-methyl benzylamine, or methoxy) at 0–5 °C and room temperature to afford di-chloro and mono-chloro derivatives, respectively. The intermediates di-chloro and mono-chloro derivatives were then processed for 2 h with hydrazine hydrate (80% in ethanol) under ultrasonic irradiation (US) to yield the hydrazine derivatives **170a–e** and **171a–g**, respectively. The obtained compounds **170a–e and 171a–g** were then reacted with acetylacetone to render the target products **172a–e** and **173a–g**, respectively. The synthesized pyrazolyl-s-triazine derivatives were tested in four human cancer cell lines, human breast carcinoma (MCF 7 and MDA-MB-231), hepatocellular carcinoma (HepG2), colorectal carcinoma (LoVo), and leukaemia (K562). It was observed that most of the s-triazine compounds were cytotoxic to the four human cancer cell lines, and compounds **172a** and **173g**, which possess piperidine moiety came out to be most efficacious with IC50 values of 5–9 μM. In K562 cells, the hit molecules **172a** and **173g** elicited cytotoxicity via S and G2/M

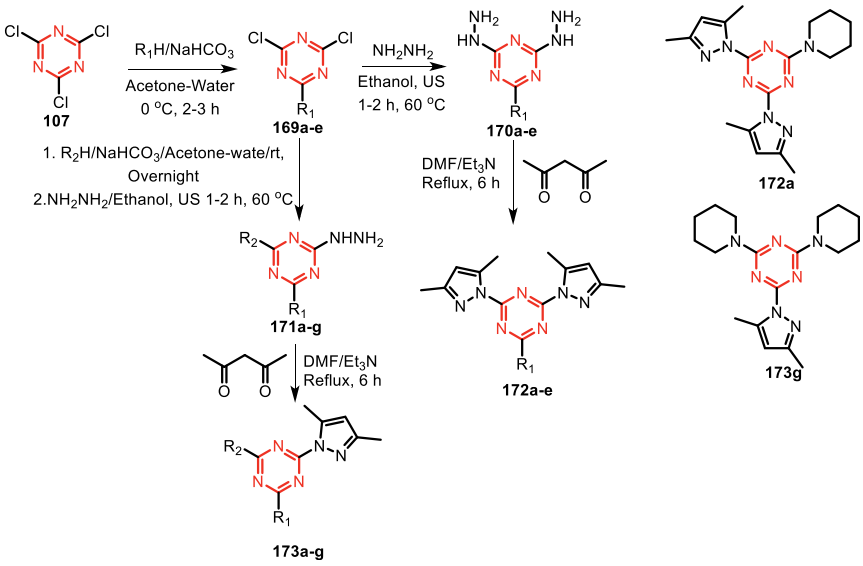


Figure 11.24: Pyrazolo – 1,3,5-triazine derivatives.

phase cell cycle arrest. The synthesized pyrazolyl-s-triazine derivatives were further examined in zebrafish embryos for *in vivo* and developmental effects. None of the derivatives were found to be deadly in the dose range tested [42].

Using a one-pot, microwave-assisted method a library of 126 compounds was ere prepared as shown in Figure 11.25. Cyanoguanidine **176**, aromatic aldehydes **175**, and arylamines **174** were condensed in the presence of HCl, further; the reaction mixture was treated with base, which encouraged a redisposition of the dihydrotriazine ring and its dehydrogenative aromatization. The created compounds were assessed for *in vitro* antiproliferative activity against DU145 (prostate-cancer) cell line and found that compounds **177a, 177b, 177c,** and **177d** showed more potency than standard nilotinib. Further, the antiproliferative properties of the prepared compounds were evaluated in normal cells as well as three (MDA-MB231, SKBR-3, and MCF-7) breast cancer cell lines. The results revealed that the compounds were selectively more toxic to the triple-negative MDA-MB231 breast cancer cells and the nontoxic to noncancerous MCF-10A breast cell line [43]. A 3D-QSAR model was built based on the structure-activity relationship studies of the synthesized compounds. Further, more potent anticancer compounds were designed and synthesized in one-pot, three-component condensation of cyanoguanidine, benzaldehydes, and anilines as shown in Figure 11.26. The compounds were tested for antiproliferative activity against three breast cancer cell lines (MDA-MB231, SKBR-3, and MCF-7), as well as noncancerous MCF-10A epithelial

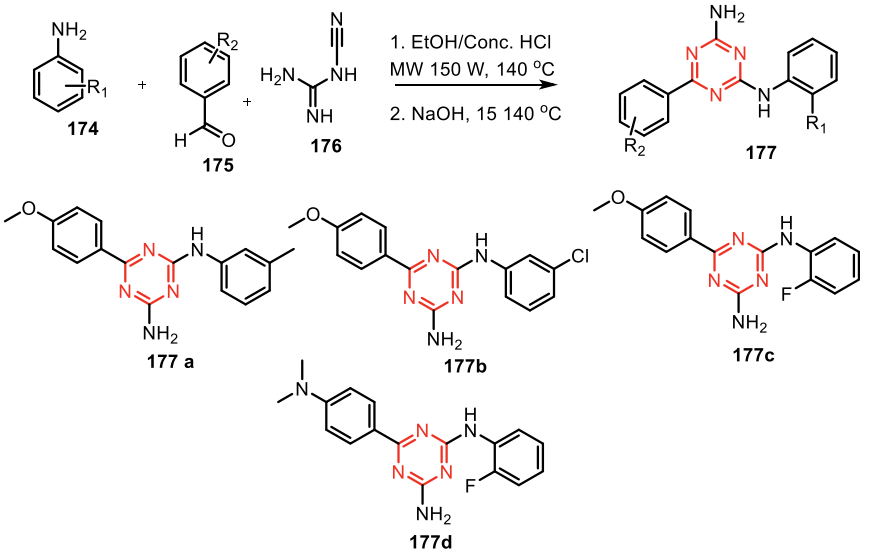


Figure 11.25: Diamino-1,3,5-triazine derivatives.

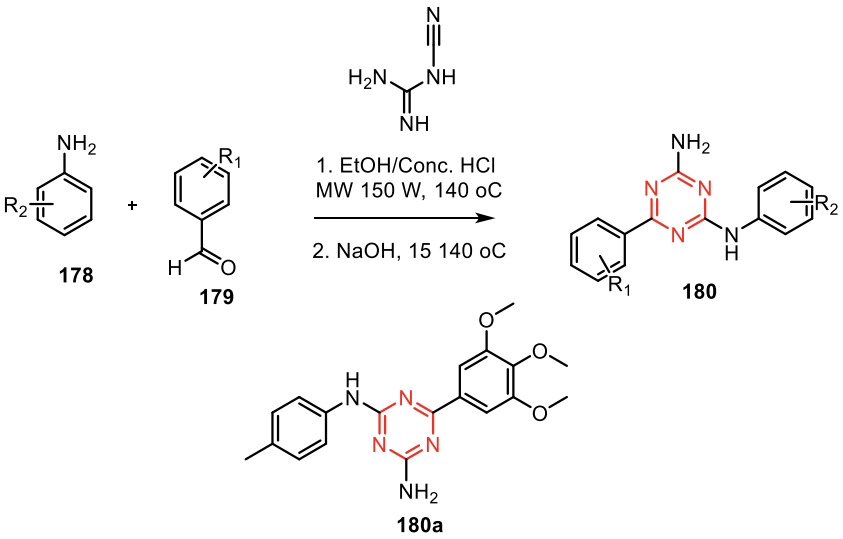


Figure 11.26: Diamino-1,3,5-triazine derivatives.

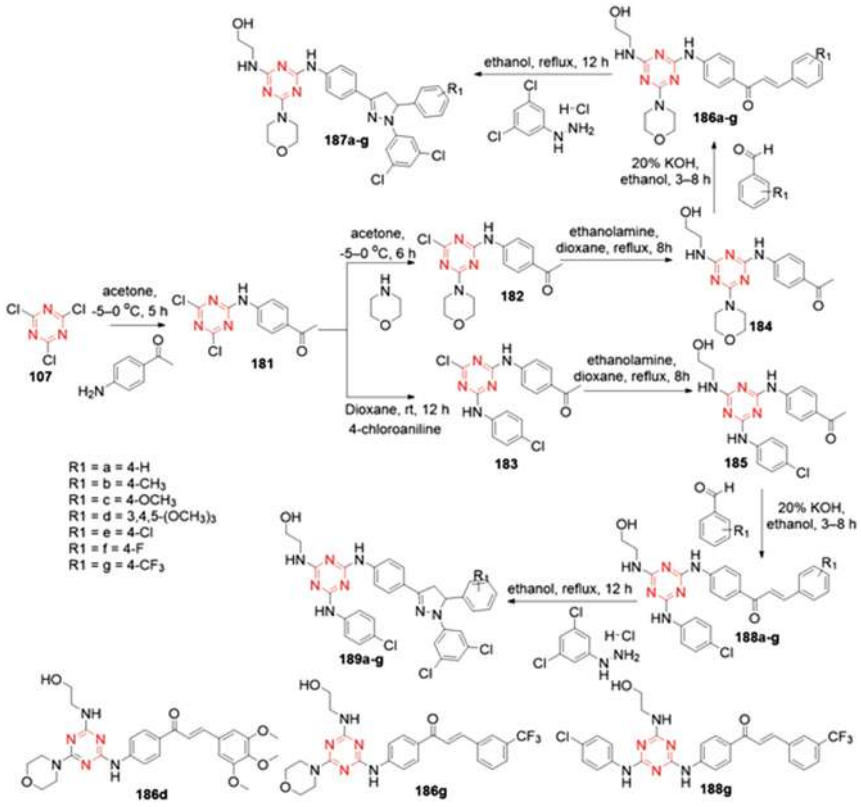


Figure 11.27: 2,4,6-Trisubstituted triazine derivatives.

breast cells, and it was discovered that the synthesized compounds had selective antiproliferative activity against triple-negative MDA-MB231 breast cancer cells over normal breast cells. The most active compound in the series inhibited MDA-MB231 breast cancer cell toxicity by apoptosis mechanism with a GI50 value of 1 nM [44].

The 1,3,5-triazine compounds with 2,4,6-trisubstitutions **187a–g** and **189a–g** were synthesized as described in Figure 11.27. Initially, the intermediates **184** and **185** were synthesized from 2,4,6-trichloro-1,3,5-triazine **107** by sequential nucleophilic substitutions: first with 4-aminoacetophenone, followed by morpholine or 4-chloroaniline, and finally with ethanolamine. Compounds **186a–g** and **188a–g** were obtained from triazine-ketones **184** and **185**, respectively, by Claisen–Schmidt condensation reaction with substituted benzaldehydes. Compounds **187a–g** and **189a–g** were produced by reacting the chalcones **186a–g** and **188a–g** with 3,5-dichlorophenylhydrazine hydrochloride in ethanol under reflux conditions. The prepared compounds were measured for their anticancer activity against nine different cancer strains and found that compounds **186d, g**, and **188g** displayed more potent *in vitro* anticancer activity, with GI50 0.422–14.9 μM and LC50 5.08 μM to >100 μM. Further mechanistic studies revealed that the hit compounds could modulate of the human thymidylate synthase enzyme [45].

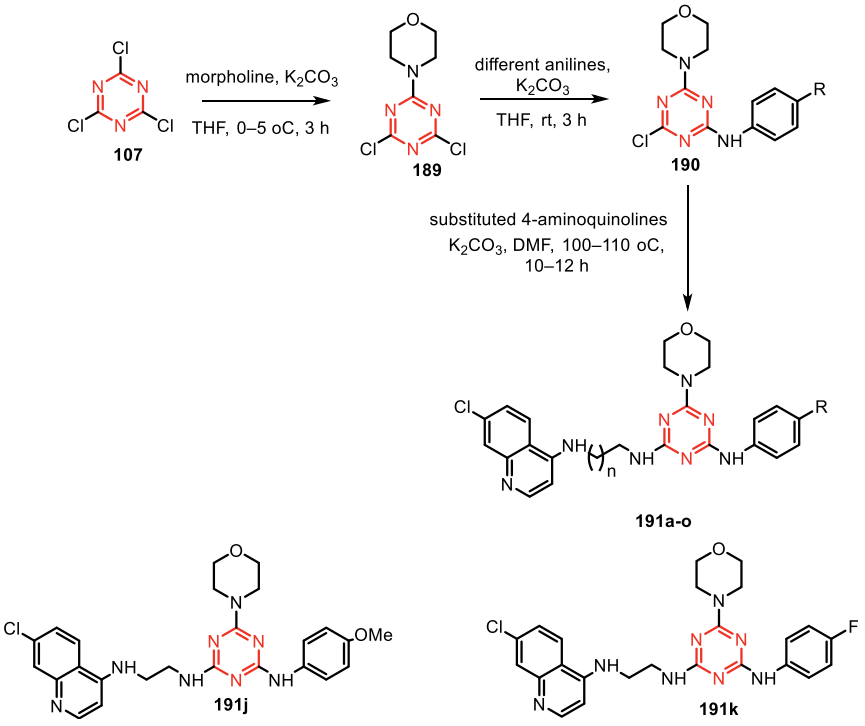


Figure 11.28: 7-Chloro-4-aminoquinoline – 1,3,5-triazine derivatives.

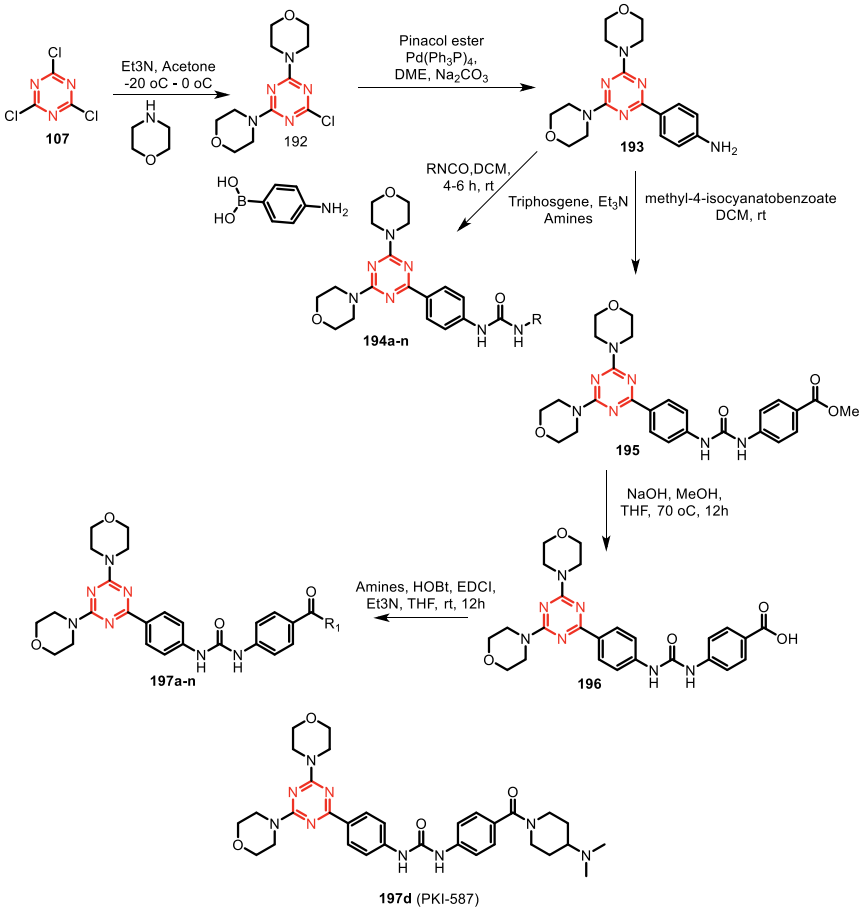


Figure 11.29: Dimorpholino-1,3,5-triazine derivatives.

As per the synthetic process described in Figure 11.28, targeted compounds were synthesized by sequential nucleophilic substitution reaction on commercially available cyanuric chloride **107**. In the first step, monosubstituted triazine **189** was synthesized by reacting compound **107** with morpholine. Further, it was utilized for the synthesis of disubstituted triazines **190** reactions with different anilines. In the final step 7-chloro-4-aminoquinoline-triazine hybrids **191a–o** were obtained by reacting 7-chloro-4-aminoquinoline with different carbon alkyl chain lengths with compound **190**. The synthesized compounds were screened *in vitro* against 60 human cancer cell lines (NCI 60) and found that compounds are causing cytotoxicity by apoptosis mechanism [46].

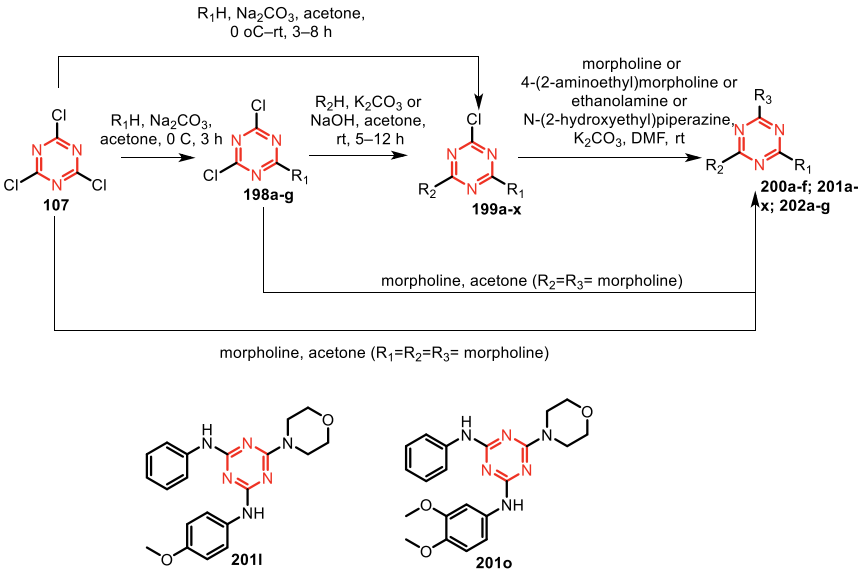


Figure 11.30: Morpholino, 1,3,5-triazine derivatives.

2,4-Dimorpholino-1,3,5-triazine derivatives **194a–n, 195, 196 & 197a–n** were synthesized as outlined in Figure 11.29. In the first step intermediate **192** was synthesized by reacting the cyanuric chloride **107** with 2 eq. of morpholine at 0 °C. Further, it was utilized for the synthesis of compound **193** by Suzuki coupling reaction with 4-aminophenyl boronic acid pinacol ester. Compounds **194a–n** and **195** were prepared from **193** by reacting with various alkyl, aryl isocyanates, and urea derivatives. Ester hydrolysis was carried out on compound **195** to get compound **196**. Further, the acid group of compound **196** was coupled with different amines to obtain **197a–n**. The synthesized compounds were screened for antitumor activity and found that compound **197d** has displayed splendid activity in *in vitro* assessments and *in vivo* models with antineoplastic efficacy in both subcutaneous and orthotopic xenograft tumor models when administered intravenously [47].

Compounds **200a–f; 201a–x; and 202a–g**, were synthesized as per the Figure 11.30. Initially, derivatives **198a–g, 199e, 199g,** and **199s** were synthesized by reacting cyanuric chloride **107** with commercially available arylamine (1 & 2 eq.).Compounds **198a–g** were reacted with arylamines, 4 eq., and 6 eq. morpholine to give compounds **199a–d, 199f, 199h–r, 199t–x 200a–e,** and **200f,** respectively. Subsequently, triaminotriazines **201a–x** and **202a–g** were synthesized by coupling the **199a–x** with the desired amines under basic conditions. The synthesized compounds were screened against colorectal cancer (CRC) cell lines (HCT-116 and HT-29) and found moderate antiproliferative activity at 10 μM concentration. From the *in vitro* results compounds

201l (0.76 μM) and **201o** (0.92 μM) were identified as hit molecules. Further compound **201l** was screened for *in vivo* antitumor efficacy and pharmacokinetic studies. It may be an encouraging new hit molecule that can be the basis to promote the development of antitumor agents [48].

11.2 Conclusions

The synthesis and their anticancer activities of various 1,2,3-triazines, 1,2,4-triazines and 1,3,5-triazines on different cancer cell lines was described. Particularly, triazine induced inhibition activity towards several types of kinases was reported. Active compounds were identified as hits and leads. A few compounds were taken path of trials. Quite a few triazine compounds are highlighted as possible promising candidates having therapeutic value. The synthetic procedures are simple, isolation of new chemical entites made easy and characterization using spectral data is also set in this chapter. Structure Activity Relations can be evolved with the various triazines reported and newer with better activity can be identified. This chapter provides the reader a confidence that one can explore triazines for medicinal value.

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12 Anticancer properties of arylchromenes and arylchromans: an overview

Abstract: Cancers are a set of pathologies originated by cells that have the ability to divide and multiply uncontrollably, associated with the capacity to invade and colonize adjacent tissues. Chemotherapy is one of the main approaches of treatment for cancer patients. Despite of the numerous antineoplastic drugs available, cancer cannot be cured; particularly at the late stages deprived of any side effect. Arylchromenes and arylchromans are a group of small molecules, of natural or synthetic origin, of great interest as prototypes for the drug development, especially against cancer. In this chapter, we will present the antineoplastic activity studies of the most promising examples of these arylchromenes and arylchroman derivatives.

Keywords: arylchromans; arylchromenes; anticancer properties.

12.1 Introduction

Cancers are a set of pathologies originated by cells that have the ability to divide and multiply uncontrollably, associated with the capacity to invade and colonize adjacent tissues [1, 2]. The pathophysiological factors that contribute to the severity of cancers are: the genomic instability that allows the propagation of mutations through cell lines; the ability of unlimited cell replication; the dysregulation of cellular energy metabolism that contributes to neoplastic proliferation; the induction of pro-inflammatory signaling pathways; the ability to induce angiogenesis in the tumor microenvironment; the activation of tissue invasion and pro-metastatic mechanisms; the resistance to cell

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death by suppressing intra and extracellular pro-apoptotic mechanisms; and, finally, the evasion of both the growth-suppressive signaling pathways and the cell death by the immune system [3, 4].

According to the report published in 2020 by the World Health Organization, 18.1 million new cancer cases were reported in 2018, which caused the death of 9.6 million people. This number corresponds to about one-sixth of the total deaths worldwide, corresponding to one of the biggest causes of premature death for most countries. In 2018, the cancers with the highest incidence were lung cancer with 11.6% of cases; breast, with an equal percentage; colorectal, 10.2%; and prostate, with 7.1% of cases. The cancers with the highest mortality, in the same period, were lung cancers, with 18.4% of deaths; colorectal, 9.2%; and stomach and liver with 8.2% of deaths attributed to each. According to that same report, it is estimated that, in 2040, the number of new cases of cancer will reach 29.4 million [5].

Currently, there are several drugs approved for the treatment of neoplastic diseases. These drugs can be classified according to their mechanism of action. They are: anti-metabolites; DNA adduct-forming agents; enzyme inhibitors responsible for gene duplication, transcription and expression; receptor inhibitors or blockers of mitogenic pathway signaling; or disrupting agents of microtubule functions [6]. Despite the therapeutic success that these classes can provide to patients in cancer remission, some drugs have a small therapeutic index, thus contributing to the emergence of harmful toxic effects [6]. Furthermore, many cancers can show *intrinsic resistance* to a class of drugs, or they can develop with the course of treatment by the induced selection of a subpopulation of mutant cells, also called *acquired resistance* [7]. Resistance to anticancer drugs can occur due to: changes in pharmacokinetic properties, such as absorption, distribution, metabolism and elimination of the drug; or to genetic alterations, for example, amplification, depletion and mutation of genes for therapeutic targets or transporters, such as efflux pumps [6, 7].

In this sense, arylchromenes and arylchromans are a group of small molecules of natural or synthetic origin, of great interest as prototypes for the drug development, especially against cancer. This interest, in addition to being linked to the synthetic facility of these substances, is associated with their high efficacy and selectivity against cancer cells, especially multidrug-resistant lines, as well as their low toxicity in *in vitro* studies [8, 9]. Arylchromenes and arylchromans can be defined as tricyclic substances containing a pyran ring (2*H*-pyran, 4*H*-pyran or dihydropyran) condensed with a benzene ring, in addition to an aryl group linked to positions 2, 3 or 4 (Figure 12.1). In this chapter, we will present the antineoplastic activity studies of the most promising examples of these arylchromene and arylchroman derivatives.

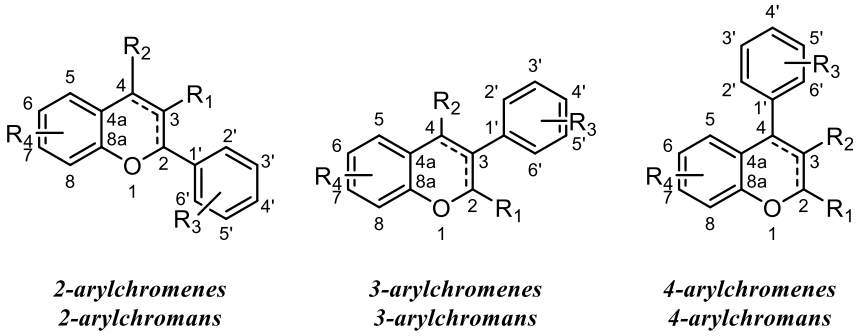


Figure 12.1: Common chemical structure of arylchromene and arylchroman derivatives.

12.2 2-Arylchromene and 2-arylchroman derivatives

The 2-arylchromene and 2-arylchroman derivatives are widely known for their biological properties. They can be obtained from synthetics approaches; however, most compounds in these classes are from natural sources. Flavans, homoisoflavonoids, epicatechin and epigallocatechin are examples of 2-arylchromans, which are abundantly present in green teas and found in a wide variety of plants. The biological activities of such natural products have been well known since a long time and the most representative ones included the antioxidant [10], antibacterial and antibiofilm [11] and anticancer [9] activities. In this session, we will, particularly, address the anticancer activity of 2-arylchromenes and 2-arylchromans, showing the most relevant results on their antiproliferative activity in different cancer cell lines.

As previously mentioned, 2-arylchrome(a)nes were isolated from plants that are popularly known to have medicinal effects, including in the treatment of cancer. In this sense, based on these natural anticancer 2-arylchrome(a)nes many derivatives have been synthesized so far. For instance, Rahmani–Nezhad and collaborators [12] synthesized and evaluated the antiproliferative activity of a series of 2-aryl-3-nitro-2*H*-chromenes in breast cancer cells MCF-7, T-47D, and MDA-MB-231, using etoposide as a positive reference. In general, all the synthesized compounds proved to be as potent as or more potent than the control drug in inhibiting the breast cell lines. For MCF-7, the 4-chloride derivative **1** and the 4-methoxy derivative **2** (Figure 12.2) were the most active, exhibiting GI_{50} values of 0.2 and 1.6 μM, being 36 and 4.5 times more potent than etoposide (GI_{50} of 7.2 μM), while for T-47D cells, the 3,4,5-trimethoxyphenyl derivative **3** (Figure 12.2) showed greater activity (GI_{50} of 2.1 μM). Regarding the MDA-MB-231 cells, the 3,4-dimethoxyphenyl and 4-nitrophenyl derivatives **4** and **5** (Figure 12.2), with GI_{50} of 0.4 and 0.5 μM, respectively, were 32.0 and 25.6 times more active than etoposide, proving promising anticancer activity against the cell lines evaluated.

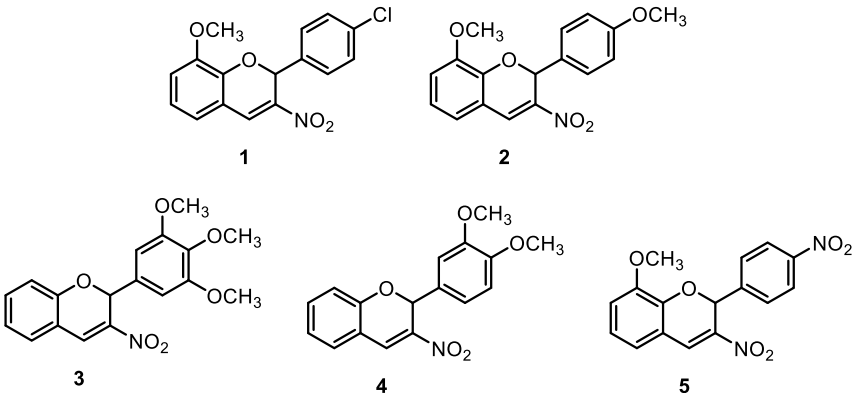


Figure 12.2: Structures of the most active 2-arylchromenes against breast cancer cells, according to Rahmani-Nezhad.

Additionally, compounds **1** e **2**, which possessed the greatest cytotoxicity against MCF-7, were chosen to assess whether cell death occurred by apoptosis or necrosis pathway, using acridine orange/ethidium bromide (AO/EB) staining, since the AO dye penetrates into living and dead cells and emits green fluorescence, whereas EB penetrates only into dead cells and emits red fluorescence after intercalation into DNA, and dominates over AO. Thus, living cells have normal green nuclei, whereas apoptotic cells have orange condensed and fragmented chromatin while cells that died from direct necrosis have a structurally normal orange nucleus. AO/EB staining analysis indicated that cells treated with the selected compounds **1** e **2** exhibited apoptotic events, resulting in reduced cell viability in the human breast cancer cell lines evaluated [12]. Furthermore, these compounds were also evaluated in caspase-3 activation in MDA-MB-231 cells. Caspase-3 is considered to be the most important of the executioner caspases and plays a direct role in proteolytic cleavage of the cellular proteins responsible for the progression of apoptosis [13]. A 3- to 4-fold increase was observed in protease induction in cells treated with the selected compounds, suggesting that the mechanism of cell death caused by 2-aryl-3-nitro-2*H*-chromenes occurs in a caspase-3 dependent manner [12].

2-Arylchroman derivatives also exhibit promising antiproliferative activities against different human cancer cells. In work published in 2016, Sun and collaborators isolated twenty-one flavans from the stem and root bark of *Daphne giraldii*, of which thirteen were not described previously in the literature [14]. The compounds were evaluated against five human cancer cell lines, including MCF-7 (breast cancer), Bcap37 (breast adenocarcinoma), HepG2 (hepatocellular carcinoma), Hep3B (hepatocellular carcinoma) and A549 (lung adenocarcinoma). Among all arylchromans tested, five (Figure 12.3) exhibited higher cytotoxic effect than the positive control 5-fluorouracil against Hep3B cells, with GI_{50} values ranging from 5.15 to 9.66 μM. In order to

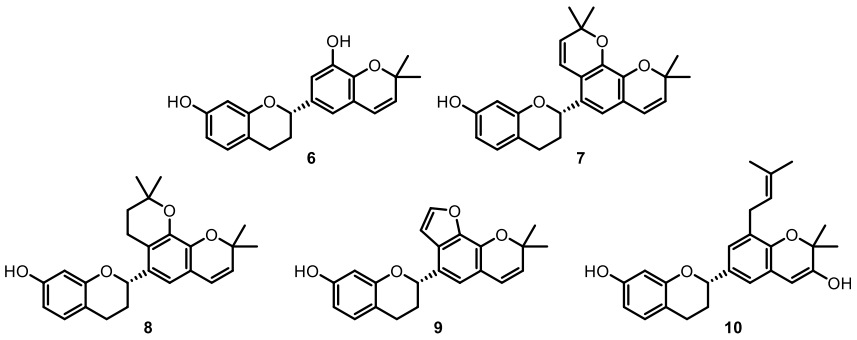


Figure 12.3: Chemical structure of flavans isolated from *D. giraldii* that showed to be potent anticancer agents.

determine a possible mechanism of action for the above-mentioned compounds, studies were carried out to check if the cell division is compromised when the cells were treated with such arylchromans and it was observed that the substances **6**–**9** increase the percentage of Hep3B cells at the G_2/M phase. Interestingly, the same was not observed for the compound **10**, which, in turn, caused a significant increase in sub-G_1 phase cells. Further experiments also showed that the treatment with **10** leads to an increase in early and late apoptotic cells [14].

Flavanols isolated from *Acacia hydaspica* (Figure 12.4) have also been described as anticancer compounds able to inhibit the growth of PC-3 and MDA-MB-231 human cancer cell lines. In general, all compounds tested inhibited cell survival and growth and there was evidence of chromatin condensation, cell shrinkage and apoptotic bodies. Compounds 7-*O*-galloyl catechin (**11**), catechin (**12**), and catechin-3-*O*-gallate (**13**) inhibited prostate cancer PC-3 cell growth in a dose-dependent manner, whereas **13** inhibited the growth of breast cancer MDA-MB-231. After 24 h treatment with 50 μM of **11**, the per cent survival of PC-3 cells was 45.5%, while for MDA-MB-231, the compound **13** at a dose of 50 μM, promoted an 84.5% inhibition of cell viability after 48 h exposure. Phase-contrast microscopy and AO/EB staining analyzes performed on cells treated with the flavonols showed low cell confluence and membrane blebbing, in addition to

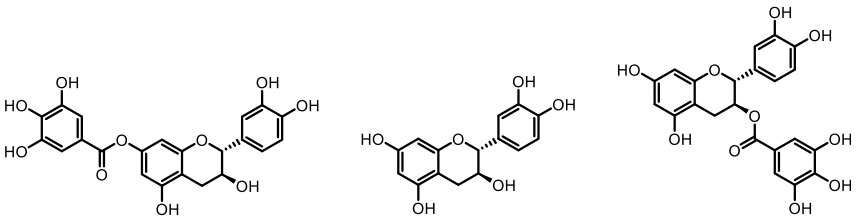
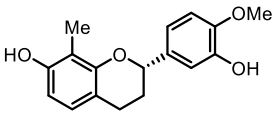


Figure 12.4: Structures of flavanols isolated from *A. hydaspica* with a promising anti-cancer activity.

highly fragmented and condensed chromatin, indicating that the cell growth inhibition is due to apoptosis [15]. In addition, chromans **11**–**13** exerted influence on several signalling factors, such as PI3-K, JAK2, Akt-1, STAT3, MAPK (ERK1/2, pERK1/2), NFκB, Bcl-xL and survivin. Moreover, it was also demonstrated that these compounds significantly suppress cell proliferation and sensitize cells to apoptosis induction which in PC-3 cells is associated with decreased expression of Bcl-2 and Bcl-xL, whereas in MDA-MB-231 cells Bcl-xL expression was reduced significantly without a significant change in Bcl-2 expression. In addition, the significant inhibition of survivin expression by these flavans provides further support for their potential role in chemoprevention or therapeutic activity [15].

Two flavans isolated from *Dracaena cambodiana* (Figure 12.5) had their cytotoxicity evaluated against leukaemia (K562) and gastric (SGC-7901) cancer cells, using mitomycin C as a reference drug. Compound **14** was more potent than the control drug in inhibiting the proliferation of K562 cancer cells, having an GI_{50} value of 5.0 μg mL^{-1}, compared to 7.1 μg mL^{-1} exhibited by mitomycin C [16].

Epigallocatechin (**16**, Figure 12.6), a substance found in large amounts in green teas, is well known for its biological properties [17]. In 2002 Vergote and collaborators studied the antiproliferative action of **16** in two different breast cancer cells lines and demonstrated that epigallocatechin strongly inhibits the growth of cells in a dose-dependent manner. A reduction in MCF-7 and MDA-MB-231 cell viability by 70% and 90%, respectively, was observed when these cells were exposed to **16** at 100 μM. On the other hand, no cytotoxic effect was observed on normal breast epithelial cells. Interesting, epicatechin, **17**, another green tea polyphenol, did not inhibit the growth of breast cancer cells. Thus, the presence of a third hydroxyl group in the pyrogallol moiety seems to be fundamental for the antiproliferative activity exerted by epigallocatechin in these cancer cells [18].



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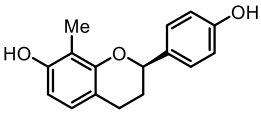

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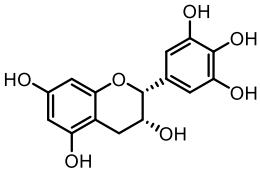
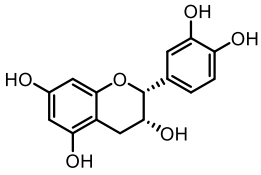
Figure 12.5: Flavans isolated from *D. cambodiana* that inhibit the growth of leukemia and gastric cancer cells.



16 (epigallocatechin)



17 (epicatechin)

Figure 12.6: Chemical structures of polyphenols epigallocatechin (**16**) and epicatechin (**17**).

Regarding the mechanism of action in cell lines, Vergote and collaborators observed that **16** does not change the progression of the cell cycle, as it was not verified any difference in the proportions of cells in the G_1, S, and G_2/M phases in the presence and absence of 100 μM of the natural product. However, at the same concentration, epigallocatechin induces apoptosis of 50% and 40% in MCF-7 and MDA-MB-231 cells. Furthermore, Vergote and collaborators report that epigallocatechin-triggered apoptosis may involve Fas signalling, as anti-Fas neutralizing antibody partially inhibited cellular death. The Bcl-2 and Bax levels were also evaluated and it was observed that Bcl-2 was decreased in MCF-7 cells whereas the level of Bax was increased after treatment with 100 μM of **16**. These results are very interesting since the ratio between Bcl-2 and Bax determines survival or cell death by modulating the release of proapoptotic factors such as cytochrome *c* from the mitochondria to the cytoplasm [18].

The antiproliferative activities of **16** and **17**, together with the others flavan-3-ols also extracted from green tea, gallocatechin **18**, epigallocatechin gallate **19**, epicatechin gallate **20** and gallocatechin gallate **21** (Figure 12.7), were also investigated against stomach cancer cells MK-1 by Kinjo and collaborators, employed the MTT assay [19]. The results showed that epigallocatechin **16** acts moderately in the reduction of cell viability in MK-1 cells (GI_{50} 14 μM), as well as its *trans* isomer **18**, indicating that the *cis*/*trans* configuration is not an essential issue to the action of these molecules in the evaluated cell line. The isomers epigallocatechin gallate **19** and gallocatechin gallate **21,** with GI_{50} values of 9 μM and 10 μM, respectively, also showed no significant difference in their cytotoxic activities against the MK-1 cells. Furthermore, it was also observed that the replacement of OH by the gallate group increases the cytotoxic potential, since compounds **19** and **21** exhibited lower GI_{50} values compared to compounds **16** and **18**. And, as seen for other tumor cell lines, the antiproliferative result for

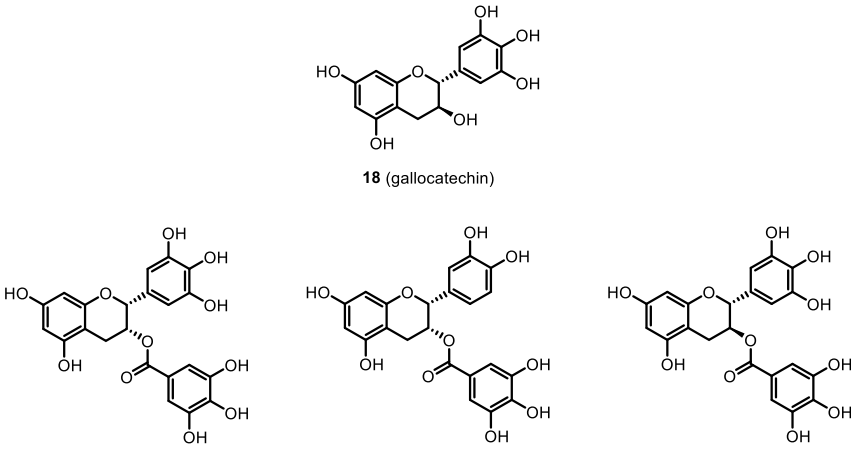


Figure 12.7: Structures of others flavan-3-ols evaluated against MK-1 cell line.

epicatechin **17** was the lowest found (GI_{50} 45 μM), indicating that the tri-hydroxylated B ring is also crucial for the action of these compounds in MK-1 cells [19].

Additionally, DPPH radical scavenging activity tests were executed and, as expected, the compounds containing a galloyl moiety showed more potent activity. In contrast, the contribution of the pyrogallol moiety in B-ring was less pronounced, although both moieties were a key factor in enhancing the antiproliferative activity [19].

A concise review about the activities of epigallocatechin gallate in signaling pathways in tumor cells has been presented by Sharifi-Rad and collaborators, in 2020, in which the effects of **19** on cell death mechanisms via the induction of apoptosis, necrosis and autophagy are reported [20].

Several biological properties have been reported for the genus *Plicosepalus*, including antihepatotoxic [21], anti-diabetic and cytotoxic activities [22]. In this context, Fawzy et al. evaluated the antiproliferative properties of two flavan gallates (**22** and **23**, Figure 12.8), isolated from *Plicosepalus curviflorus*, against five cancer cell lines including MCF-7, HepG-2, HCT-116, Hep-2 and HeLa [23]. Both compounds showed moderate cytotoxic activities against all cell lines; however, flavan **22** was slightly more active, with GI_{50} values ranging from 11.6 to 38.8 μg/ml, while the values of 39.8 to above 50 μg/ml was observed for **23**. Structurally, flavans differ by the presence of a galloyl portion at the 4′ position and an additional OH group at 5′ position of the B ring. Thus, it is possible to infer that, despite the antiproliferative activity is only moderate, the presence of a gallate group in the B ring hinders the role of these compounds in the cell death of cancer cells. Breast (MCF-7), liver (HepG-2) and colon (HCT-116) cells lines showed to be more sensitive to compound **23** than the laryngeal (Hep-2) and cervical (HeLa) cell lines. Furthermore, in the study in normal cells (Vero) the compounds exhibited low cytotoxicity, which makes them safe for normal cells while cytotoxic to the cancerous cells studied [23].

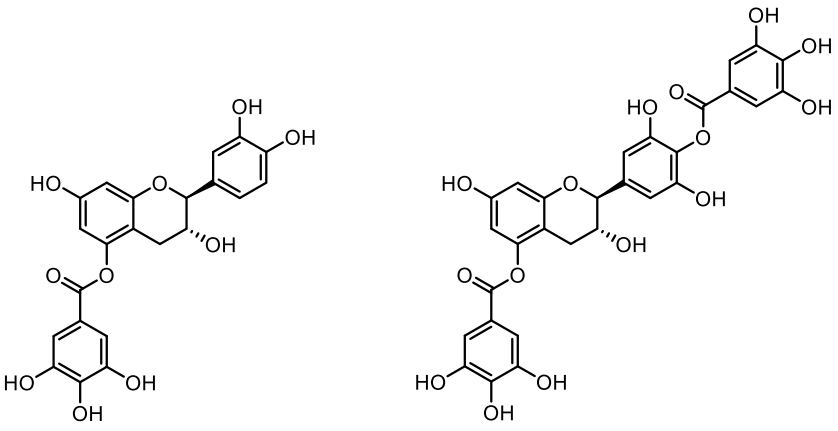


Figure 12.8: Flavans isolated from *P. curviflorus* which present promising antiproliferative activities.

In 2013, Fujii and collaborators [24] described the total synthesis of the bis-arylchromans prodelphinidin B1, B2 and B4 (Figure 12.9) and evaluated their antiproliferative activity against PC-3 prostate cancer cells. The antiproliferative activity of epigallocatechin gallate **19** and prodelphinidin B3 **26** were used as positive controls. The studies demonstrated that the synthesized bis-chromans acted in a concentration-dependent manner in PC-3 cells and reached significant cytotoxic activity, with GI_{50} values below 50 μM. Prodelphinidins B1, B2, and B4 exhibited greater antiproliferative activity than prodelphinidin B3 and the presence of an extra pyrogallol group in the former may be the reason for the improvement in anticancer activity under PC-3 cells. Compared to **19**, the synthesized molecules were also slightly more potent. More comprehensive studies on the cytotoxicity of these compounds are needed, however, it is undeniable that the results are encouraging.

Structurally similar to prodelphinidin B2 (**25**), the two-unit gallate-group analogue prodelphinidin B2 3,3′-di-*O*-gallate (**28**, Figure 12.10) had its antiproliferative activity in lung cancer cells (A549) investigated by Kuo and collaborators [25]. Indeed, compound **28** decreases cell viability in a concentration-dependent manner and shown to be potent against the A549 cell line, exhibiting an GI_{50} value of 5.2 μM and a maximal proliferation effect of 79% by 10 μM at 72 h. The influence on cell cycle arrest and apoptosis was evaluated, and growth in cell population in the G_0/G_1 phase of 63.9%

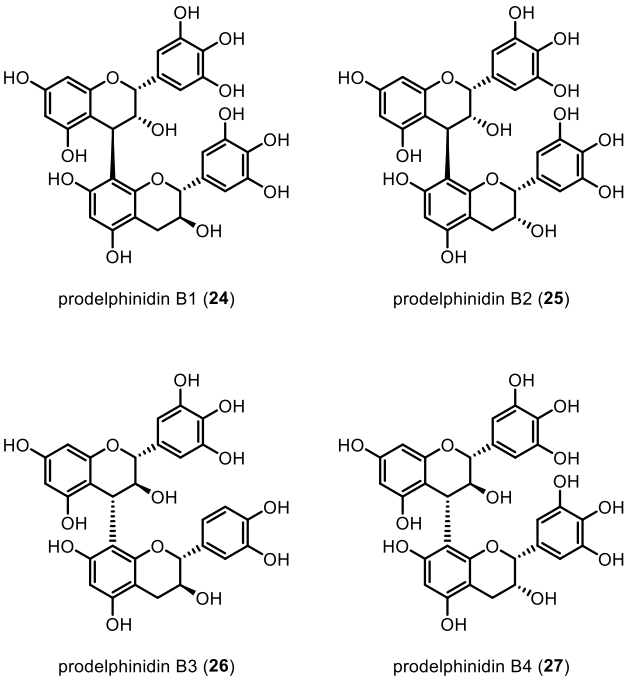


Figure 12.9: Chemical structures of prodelphinidin B1–B4.

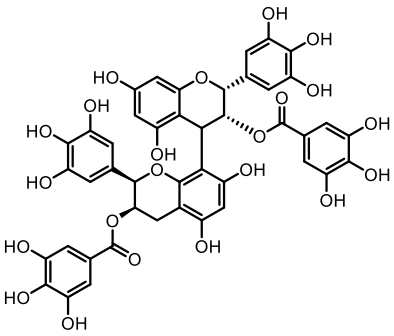


Figure 12.10: Structure of prodelphinidin B2 3,3′-di-*O*-gallate.

was observed. In addition, DNA fragmentation in cells was noted with continuous treatment with prodelphinidin B2 3,3′-di-*O*-gallate at 5 and 10 μM, with an increase in the number of cells undergoing apoptosis of 6.1–10.2-fold. The results showed that the presence of **28** interrupts the cell cycle in the G_0/G_1 phase and the authors suggest that this interruption is due to increased amounts of $p21^{WAF1}$ protein. This protein inhibits the activity of several cyclin-dependent kinases, in addition to phosphorylation of the retinoblastoma protein, which, in turn, leads to inhibition of the G_1-S phase [26, 27]. According to Nagata, Fas/FasL system is the key to the transduction pathway of apoptosis in cells and tissues. Furthermore, the binding of Fas by agonistic antibody leads to receptor oligomerization and death signalling the complex formation which follows in order to activate caspase-8 which triggers the activation of other caspases leading to apoptotic cell death [28]. In this work the authors reported that there was an increase in the expression of Fas/APO-1 receptor and soluble/membrane-bound Fas ligand levels and in the caspase-8 activity in A549 cells treated with prodelphinidin B2 3,3′-di-*O*-gallate. Additionally, when the Fas/FasL system was blocked by antibody ZB4, it was observed a decrease in cell growth inhibition and pro-apoptotic effect of **28**. Thus, the authors conclude that prodelphinidin B2 3,3′-di-*O*-gallate induces apoptotic cell death by the initiation of the Fas/FasL death receptor system in A549 cells, being a promising anticancer agent [25].

Bisflavanols also exhibited anticancer activity in S180 sarcoma cells. Zhang and collaborators [29] demonstrated that daphnodorin D1 and D2 (**29** and **30**, Figure 12.11) have significant anticancer activity. *In vivo* assays performed with female Kunming mice showed that these compounds are able to inhibit the growth of sarcoma S180 cell viability by 43% and 45%, respectively, at a concentration of 10 mg/kg. However, the mechanism of action has not been studied.

In summary, examples of 2-arylchromenes and 2-arylchromans, from the most varied structural skeletons, were presented, such as their potential as anticancer agents for a wide range of cells. Many of them show promising behavior in the

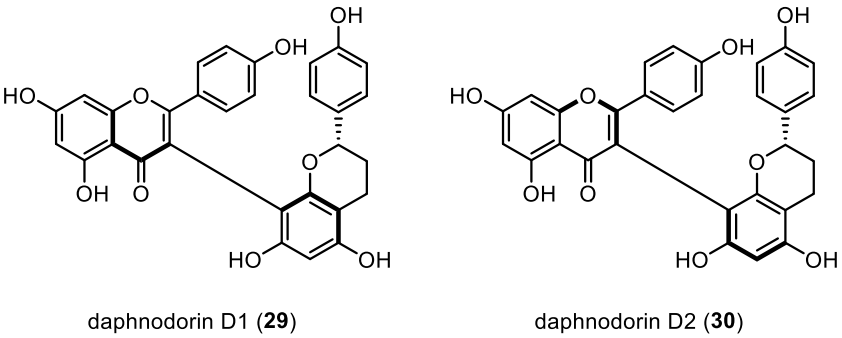


Figure 12.11: Chemical structure of daphnodorin D1 and D2.

treatment of cancer and can also be designed for joint treatment with drugs already approved. However, it is not possible to safely present a comparison between them due to the particularities of each experiment and the uniqueness of how they are performed. More comprehensive studies would be needed so that a point of view of comparing potentials and promising uses could be seen with clarity and richness of details.

Nevertheless, it is undeniable that we have a vast library of 2-arylchromenes and chromans, natural and synthetic, which present exciting possibilities in the treatment of cancer.

12.3 3-Arylchromene and 3-arylchroman derivatives

As with the 2-aryl motif, chromans and chromenes 3-aryl substituted compounds are comprised of naturally occurring molecules, such as licoridicin, equol and phenoxodiol, and synthetic ones, such as centchroman, in various stages of development.

Licoricidin (**31**, Figure 12.12), an isoprenylated polysubstituted isoflavane founded in licorice extracts (Glycyrrhiza sp.), exhibited potent anti-proliferative activity against multiple colorectal cancer cell lines, such as LoVo, HCT116 and the metastatic pair SW480 (primary) and SW620 (lymph node), all with GI_{50} below 10 μM (Table 12.1) [30, 31]. There are also reports of **31** inhibiting metastasis of DU145 prostate cancer cells

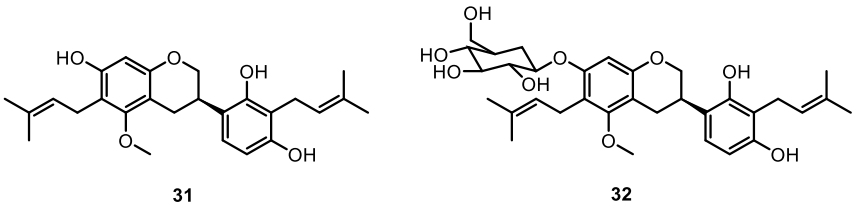


Figure 12.12: Licoricidin (**31**) and its glucoside (**32**) structures.

Table 12.1: Licoricidin anti-proliferative activity and growth inhibitory concentrations for 50% (GI_{50}).

Cancer type	Cell line	GI_{50} (μM)	Reference
Colorectal	SW480	7.2	[30]
	SW620	4.5	
	HCT116	5.4	
	LoVo	5.1	

and 4T1 mammary cells [32, 33]. A more detailed study of licoricidin on SW480 cell line showed that the isoflavane delayed cell cycle, arresting in G_1/S, accompanied by a reduction in levels of cyclins B1, and CDK1 dose- and time-dependent.

In addition to arresting cell mitosis, **31** also promoted cell death due to both apoptosis and autophagy. After treatment, caspase-3 was activated together with increased levels of cleaved PRAP, a substrate of caspase-3, and diminished concentration of Bcl-2, a pro-survival protein, therefore resulting in apoptotic cell death. Similarly, cleavage of LC3, a protein presented in the autophagosome membrane and one indicator of autophagy, was increased. This route is also supported by activation of AMPK concurrently with dephosphorylation of PKB, resulting in mTOR pathway inhibition, followed by activation of ULK1 – initiating autophagy [30]. Solubility of **31** can be improved by glycosylation, forming a 7-*O*-β-D-glucoside (**32**, Figure 12.12) [34], and its toxicity against SW480 cells, in *in vivo* xenograft model, remained unchanged. Both **31** and **32** displayed comparable activity to the control, etoposide, albeit with lesser model's weight loss, an indication of diminished toxicity against normal cells.

The anti-metastatic capabilities of **31**, appears to be due to lower secretion of MMP-2 and MMP-9, metalloproteinases associated with cell motility, diminished expression of integrin-α2, ICAM-1 and VCAM-1, associated with cell adhesion, reduced VEGF-A/R2 and CD31 secretion, related to angiogenesis, decreased lymphangiogenesis, through LYVE-1, VEGF-C/R3, and increased levels of both TIMP-1 and 2, tissue inhibitors of MMPs. Furthermore, licoricidin caused a decrease in inflammation related markers, HIF-1α, iNOS and COX-2, reduced and macrophage infiltration markers, CD45, F4/80 and CD203 [32, 33]. Although being capable of impeding cell metastasis, licoricidin did not alter 4T1 and DU145 cell viability at these lower concentrations.

Other bioactive isoflavane is equol (**33**, Figure 12.13), a selective estrogen receptor modulator (SERM) in which the *S* isomer is ERβ selective whereas the *R* enantiomer is ERα selective [35].

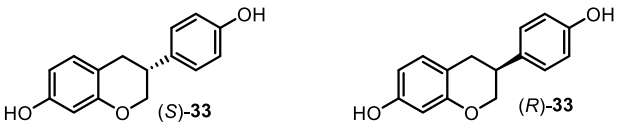
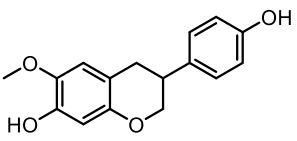


Figure 12.13: Equol isomers, *S* and *R*.

The effect of equol on prostate cancer is moderately weak, with GI_{50} over 100 μM. At these high doses, however, equol activated and upregulated the FOXO3a, a transcription factor and suppressor of primary tumor growth through upregulation of p21 and p27 cell-cycle inhibitors and upregulating pro-apoptotic proteins such as FasL and Bim. Equol also maintained FOXO3a in its active non-phosphorylated nuclear form by inhibiting the PI3K/PKB pathway, thus resulting in cell arrest in G_2/M phase following treatment in high dosages [36]. On MGC-803 gastric cancer cells, equol, here as a racemate, exhibited very similar behavior as previously, but its time-dependency became clearer; at a 24 h span from the treatment, the GI_{50} of **33** was 180 μM, but at 48 h the inhibitory concentration decreased more than ten-fold to 14 μM [37]. The cell cycle was also arrested, but in G_0/G_1 phase with downregulation of cyclins D1 and E1, CDKs 2 and 4, furthermore, the percentage of apoptotic cells was increased, as did the levels of caspase-3 and PARP. In cervical cancer HeLa cells, equol time- and dose-dependently induced cell apoptosis. The isoflavane promoted activation of caspase-8/9 alongside increased ROS generation, disruption of mitochondrial membrane polarization, downregulation of Bcl-2 and upregulation of Bax – pro-apoptotic protein – therefore increasing the Bax/Bcl-2 ratio, a marker for intrinsic apoptosis pathway [38].

Breast cancer, however, is the most studied anti-proliferative target of equol, with several cell lines investigated, like the oestrogen positives MCF-7, $ErbB2^+$ and MDA-MB-453 lines, triple-negatives (ER^-, PR^-, $ErbB2^-$) MDA-MB-468/231 and HS578t. The results are somewhat conflicting, ranging from having anti-cancer properties to being oncogenic depending on the concentration of the compound and cell line – a more detailed systematic review of these findings is available [39]. In MCF-7 line, **33** increased the expression of miR-10a-5p, downregulated microRNA in cancer that post-transcriptionally mediates gene expression, that in turn inhibited the expression of PI3K p110α, thus inhibiting the activation of PKB and therefore downregulation the PI3K/PKB pathway and leading to an increase in caspase-3/9 activity and apoptosis [40]. In MDA-MB-453 cells, equol activated the intrinsic apoptosis pathway, with cytochrome *c* release and caspase activation [41]. Cell death can also be triggered by cell cycle arrest, in which MDA-MB-453 cells were markedly more sensible than MCF-7 ones, the latter's proliferation being promoted at low concentrations of **33** due to ER binding [42]. Other effects include the diminished invasive capabilities of MDA-MB-231 cells though downregulation of MMP-2 [43] and radiosensitization of both ER^+ and ER^- cells [44].



34

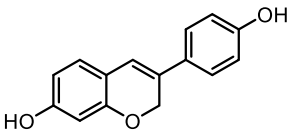
Figure 12.14: 6-Methoxyequol structure. Chiral center not assigned.

An analogue of equol, 6-methoxyequol (**34**, Figure 12.14) displayed specific antimitotic properties against FGF2-induced endothelial cell proliferation, GI_{50} of 3 μM, while being inactive against HeLa, T24 (bladder carcinoma) and MCF-7 cells. **34** exerts its activity by inhibiting VEGF/FGF-2 induced phosphorylation of MEK1/2 and its downstream kinases, ERK1/2, moreover the induction of DUSP1/5, phosphatases that act as regulators of ERK, were blocked; however, no alterations were detected in the PI3K/Akt pathway or VEGF survival mechanisms. *In vivo* evaluation of **34** in A-431 (epidermoid carcinoma) xenograft model showed poor oral bioavailability, but direct injection to the tumor lead to significant size reduction of the malignancy and increased survival [45].

Another structural analogue of equol, phenoxodiol or idronoxil (**35**, Figure 12.15), is an isoflav-3-ene and proposed intermediary on the biotransformation of daidzein to equol, being also found naturally in *Lespedeza homoloba*, receiving the name of haginin E [46, 47]. The same substance can also be synthesized in 6 steps from resorcinol at a global yield of approximately 27% [47]. **35** has demonstrated antiproliferative properties against a large range of cancerous cell lines (Table 12.2), being evaluated in several clinical trials, from phase I to phase III, being the main hurdle its low bioavailability when administered orally [48].

A plethora of cytotoxic effects have been attributed to **35**, including cell cycle arrest, activation of intrinsic and extrinsic apoptotic pathways (Figure 12.16) [48] and reversal of chemoresistance from certain cell lines towards classical therapeutic agents such as gemcitabine [49], docetaxel [50], doxorubicin [51], and platinum-based drugs [52, 53]. Phenoxodiol's cell cycle arrest is dose- and time-dependent, but the cycle's phase is variable [46, 54]. Predominant G_1-S arrest by **35** has been reported in several lines of head and neck squamous cancer cells due to increased expression of $p21^{WAF1}$, an endogenous cyclin-dependent kinase inhibitor protein, thus leading to CDK2 inhibition in a p53 independent manner [54].

Cell apoptosis *via* the intrinsic pathway has been described in several cancer types and cell lines [55–57]. In Me4405 melanoma cell line, activation of the apoptosis seems a multifactor mechanism, in which increases in p53 and p21 proteins expression were detected and similarly the expression of pro-apoptotic Bim, Noxa and PUMA proteins – that inactivate the mitochondria protecting proteins such as Mcl-1 and Bcl-XL, whose levels were decreased along with the X-linked inhibitor of apoptosis protein (XIAP). The more resistant Mel-RM cell line did not suffer gross alterations in these proteins' levels. Following the reduction in mitochondrial membrane potential ($\Delta\Psi_m$),



35

Figure 12.15: Phenoxodiol structure.

Table 12.2: Phenoxodiol's growth inhibitory concentration for 50% for cancerous cells.

Cancer type	Cell line	GI_{50} (μM)	Reference
Breast	MCF-7	25.0	[46]
	MDA-MB-468	7.9	[46]
	MDA-MB-231	31.3	[59]
Colorectal	HCT-15	20.3	[46]
	HT-29	41.0	[46]
Glioblastoma	U87	>100	[59]
Glioma	HTB-138	9.8	[46]
Head and neck squamous carcinoma	HaCaT	1.2	[54]
	HN17	3.5	[54]
	HN12	26.2	[54]
Leukemia	CCRF-CEM	1.7	[46]
	RPMI-8226	3.7	[46]
	–	5.4	[60]
Lung	NCI-H23	6.0	[46]
	NCI-H460	9.4	[46]
Melanoma	MM200	3.0	[46]
Mesothelioma	N036	3.4	[46]
	LO68	8.5	[46]
Neuroblastoma	SKN-BE(2)C	4.5	[59]
Ovarian	A2780	1.7	[46]
	CP70	1.35; 1.7	[46, 56]
Pancreatic	HPAC	46.6	[46]
Prostate	DU145	6.0–8	[46, 52]
	LNCaP	4.8	[46]
	PC3	7.0; 38	[46, 52]
Renal	769-P	50.6	[55]
	786-P	19.9	[55]
	Caki-2	28.8	[55]
Rhabdomyosarcoma	KYM-1	0.8	[46]

cytochrome *c* and Smac/DIABLO, pro-apoptotic mediators, were released in the cytoplasm, initiating activation of the caspase downstream [58]. Activation and cleavage of PARP-1 by caspase-3 triggers DNA repair and depleting intracellular NAD+, leading to energy starvation and death of the cell [57].

The extrinsic pathway can be activated following treatment with phenoxodiol, by reducing concentrations of c-FLIP, cellular FLICE-like inhibitory protein that competes with caspase-8 on the Fas associated death domain adapter protein (FADD)-pathway and blocks death inducing signaling. **35** diminished cellular phosphorylated Akt levels, without altering total Akt, thus reducing c-FLIP expression and allowing the death inducing signaling to proceed to activate caspase-8 and the effector caspases [55]. Cell death can also be triggered by phenoxodiol *via* the sphingomyelinase pathway by inhibiting sphingosine kinase (SphK), that downstream, reduces the level

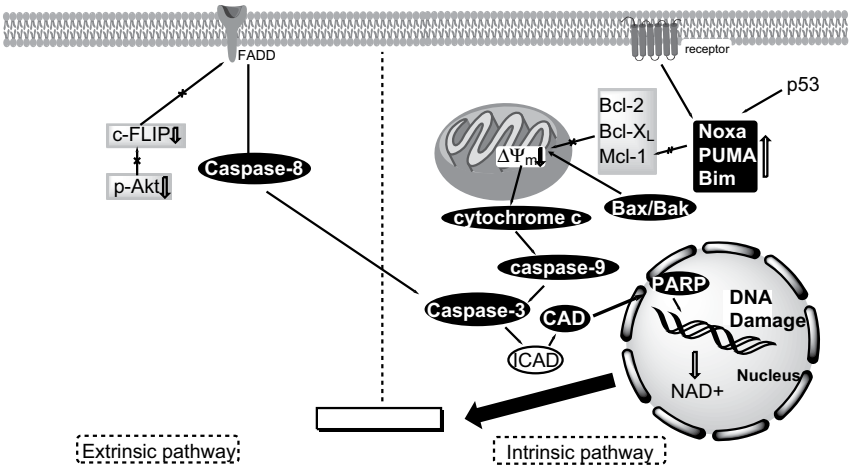


Figure 12.16: Simplified apoptosis pathways for phenoxodiol.

of sphingosine-1-phosphate, thus leading to accumulation of ceramide in the membrane and downregulating Akt - that itself regulates the pro-survival XIAP. This activation seemed specific to tumor cells, as it had no effect on survival or levels of sphingosine kinase on regular cells [46, 51].

The chemosensitizer action of **35** on refractory tumors is linked to the above apoptotic mechanism, since high levels of pro-survival proteins are usual in chemoresistant cells. Following the isoflavene treatment, XIAP levels in docetaxel resistant SKOV3 ovarian carcinoma cells were reduced, accompanied by increased levels of the caspase cleaved p30 form of it and activation of the intrinsic pathway caspases [50]. In U2OS osteosarcoma cells, unusually high levels of SphK, leading to upregulation of Akt and anti-apoptotic proteins. In combination with doxorubicin, **35** promoted a marked decrease in the SphK levels, promoting the accumulation of ceramide and inactivation of Akt pathway, leading to apoptosis.

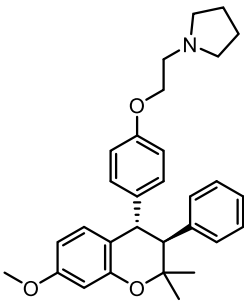


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Figure 12.17: Centchroman's structure, as free base.

Table 12.3: Centchroman's growth inhibitory concentration for 50% against multiple cancer cell lines.

Cancer type	Cell line	GI_{50} (μM)	Reference
Breast	MDA-MB231	10.8	[63]
Breast	SK-BR-3	6.7	[63]
Breast	MCF-7	12.3	[63]
Breast	MDA-MB-468	18.5	[63]
Head and neck squamous carcinoma	FaDu	7.5	[65]
Head and neck squamous carcinoma	CAL-27	7.5	[65]
Head and neck squamous carcinoma	SCC-9	10	[65]
Head and neck squamous carcinoma	SSC-25	10	[65]
Prostate	PC3	22	[64]
Prostate	DU145	17	[64]

Centchroman (**36**), also known as ormolexifene (Figure 12.17), is an Indian approved SERM for use as a female non-steroidal contraceptive [61]. It also showed anti-proliferative activity against breast [62, 63], prostate [64] and head and neck cancers [65] (Table 12.3). One mechanism of centchroman's cytotoxicity, observed in head and neck squamous cancer cells, is the modulation of PI3K/Akt/mTOR pathway, which plays a role in cell survival and proliferation, by inhibiting the phosphorylation of Akt with no alteration in PI3K levels. Furthermore, **36** inhibited activation of 70S6K, ribosomal S6 protein and STAT3, downstream targets of mTOR pathway, with a concurrent decrease in cyclin D1 and increased p21 expression, leading to cell cycle arrest and eventual apoptosis [65].

In PC3 and DU145 prostate cancer cells, **36** arrested cell cycle at G_0-G_1 phase, with activation of the intrinsic apoptosis pathway [64]. Additionally, centchroman suppresses metastasis by inhibiting epithelial-mesenchymal transition (EMT) progression and β-catenin promotion of oncogenes [63, 64, 66]. The catalytic activity of the GSK3β is enhanced by **36** by stabilization of the complex with ATP, leading to augmented β-catenin phosphorylation, that results in its higher proteasomal degradation and prevents translocation into the nucleus. Furthermore, **36** binds to β-catenin's Arg469, which is also the binding site to the TCF7L2 genetic promoter, thus preventing the expression of metastasis-related oncogenes [64]. Prevention of migration and invasion was also observed in mammary cancer cells, in which TGFβ and TNFα induced EMT was negated by centchroman [63]. In the highly invasive breast cancer cell lines, MDA-MB-231 and HER2-overexpressing SK-BR-3, **36** reduced phosphorylation on the mitogen-activated kinases ERK1/2 and p38, downstream members of the HER2 signaling pathway, causing downregulation on the MMP-9 activity, and reducing HER2 expression.

In addition to the antiproliferative capabilities of centchroman, the fact that it is an already approved drug with good bioavailability, favorable pharmacokinetics and safety profile [61] makes it a promising candidate for repurposing - saving time and money in the approval process [67] for an anti-cancer chemotherapeutic.

12.4 4-Arylchromene derivatives

4-Aryl-4*H*-chromenes are a class of structural prototypes that are easy to synthesize and have several biological applications, such as drug design in models of antimicrobial, anticholinesterase, antirheumatic, anticonvulsant, anti-inflammatory, antidiabetic activities and, finally, for the purpose of this chapter, anti-cancer and antiproliferative activities [9].

Kemnitzer and collaborators synthesized a series of compounds similar to 2-amino-4-aryl-3-carbonitrile-7-(dimethylamino)-4*H*-chromenes and identified potent apoptosis inducers (Figure 12.18) [68], by high-throughput screening assay (HTS), against tumor cell lines T47D (breast), H1299 (lung) and DLD-1 (colorectal). According to these authors, the evaluated analogues represented in Figure 12.18 were equipotent or slightly more potent than the antimitotic paclitaxel, colchicine and vinblastine, in caspases activation, cysteine-aspartate proteinases that degrade several cytoplasmic proteins, a process that

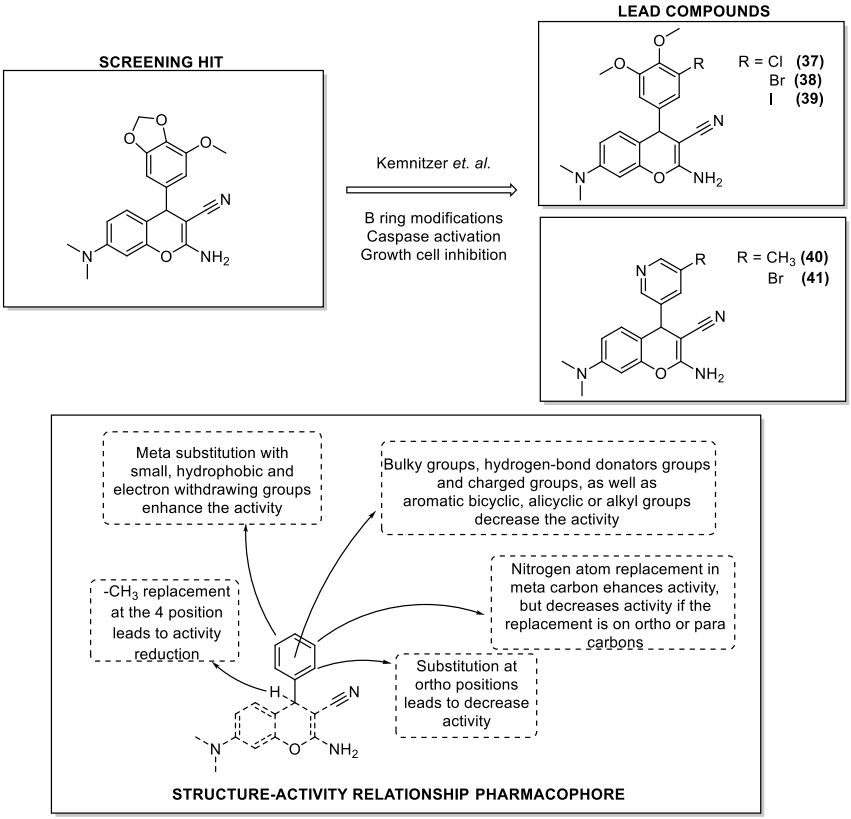


Figure 12.18: Kemnitzer's structure-activity relationship (SAR) study for B-ring aryl group and lead compounds.

culminates in cell death. Furthermore, it was found that T47D cells were more sensitive to analogues than the H1299 and DLD-1 cell lines. The results of caspase activation assays showed, in general, a direct correlation with the results of the traditional cell proliferation inhibition assay [68].

According to the studies of structure-activity relationships carried out by Kemnitzer et al., substitution of any nature in position 2′ on the aryl ring led to a reduction activity of caspases activation, compared to non-substituted analogous. In addition, the replacement of the hydrogen atom at the 4-position of compound **38** by a methyl group leads to a reduction of activity by about 178 times. Both effects were justified by the authors due to steric restriction of the pharmacophore or due to a possible unfavorable conformational change of the B ring [68].

In general, phenyl or pyridyl groups in carbon 4 with bulky, hydrogen-bond donors and/or charged substituents at any positions, as well as aromatic bicyclic, alicyclic or alkyl groups, reduce activity; whereas substitutions at the 3′ and 5′ positions of the monoaryl groups are important for activity. When the phenyl group is replaced by a pyridyl group, there is an increase in activity if the nitrogen is at 3′ position of the B ring, however, when nitrogen was either in 2′ position or 4′ position, there was a reduction in the caspase activation. Nevertheless, the structure-activity relationship for substituents at the 4′ position is not assertive, since they showed variation in activity [68].

Notably, the analogue **38**, when tested against sensitive uterine sarcoma cells (MED-AS) and against its multidrug-resistant cell line (MED-AS/DX5), presented a GI_{50} of 2 nM for both lines, while the reference drugs paclitaxel and vinblastine were, respectively, 160 and 20 times less active against MED-AS/DX5 cells. Furthermore, in the tubulin polymerization inhibition assay, analog **38** demonstrated similar potency to colchicine [68].

In the interaction assay between tubulin and tritium-labelled colchicine, the compound **38** displaces the labelled drug from the β-tubulin active site, suggesting that the colchicine and **38**, as well as other 4-aryl-4*H*-chromenes compounds, share the same active site or very close sites. Thus, the 4-aryl-4*H*-chromenes analogues present pro-apoptotic activity via inhibition of tubulin polymerization [68].

The following year, Kemnitzer and collaborators synthesized a new series of 4-aryl-4*H*-chromenes with variations centered on the A ring (Figure 12.19) [69]. It was identified that the monosubstitution in carbon 7 by small hydrophobic electron-density donor groups, such as dimethylamino (**38**), ethylamino (**42**) or methoxy (**43**), are responsible for the apoptosis-inducing activity. However, when these groups are replaced by more hydrophilic groups, such as amino (**47**) and hydroxy (**48**); or by larger groups, such as diethylamino (**45**) and ethoxy (**46**);or by electron-withdrawing groups, such as chlorine (**49**) or bromine (**50**), there is a reduction in activity. When these groups are even larger at carbon 7, such as the phenylamino (**51**) or *N*-morpholino (**52**) groups, the substances were inactive below 10 μM, confirming that this position has steric restriction in the pharmacophore [69].

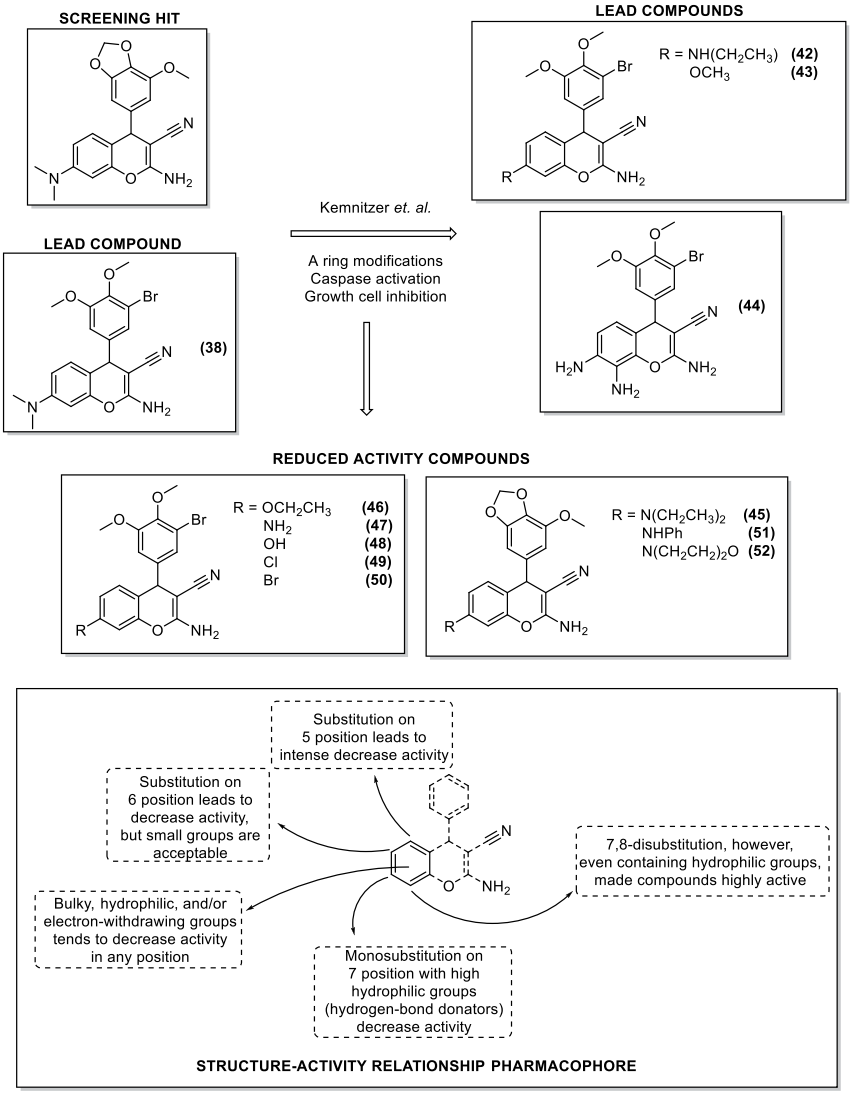


Figure 12.19: Kemnitzer's structure-activity relationship (SAR) study for A-ring aryl group and lead compounds.

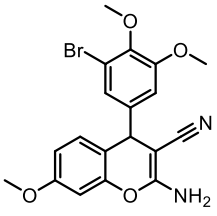
In addition, Kemnitzer et al., evaluated the influence of the disubstitution at positions 5,7, 6,7 and 7,8 [69]. 5,7-Disubstituted derivatives are generally inactive up to 10 μM, indicating that groups at position 5 are not permissible, due to steric restriction of the pharmacophore or due to unfavourable conformational change of the C ring, as well as the substitutions at positions 4 and 2' previously studied [68, 69]. Substituents at position 6 also tend to reduce activity, but less bulky groups are acceptable, whereas

small groups at position 8 have produced promising analogues. 7,8-Disubstituted compounds, even containing hydrophilic groups at the 7 position, were more active than the 7-monosubstituted compounds, such as the 7,8-diamino derivative (**44**) whose activity was slightly lower than the 7-dimethylamino analogue, **38** [69].

To evaluate the influence of A ring substituents with several C aryl groups, Kemnitzer et al. synthesized and tested different 4-aryl-4*H*-chromenes with the 7-ethylamino, 7-methoxy and 7,8-diamino groups to compare them to the first study involving 7-dimethylamino derivatives [68, 69]. It was observed that all 7-methoxy derivatives (Figure 12.20) containing electron-withdrawing groups at 3′ position (e.g. **53**), as well as the C ring analogues 3′,5′-dimethoxyphenyl, 3′,4′,5′-trimethoxyphenyl, 5′-bromo-3′,4′-dimethoxyphenyl (e.g. **43**) and 5′-methoxy-3′-pyridyl were active against T47D and H1299 at a potency range from 15 to 89 nM [69].

The 7-ethylamino, 7,8-diamino and 7-methoxy derivatives showed similar behavior to that observed for 7-dimethylamino, being the series of 3′-monosubstituted compounds less potent in the caspase activation assay than the 3′,4′,5′-trisubstituted derivatives, especially containing the group 5′-bromo-3′,4′-dimethoxyphenyl [68, 69]. According to the researchers, the 7,8-diamino derivatives are promising for having two hydrophilic groups that would become more water-soluble [69]. In the cell proliferation inhibition assay, these derivatives showed activity proportional to caspases activation assay, with the exception of **43** and **53** analogues, which were more potent in inhibiting cell proliferation than the caspase activation [69]. The authors did not suggest explanations for the greater potency of these substances in the growth inhibition assay compared to the caspase activation assay.

Kemnitzer also synthesized and evaluated condensed cyclic analogues at the 7,8 positions (Figure 12.21) and found that aromatic rings, such as pyrrolo, benzo and pyrido rings, in general, produce equipotent or more potent caspase activators, compared to the 7,8-diamino derivative [70]. The alicyclic analogue, tetrahydrobenzo, and substituted aromatic ring analogues demonstrated a reduction in activity, which, according to the researchers, that is justified by the steric restriction in the pharmacophore around these positions. However, the pyrrolic ring derivatives, **54** and **55**, were very active in the caspase activation assay with T47D cells which presented EC_{50} values of 16 nM and 5 nM, respectively, being more potent than the 7,8-diamino lead compound [70].



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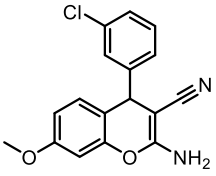

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Figure 12.20: Molecular structures of two Kemnitzer's compounds that combine the influence of A-ring substituents with several C-aryl groups.

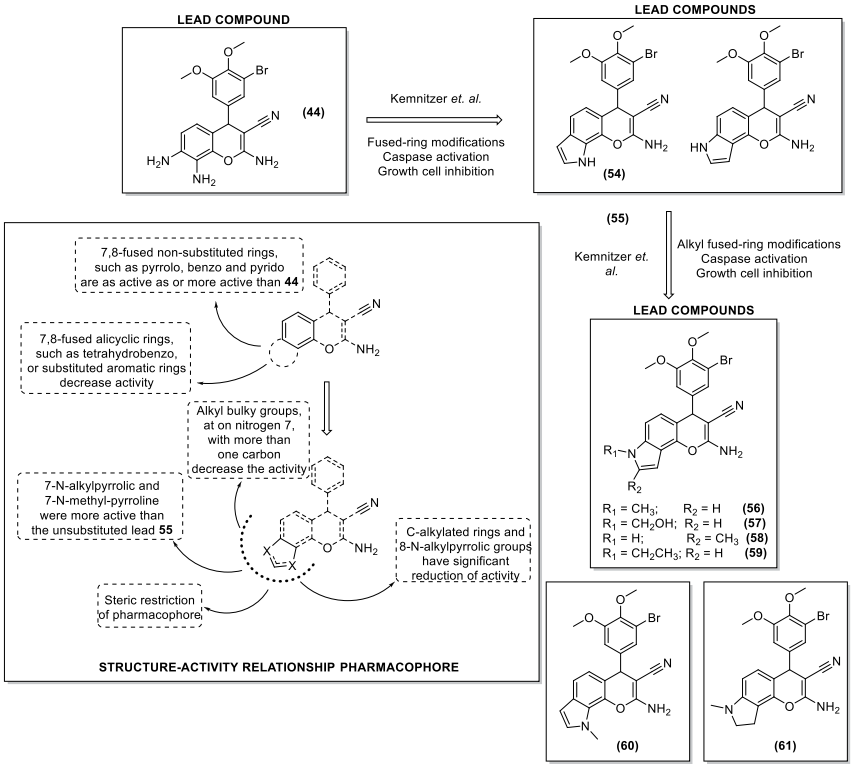
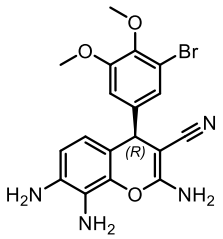


Figure 12.21: Kemnitzer's structure-activity relationship (SAR) study for 7,8-fused rings and alkylated derivatives and lead compounds.

In another paper, Kemnitzer and collaborators synthesized and tested new analogues of condensed pyrrole rings with alkyl substituents and found that the pyrrolic derivatives (Figure 12.5) 7-*N*-methyl (**56**), 7-*N*-methylhydroxy (**57**) and the partially saturated derivative pyrroline (**61**) were more active than the unsubstituted **55** prototype in the HTS assay of caspase activation with T47D cells, with human colon cancer cells (HCT116) and hepatocellular carcinoma cells (SNU398), with EC_{50} of 2–4 nM [71]. Furthermore, derivatives **56** and **57** were highly potent in the T47D cells growth inhibition assay, with GI_{50} of 0.3 nM and 3 nM, respectively. The 7-*N*-methyl pyrrole analogues, in which the B ring was modified to aryl groups that performed better in Kemnitzer's first study, were also highly potent in caspase activation assays, with $EC_{50} < 10$ nM. However, other derivatives C-alkylated and *N*-alkylated, for example **58**, **59** and **60**, achieved a significant reduction of activity, due to the steric restriction of the pharmacophore, as also proposed by the research group in the study of structure-activity relationship for positions 7 and 8 [71].

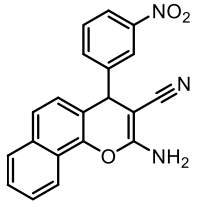


EPC2407 (Crolibulin)

Figure 12.22: Molecular structure of lead compound Crolibulin.

In studies conducted by pharmaceutical companies *Maxim Pharmaceuticals* and *Shire BioChem*, Kasibhatla (*Maxim Pharm.*) and collaborators established that derivatives **38**, **54** and **55** were potent β-tubulin polymerization inhibitors in the order of 10^2 nM [70, 72]. In addition, in the cell cycle analysis assay, **55** (**MX-76747**) induced apoptosis of mammary carcinoma cells (MCF-7) via arrest of the G_2/M phase of the cell cycle [70, 72]. In another article published by this partnership, Gourdeau (*Shire BioChem*) and collaborators identified that the *R* enantiomer of the 7,8-diamino derivative **44**, called Crolibulin (**MX-116407** or **EPC2407** in Figure 12.22), although less active *in vitro* to inhibit cell proliferation, demonstrated, *in vivo*, potent activity of selective interruption of tumor blood flow and, consequently, the induction of tumour necrosis [70, 73]. In this sense, accordingly to Kasibhatla and Gourdeau, **MX-116407** presented tumour antiproliferative activity *in vivo* at lower doses than the toxic dose, suggesting that the derivative may have greater clinical efficacy and, therefore, would be a promising candidate to become a marketed-drug [72, 73]. Vascular disruptor agents (VDAs), as the drugs that have these properties, are considered promising for cancer pharmacotherapy, especially in combination with another antitumor drugs [74, 75].

In a study published by Smith et al., a series of 2-amino-4-aryl-3-carbonitrile-naphthochromenes (Figure 12.23) was synthesized and tested for the prevention of articular cartilage wear, associated with several degenerative pathologies such as rheumatoid arthritis [76]. Although Smith's paper aimed at evaluating the antirheumatic activity of these derivatives, the matrix metalloproteinases (MMP) are involved in



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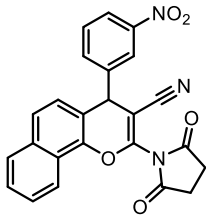

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Figure 12.23: Molecular structures of Smith's naphthochromenes derivatives with antisecretory activity of extracellular matrix-degrading metalloproteinases (MMP).

several aspects of the cancer pathogenesis, such as tissue invasion and metastasis [77]. According to Smith et al., the analog **62** demonstrated inhibition of secretion, but no activity of extracellular matrix-degrading metalloproteinases (MMP) [76]. However, naphthochromene derivatives that had the enamine group showed instability in an acidic environment, producing multiple degradation products [76]. To study the acid–base stability of these compounds, and to increase the oral bioavailability, Smith and coworkers have synthesized derivatives that are stable in acid medium with the group succinimide group at position 2 (**63** and others), which remained *in vitro* antisecretory activity [76].

Furthermore, due to the structural similarity with the described antiproliferative chromenes, Kemnitzer et al. synthesized a new series of analogues with structural changes in 2 and 3 positions, aiming at acid-base stability (Figure 12.24) [78]. Unlike what was demonstrated by Smith for antirheumatic naphthochromenes, replacing 2-amino group by succinimide group (**69**) and by phenylurea group (**70**) resulted in reduced antiproliferative activity compared to **38**. Derivatives with substitution of the 3-carbonitrile group by esters (**67** and **68**) led to a great reduction in activity both in the caspase activation assay and in the cell growth inhibition assay. In addition, the deaminated analogues **64** and **65** showed

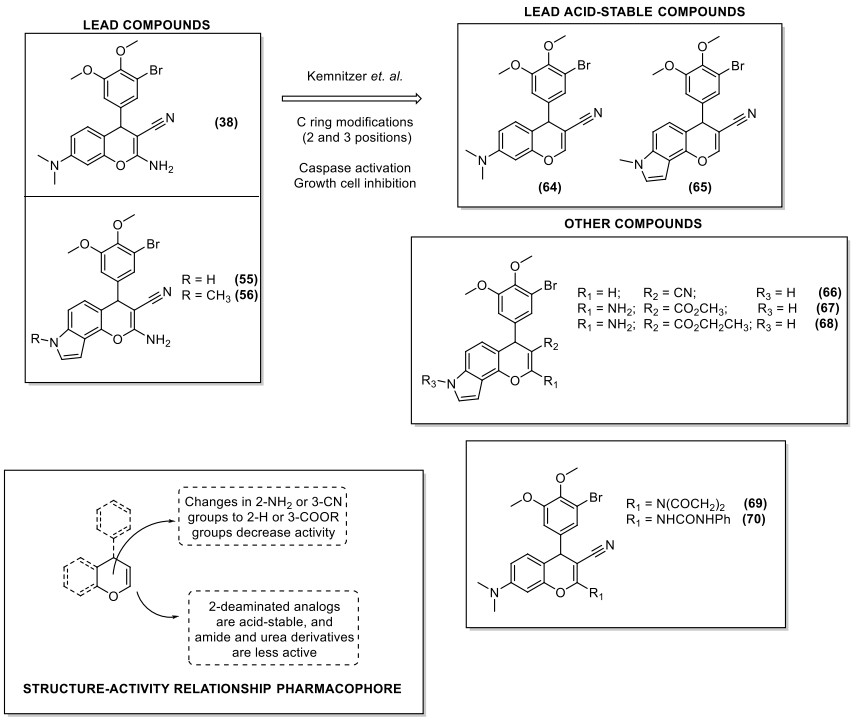

Figure 12.24: Kemnitzer's structure-activity relationship (SAR) study for 2,3-positions of C-ring and lead acid-stable compounds.

a 4- to 10-fold reduction in activity compared to 2-amino derivatives **38** and **56**, however **65** showed greater stability in acidic solution (pH = 4) compared to the amino derivative. In this sense, in addition to prove the attribution of the enamine group as responsible for acid-base instability of this class of chromenes, Kemnitzer demonstrated that the deaminated derivatives are stable in acid solution, suggesting that these could have greater potential for high oral bioavailability [78]. The values of the caspase activation and cell growth inhibition assays of the 4-aryl-4*H*-chromenes derivatives from the collection of works published by Kemnitzer (**37–70**) are grouped in Table 12.4.

Shestopalov's and Chernysheva's research groups synthesized a new series of 4-aryl-4-*H*-chromenes (Figure 12.25) and evaluated the antimitotic activity, through the screening of NCI60 human tumour cells, and microtubule destabilizing activity, through the phenotypic sea urchin embryo assay [79, 80]. The podophyllotoxin (PT) molecular simplification analogues **71–73** showed microtubule destabilizing activity, with EC values between 2 and 10 nM, compared to 20 nM of the standard reference compound PT, on the sea urchin embryo model [80]. Furthermore, these analogues showed average growth inhibition activity around 800 to 20 nM (GI_{50}, NCI60 cancer cells panel), however the ovarian cancer multidrug-resistant cell line with overexpressed P-glycoprotein (NCI/ADR-RES) showed greater sensitivity to these substances than the non-multidrug-resistant strain (OVCAR-8) [80].

The molecular hybridization analogues **74–78** exhibited cell growth inhibition ranging from 400 to 40 nM (GI_{50}, NCI60). However, as demonstrated by Chernysheva et al., the NCI/ADR-RES cells were more sensitive to these substances [79].

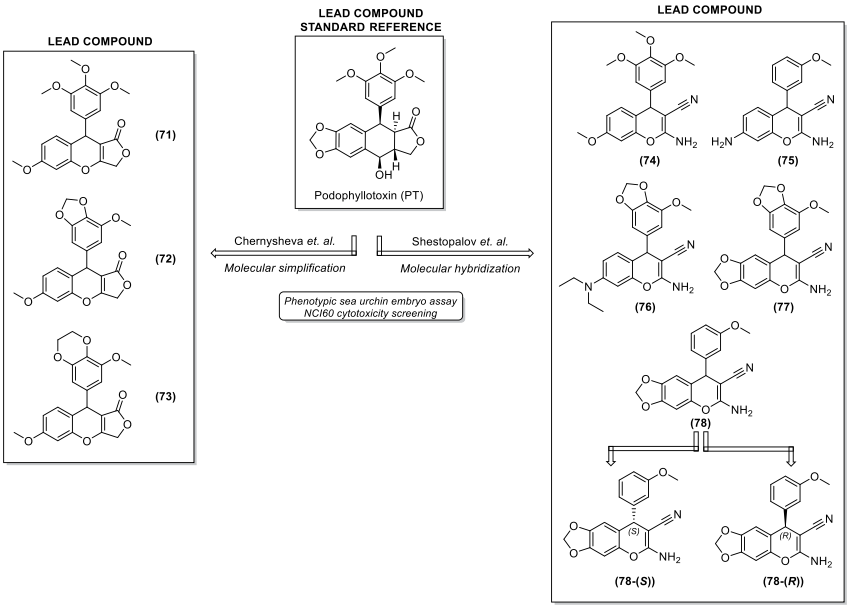

Figure 12.25: Chernysheva's and Shestopalov's 4-aryl-4*H*-chromenes derivatives.

Table 12.4: Effects of Kemnitzer's 4-aryl-4*H*-chromenes derivatives on caspase activation assay and cell growth inhibition assay.

Authors	Compounds	Caspase activation assay (EC_{50} nM)[a]					Cell growth inhibition assay (GI_{50} nM)[b]				
		T47D[c]	HCT116[d]	H1299[e]	DLD-1[f]	SNU398[g]	T47D[c]	H1299[e]	DLD-1[f]	HCT116[d]	SNU398[g]
Kemnitzer et al.	**37**	18	NT	36	65	NT	NT	NT	NT	NT	NT
	38	19	NT	43	66	NT	92	NT	70	NT	NT
	39	18	NT	34	64	NT	NT	NT	NT	NT	NT
	40	11	NT	27	18	NT	NT	NT	NT	NT	NT
	41	33	NT	46	26	NT	NT	NT	NT	NT	NT
	Screening hit	73	NT	93	83	NT	NT	NT	NT	NT	NT
	Colchicine	14	NT	34	190	NT	NT	NT	NT	NT	NT
	Vinblastin	25	NT	73	129	NT	NT	NT	NT	NT	NT
	Paclitaxel	30	NT	163	75	NT	NT	NT	NT	NT	NT
	42	14	NT	27	NT	NT	NT	NT	NT	NT	NT
	43	17	NT	34	NT	NT	8	9	NT	NT	NT
	44	34	NT	81	NT	NT	150	31	NT	NT	NT
	45	480	NT	530	NT	NT	NT	NT	NT	NT	NT
	46	64	NT	92	NT	NT	NT	NT	NT	NT	NT
	47	33	NT	67	NT	NT	77	76	NT	NT	NT
	48	130	NT	210	NT	NT	800	190	NT	NT	NT
	49	160	NT	290	NT	NT	64	55	NT	NT	NT
	50	140	NT	280	NT	NT	NT	NT	NT	NT	NT
	51	>10000	NT	>10000	NT	NT	NT	NT	NT	NT	NT
	52	>10000	NT	>10000	NT	NT	NT	NT	NT	NT	NT
	53	80	NT	160	NT	NT	NT	NT	NT	NT	NT
	54	16	69	53	NT	89	9	6	NT	NT	NT
	55	5	14	13	NT	15	8	19	NT	20	14
	56	2	3	NT	NT	2	0.3	NT	NT	1.4	0.2
	57	3	3	NT	NT	2	2	NT	NT	4	1
	58	30	54	57	NT	27	89	140	NT	NT	NT

Table 12.4: (continued)

Authors	Compounds	Caspase activation assay (EC_{50} nM)[a]					Cell growth inhibition assay (GI_{50} nM)[b]				
		T47D[c]	HCT116[d]	H1299[e]	DLD-1[f]	SNU398[g]	T47D[c]	H1299[e]	DLD-1[f]	HCT116[d]	SNU398[g]
	59	29	44	NT	NT	25	NT	NT	NT	NT	NT
	60	15	17	NT	NT	13	NT	NT	NT	NT	NT
	61	2	4	NT	NT	1	7	NT	NT	4	1
	Vinblastin	43	44	NT	NT	38	7	NT	NT	NT	NT
	Vincristin	43	58	NT	NT	12	NT	NT	NT	NT	NT
	Colchicine	9	15	NT	NT	4	7	NT	NT	NT	NT
	Taxol	36	26	NT	NT	11	NT	NT	NT	NT	NT
	64	65	93	NT	NT	77	66	NT	NT	51	17
	65	29	38	NT	NT	34	6	NT	NT	6	4
	66	47	66	NT	NT	52	73	NT	NT	53	34
	67	1400	660	NT	NT	1000	NT	NT	NT	NT	NT
	68	3200	3000	NT	NT	2200	3300	NT	NT	4300	1600
	60	250	420	NT	NT	190	NT	NT	NT	NT	NT
	70	68	84	NT	NT	93	NT	NT	NT	NT	NT

[a]Effective concentration at which the stimulus response corresponds to half of the maximum response (EC_{50}). [b]Concentration required to inhibit the growth of 50% of the cell population (GI_{50}). [c]Human breast cancer cell line (T47D). [d]Human colon cancer cell line (HCT116). [e]Human non-small cell lung carcinoma cell line (H1299). [f]Human colorectal adenocarcinoma cell line (DLD-1). [g]Human hepatocellular carcinoma cell line (SNU398). NT = not tested.

Table 12.5: Effects of Chernysheva and Shestopalov 4-aryl-4*H*-chromenes derivatives on the sea urchin embryo and on human cancer cells (OVCAR-8, NCI/ADR-RES and NCI60 cancer cells panel).

Authors	Compound	Phenotypic sea urchin embryo assay (EC nM)[a]			Cell growth inhibition assay (GI_{50} nM)[b]		
		Cleavage alteration	Cleavage arrest	Embryo spinning	OVCAR-8[c]	NCI/ADR-RES[d]	NCI60 (average)[e]
Chernysheva et al.	**71**	5	50	200	788	334	631
	72	2	10	100	55.6	18.6	NT
	73	10	50	500	49.5	23.5	NT
	Podophyllotoxin	20	50	200	NT	NT	NT
Shestopalov et al.	**74**	10	50	500	34.5	19.9	45
	75	100	1000	5000	701	240	257
	76	20	50	200	36.8	<10	45
	77	5	50	200	435	101	389
	78	5	50	200	97.1	30.2	65
	78-(*S*)	100	1000	5000	NT	NT	NT
	78-(*R*)	2	20	100	NT	NT	NT
	Podophyllotoxin	20	50	500	NT	NT	NT

[a]Effective threshold concentration (EC). [b]Concentration required to inhibit the growth of 50% of the cell population (GI_{50}). [c]Ovarian cancer cell line (OVCAR-8). [d]P-glycoprotein-overexpressing multidrug resistant derived from ovarian cancer cell line (NCI/ADR-RES). [e]Average of GI_{50} values obtained from NCI60 drug-screening cancer cells panel. NT = not tested.

Furthermore, Shestopalov's research group evaluated the stereochemistry influence on the activity of the **78** analogue and reported that the *R* stereoisomer was twice as potent in antimitotic activity as the racemic mixture, with the *S* stereoisomer being about 50 times less active [79]. This suggests that the absolute configuration of the asymmetric carbon 4 has a significant influence on the antiproliferative activity of 4-aryl-4-*H*-chromenes. The values of the cell growth inhibition assay and the sea urchin embryo phenotypic assay of the 4-aryl-4*H*-chromene derivatives of the works published by Shestopalov and Chernysheva are grouped in Table 12.5.

12.5 Conclusions

Arylchromenes and arylchromans, from the natural or synthetic origin, are undoubtedly an inspiring prototype for drug development, especially against cancer. As pointed out in this chapter, some representatives of these classes of substances demonstrated selectivity against cancer cells, especially multidrug-resistant lines, as well as present low toxicity in *in vitro* studies. Moreover, compound **35** and **MX-116407** have reached the clinical phase of the drug development reinforcing the great potential of arylchromenes and arylchromans as chemical prototypes as anticancer drugs. Despite the potential of arylchromenes and arylchromans as anticancer agents, it is noteworthy to mention that some pharmacokinetics properties of these compounds (for instance, the low solubility, biodisponibility etc.) must be overcome in order to further develop into new antineoplastic agents.

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Shashi Kiran Misra, Devender Pathak and Kamla Pathak*

13 Anticancer potential of indole derivatives: an update

Abstract: The heterocyclic indole is one of the most prevalent pharmacophores in nature. It has been a highly privileged scaffold for designing targeted and anticancer therapeutics. Countless fused heterocyclic templates have been developed with diverse physicochemical and biological properties. Due to their versatile ethanobotanical and pharmacological values, indole and its derivatives seek high demand in the chemical and healthcare sectors. Extensive anticancer research has been conducted in this decade to evaluate their efficacy for diverse malignancies. The chapter explores the anticancer activity of natural and synthetic indole derivatives expressed through targeting different biological receptors and enzymes.

Keywords: anticancer agents; heterocyclic; indole derivatives; receptors.

13.1 Introduction

Heterocyclic compounds have always been a fascinating framework for designing effective pharmacophores and are extensively utilized in pharmaceutical sciences as therapeutic agents owing to the basic nucleus structure similar to that of enzymes, amino acids, peptides, and proteins [1]. Countless fused heterocyclic templates have been developed with diverse physicochemical and biological properties. Their well-defined polycyclic geometrical structures facilitate three-dimensional substitutions thus presenting a promising approach for designing novel organic and medicinal compounds. A survey reveals a high contribution (approximately 80%) of heterocyclic compounds for the synthesis of bioactive agents in the healthcare sector due to their prominent reactivity. These molecules have been explored for designing diverse medicines belonging to tranquilizers (chlordiazepoxide), antidepressants (imipramine), diuretic (indapamide), antihypertensive drugs (guanethidine), and various antibiotics such as penicillin, norfloxacin, and cephalosporin [2].

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Aromatic nitrogen-based heterogeneous indole is abundantly found in nature i.e. plants, bacteria, fungi, coal tar, and in excretory fecal matter. Few flowers oil such as jasmine and orange blossom, are enriched with indole heterogenous compounds. Bicyclic indole is notified as an intracellular signal in bacteria (Gram positive and Gram negative) which manages several ongoing physiological processes such as plasmid stability, biofilm formation, spore generation, virulence, and drug resistance. As an intercellular signal in both Gram-positive and Gram-negative bacteria, it has been shown to control a number of bacterial processes such as spore formation, plasmid stability, drug resistance, biofilm formation, and virulence. Most organisms such as *Bacillus alvei, Vibrio cholera, Escherichia coli,* and *Enterococcus faecalis* require a tryptophan biosynthesis pathway and can synthesize indoles [3].

Heterocyclic indole (C_8H_7N) consists of a six-membered benzene ring assembled with five-membered pyrrole moiety hence is named as benzopyrroles. It is a colorless crystalline solid at room temperature with a scented fragrance. In the year 1866, Adolf Von Baeyer very first synthesized indole and elaborated its nucleophilic substitution reactions under basic conditions [4]. Figure 13.1 summarizes the physicochemical properties of indole.

Molecular formula	C_8H_7N
Molecular weight	117.151 g/mol
Physical state	Solid
Melting point	52-54 °C
Solubility	0.19 g/100 ml water
Density	1.17 g/cm^3
Crystal structure	Pna2
XLog P3	2.1

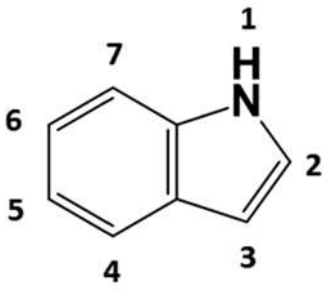

Figure 13.1: Physicochemical property of indole pharmacophore.

As a parent moiety, indole exists in numerous compounds that occur abundantly in nature such as skatol (feces), tryptophan (amino acid) and hereroauxin (plant hormone), serotonin (vasoconstrictor hormone), bufotenine (toad and toxic mushroom), vincristine, and vinblastine (antileukemic alkaloids), etc. Cruciferous vegetables containing indole-3-carbinol have been utilized for the cure and management of several chemotherapies related to colorectal, breast, and prostate cancers [5]. Indole-based tryptophan, an essential amino acid is a vital growth factor for infants. It balances nitrogen requisite in adult humans. L-tryptophan is explored for the synthesis of serotonin, a neurotransmitter, Vitamin B3, and melatonin an epiphyseal hormone [6]. Few natural occurring indole-based compounds are exemplified in Figure 13.2. Few fungi such as *Fusarium semitectum, Aspergillus fumigatus, Penicillium chrysogenum,* and *Claviceps purpurea (Ergot)* are extensively explored for the synthesis of indole derivatives for the utilization in the healthcare sector [7].

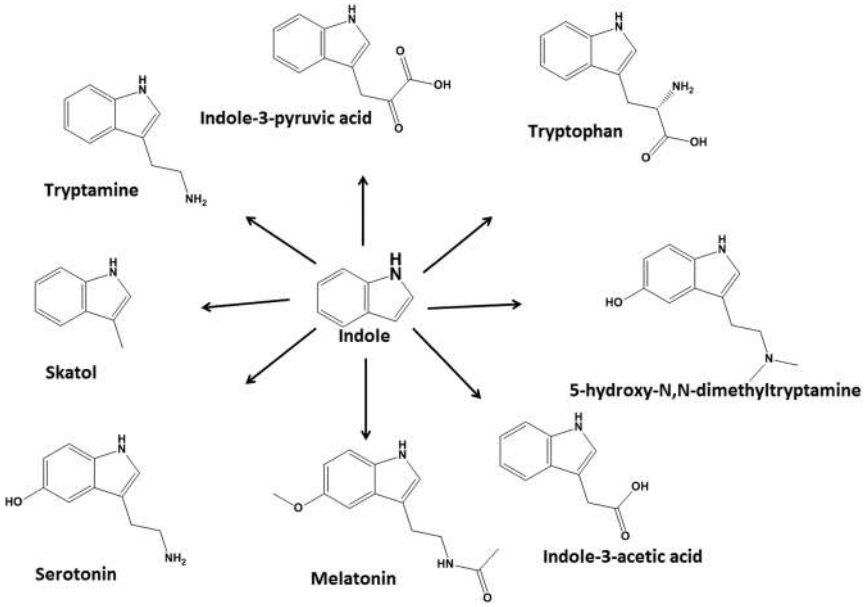

Figure 13.2: Natural derivatives comprised indole parent core.

Indole alkaloids, the largest class of secondary metabolites are widely distributed in *Vinca*, *Rauwolfia*, *Nux vomica*, *Physostigma*, wild cabbage, and coffee belonging to Apocynaceae, Loganiaceae, Brassicaceae, and Rubiaceae. One of the medicinal plants *Catharanthus roseus* (Family: Apocynaceae) has been evaluated for its potent cytotoxic action owing to the presence of indole-based alkaloids such as vincristine, vinblastine, catharanthine, ajmalicine, vinflunine, and tabersonine [8]. Heterocyclic indole alkylamines psilocin and psilocybin have been identified in Mexican mushrooms i.e. *Psilocybe cubensis* and *Psilocybe mexicana* [9]. Indole derivative strychnos of the Loganaceae family was investigated for its noticeable ethanobotanical and medicinal significances. In spite of possessing arrow/ordeal poisons (curarine and calebassine), Strychnos exhibits curative efficiency for rheumatic fever, ulcers, leprosy, and worms. Approximately 300 different Strychnos alkaloids have been evaluated for their ethanomedicinal applications in diverse arenas of parasitology (Usambarensine and 18-hydroxyisosungucine), antimicrobial, chemotherapy (isostrychnopentamine and sungucine), inflammation (brucine and brucine *N*-oxide), and neurology [10, 11].

Saidou et al. reported pro-apoptotic effects induced by isostrychnopentamine strychnos alkaloid against human colon cancer cell lines (HCT 116), glioblastoma cells (U373), nonsmall cell lung cancer (NSCLC) cells (A549), and PC-3 cancers cells of the prostate [12]. Evodiamine, a naturally occurring indole-based alkaloid is prominently found in fruits of the multipurpose herb *Evodiae fructus* (Chinese fruit "Wu-Zhu-Yu"). This potent component is active against versatile ailments including cancers,

neurodegenerative, and cardiovascular disorders [13]. Physostigmine or eserine, a parasympathomimetic indole alkaloid is found in the Calabar bean. Highly toxic, physostigmine is an important choline esterase inhibitor and employed in the management of glaucoma and Alzheimer's disease [14]. Numerous indole-based simple and substituted derivatives are also isolated from marine sources (algae, tunicates, worms, and sponges). Naturally, these complex molecules are involved in their defense mechanism and are been utilized as promising drug delivery tools for the management of several human diseases. Marine natural component aplysinopsins, a tryptophan-derived indole derivative is isolated from corals, sea anemones, and sponges. Reports revealed its neurotransmitter modulation, anticancer, antimicrobial, and antiplasmodial activities [15].

Nitrogen-based indole heterocyclic compounds are frequently utilized in the management of chemotherapy through targeting various enzymes (PIM kinase, histone deacetylases, and DNA topoisomerases) or receptors (sigma receptor) [16]. Heterocyclic indoles and their derivatives with pyrazone are highly involved in the inhibition of physiological enzyme-mediated channels such as inhibition of nuclear factor kappa B (NFkB), phosphoinositide-3 kinase (PI3K), and serine/threonine-protein kinase (Akt). Several 3-substituted indole derivatives (3-pyranyl indole) are reported for anticancerous activity associated with cell proliferation and apoptosis for the mitigation of human colon carcinoma (HT-29), ovarian adenocarcinoma (SK-OV-3), and breast cancer (MCF-7). Similarly, indole-3-carbinol and its active metabolite 3,3-diindolylmethane (DIM) have an impact on Akt-NFkB signaling, cyclin-dependent kinase activity, caspase activation, estrogen receptor signaling, and breast cancer gene expression. Numerous aroylindoles, diarylindoles, arylthioindoles, and indolylglyoxamides are considered as potent tubulin polymerization inhibitors. The earliest indole-based natural antitumor agents i.e. vincristine and vinblastine are recognized for their tubulin polymerization inhibition and frequently utilized in combined therapy in Hodgkin/non-Hodgkin lymphoma. Heterocyclic indole-based nanodrug delivery systems have shown potential to overcome the most notorious downsides including narrow therapeutic effects, poor solubility, and toxicity for healthy cells. Hence indole-based molecules may be decisive anticancer agents for the management of diverse malignancies. Figure 13.3 illustrates different chemopreventive modes of action exhibited by heterocyclic indole and its derived pharmacophores.

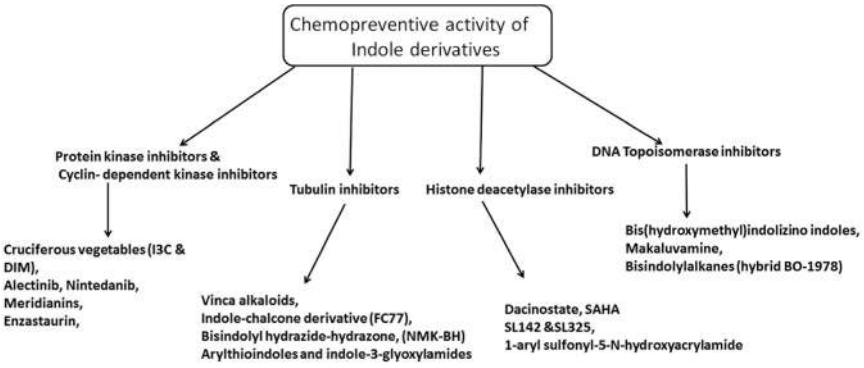

Figure 13.3: Chemopreventive modes of action exhibited by indole derivatives.

13.2 Protein kinase inhibitors and cyclin-dependent kinase inhibitors

Indole-derived molecules have been explored to target cyclin-dependent protein kinase owing to their potential action on cell division, cell proliferation, angiogenesis, and cellular signaling. Several pathways are restricted by these small molecules as they potentially inhibit Akt signaling pathway (2-oxindole derivatives), epidermal growth factor (osimertinib), glycogen synthase kinase (tivantinib), and protein kinase C (enzastaurin). Cyclin-dependent kinase plays vital roles in cell division and modulation of transcription. This enzyme family expands into three cell cycle subfamilies (CDK1, CDK4, and CDK5) and five several transcriptional subfamilies (CDK7, CDK8, CDK9, CDK11, and CDK20).

13.2.1 Cruciferous vegetables

Cruciferous vegetables are widely utilized for the isolation of indole-derived bioactive agents for the management of diverse cancers owing to potent inhibitors of cyclin-dependent kinase enzymes. Indole-3-carbinol, an important indole scaffold is derived from the breakdown of glucosinolate glucobrassicin cutting, chewing, or cooking cruciferous vegetables i.e. cabbage, turnip, Kohlrabi, broccoli, cauliflower, Brussel's sprout, mustard green, etc. This compound has been explored for designing antineoplastic agents for the management of breast, colon, cervical, and prostate cancers through targeting signaling pathways such as hormonal homeostasis and cell proliferation. The compound also finds its place for the treatment of systemic lupus erythematous, an autoimmune disease. Dietary studies have focused on high consumption or intake of cruciferous fruits and vegetables as cancer preventive measurements. Indole-3-carbinol faces intrinsic instability under acidic media resulting in active components that show high susceptibility toward the cell cycle. A series of acid-catalyzed dehydration and condensation produce different oligomeric pharmacologically active compounds such as DIM, indole-3,2b-carbazole (ICZ), linear/cyclic trimmers (LTR_1/CTr), and cyclic tetramer (CTet). Amongst these, DIM exhibits significant chemopreventive activity that involves numerous cell responses such as apoptosis induction, cell cycle arrest, suppression of estrogen receptor alpha (ERα)-dependent gene expression, and inhibition of angiogenesis [17]. Possession of two indole molecules in its chemical configuration explains a high degree of action against angiogenesis and targeting diverse cellular responses. Figure 13.4 illustrates several vital components derived from cruciferous sources.

Another highly active tetrameric component (CTet metabolite) has been explored in the mitigation of breast cancer through inhibiting overexpression of cell cycle regulatory enzymes such as cycline-dependent kinase and CDK6 [18].

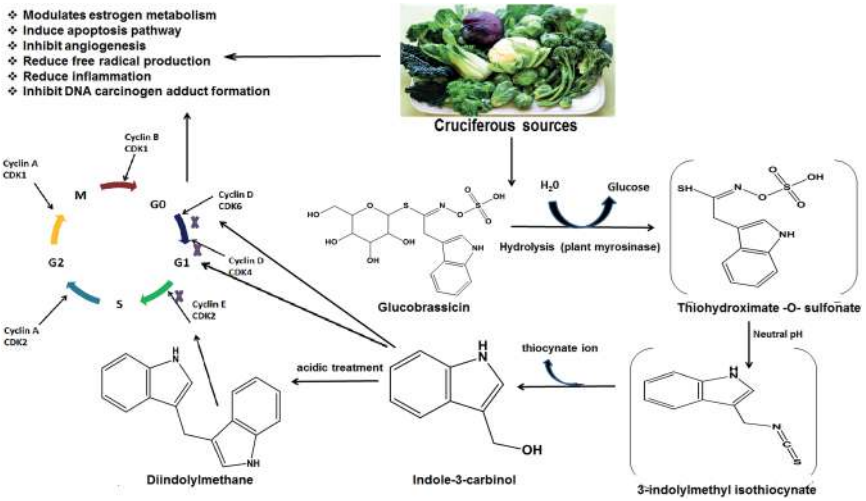

Figure 13.4: Cruciferous indole derivatives and their mode of action.

Both I3C and DIM are extensively involved in G1 phase cell cycle arrest that comprises upregulation of cycline-dependent kinase enzyme inhibitors (p21 and p27). The mentioned process is highly communicative in breast and prostate malignancy. At the cellular level, I3C and DIM inhibit the kinase activity of CDK2/cyclin E, CDK4/cyclin D, and CDK6/cyclin D that reduces Rb phosphorylation. Consequently, more of the Rb protein attaches over the E2F transcription factor, inhibiting cycling of S phase through blocking G1 phase in the cell cycle [19]. Grubbs et al. discussed chemopreventive roles of indole-3-carbinol in the process of tumorigenesis at visceral organs such as lungs, mammary glands, cervix, and gastrointestinal tract [20]. A series of spontaneous and chemical-induced malignancies in female Sprague Dawley rats were performed that revealed suppression of cancer cells proliferation by indole-3-carbinol in a dose of 50–100 mg/day, five times a week. Earlier studies demonstrated indole-3-carbinol as an effective cancer preventive tool that showed approximately 96% of reduction in cancer proliferation. Subsequently, after seven days of the administration, higher induction of hepatic phase I and phase II metabolic enzymes was determined. Substantial reduction in mammary tumor cells (65%) was reported at the end of the study that revealed the potential application of indole-3-carbinol for the prevention and management of mammary carcinogenesis. Malejka-Giganti et al. examined a detailed study of chemopreventive responses of tamoxifen (10 μg), indole-3-carbinol (250 mg), and a combination of both in 7, 12-dimethylbenzanthracene treated female Sprague Dawley rats for 20 weeks [21]. Previous studies suggested a significant decrease in mean tumor number per rat and increased the circulating estrogen level due to mediation of hepatic cytochrome 450 enzyme. The average ratio of the uterus to body weight was measured and found to be dropping after subsequent treatments. The rats receiving bare indole-

3-carbinol doses did not exhibit noticeable efficacy against cancerous cells. Although, synergistic chemopreventive activity was reported by tamoxifen+ indole-3-carbinol administered in rats, owing to induced caspase enzyme in mammary glands by indole-3-carbinol compound. Indole-3-carbinol derived antineoplastic therapeutic agents such as cisplatin causes downregulation of Bcl-2 expression in cervical cancer and inhibition of Akt/NF-kB signaling in prostate cancer cells [22]. Similarly, tamoxifen makes down-regulation of kinase enzyme CDK6/cyclin D at G1 phase thus targets MCF-7 breast cancer cells [23]. Another DIM derivative Taxotere is involved in the inactivation of Akt/NF-kB signaling and targets MDA-MB-231 breast cancer cells [24].

Synthetic indole-3-carbinol derived antineoplastic molecule has been designed and reported to possess more than 100 times high potency toward cell cycle arrest. In this context, acid stable OSU-A9 {[1-(4 chloro-3-nitrobenzenesulfonyl)-1H-indol-3-yl]-methanol} has been studied for enhanced apoptosis induction in cancerous cells compared to the parent one. Synthesized OSU-A9 demonstrated potential striking property for phosphorylation of diverse signaling targets such as protein kinase, cyclin D1, p21, and 27, Bcl-2 subgroups, and Akt mediated cell process. Weng et al. [25] demonstrated pleiotropic effect of OSU-A9 (10 and 25 mg/kg, intraperitoneal) on prostate cancer cells bearing athymic nude mice. The results outlined approximately 65 and 85% suppression of cancerous cells proliferation in a dose of 10 and 25 mg/kg, respectively, after 42 days. Western blotting study analyzed substantial reduction of phosphorylated biomarkers level (Akt, Bcl, and RelA) in prostate cancerous cells [25].

13.2.2 Synthetic indole derivatives: Alectinib, Osimertinib, Tivantinib, Anlotinib, Nintedanib, and Sunitinib

Popular synthetic indole derivative alectinib is successfully recommended for treating NSCLC through targeting anaplastic lymphoma kinase inhibitors. Food and Drug Administration (FDA)-approved orally active alectinib was first developed by Astra-Zeneca for the treatment of crizotinib-resistant NSCLC [26]. Fujimura et al. [27] studied combination effect of alectinib with six different chemotherapeutics and six molecularly targeted compounds (palbociclib, pemetrexed, carboplatin, gemcitabine, paclitexal, irinotecan, abemaciclib, everolimus, suberoylanilide hydroxamic acid (SAHA), luminespib, gedatolisib, and BKM10) to amplify antineoplastic efficiency against rearranged during transfection (RET) fusion cells. The outcomes analyzed through IC 50 isobologram and combination index applying LC-2/ad and Ba/F3-KIF5B-RET cells of mouse xenograft model demonstrated synergistic effects of alectinib with CDK4/6 inhibitors i.e. palbociclib and abemaciclib [27]. However, in this series diverse synthetic indole derivatives such as osimertinib, tivantinib, anlotinib, nintedanib, sunitinib, etc. were discovered that potentially inhibit subfamilies of protein kinase such as epidermal growth factor (EGFRT790M), multitargeted receptor tyrosine kinase, glycogen synthetase kinase, and multiple receptor kinases [28].

FDA approved, orally active sunitinib, a multitargeted receptor tyrosine kinase inhibitor is highly used in the management of drug-resistant gastrointestinal stromal tumors and treatment of renal cell carcinoma [29]. Several synthetic indole derivatives explored for their cytotoxic effects are compiled in Figure 13.5. Receptor tyrosine kinase inhibitor, indole-derived anlotinib is recommended by the virtue of targeting vascular endothelial growth factor type 2 and type 3 receptors (VEGFR2 and VEGFR3). It is found to inhibit angiogenesis and is indicated for the treatment of metastatic colorectal cancer [30]. Indolinone-derived nintedanib is orally active, known for its potent antineoplastic, antifibrotic, and antiangiogenic efficiencies. Nintedanib, a member of oxindoles inhibits collagen formation and hence is used in the treatment of idiopathic pulmonary fibrosis. Its combination with docetaxel is approved worldwide for NSCLC [31].

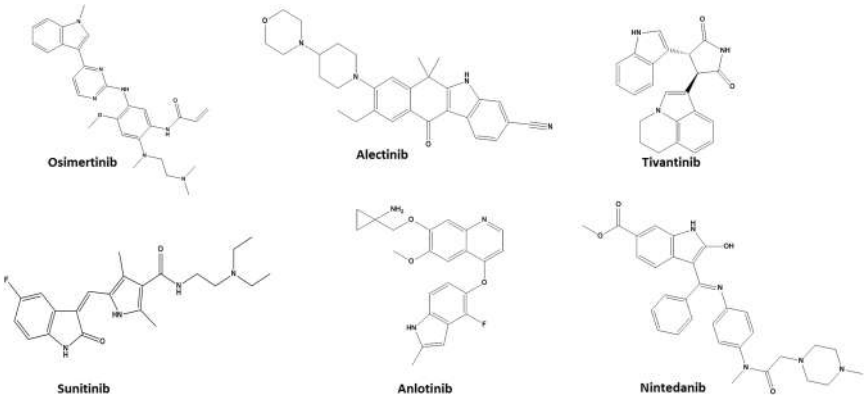

Figure 13.5: Indole-derived synthetic cytotoxic agents.

13.2.3 Meridianins

Meridianins, isolated as a secondary metabolite from marine invertebrate '*Aplidium meridianum*' is a rich source of indole alkaloid of chemopreventive activity. Meridianins (AG 1–7) exhibit promising antiproliferative action on cancer cells by the virtue of protein kinase inhibition (Figure 13.6). These molecules target CDK1 and CDK5 that suppress cell cycles and cause cell death [32].

Imperatore and coworkers reviewed the pharmacological significance of indole-derived molecules for their chemopreventive actions. Their research summarized the potential roles of South Atlantic marine ascidian 'Meridianins' for their anticancer activity. Chemically active meridianins are 3-(2-aminopyrimidine) indoles that are differentiated either by substituting bromine at the seventh or hydroxyl group at the fourth position of the indole ring. Meridianin E was substituted with bromine and was identified as a potent molecule with minimum IC 50 as illustrated in Figure 13.6. Their

report outlined a sequence of protein kinase inhibition, restriction on cell proliferation, apoptosis, and cell death [33]. Radwan and coworkers synthesized meridianin D analog from 3-cyano acetyl indole. The biological assay of cyano functionalized meridianin D analog depicted good cytotoxic action against MCF7 and HeLa cells with minimum IC 50 values of 0.85 and 2.65 μg, respectively [34]. Recently 'Aplicyanins' have been identified in Antarctic tunicate (*Aplidium cyaneum*, a marine invertebrate) containing newer indole alkaloids [35]. Chemically, these are configured with 3-tetrahydropyrimidyl indole moiety as a core skeleton. Various aplicyanins (A-F) that are supposed to be reduced forms of meridianins, were investigated. The reported indole-derived molecules demonstrated cytotoxic action by arresting tubulin polymerization and hence have been explored against the management of several malignancies such as lung carcinoma, breast adenocarcinoma, and colorectal carcinoma [36].

13.2.4 Enzastaurin

Indole-derived synthetic enzastaurin is a selective serine-threonine kinase (Protein kinase C, PKCβ) and PI3K/AKT inhibitor. It is widely used as an antitumor agent for the mitigation of relapsed glioblastoma multiforme, T/B cell lymphoma, solid tumor malignancy, and multiple myeloma. PKC is a vital channel for the survival of diverse cell types hence targeted by oncogenes that lead to malignant transformation [37]. Enzastaurin is an orally active antineoplastic agent and manufactured by renowned manufacturing company 'Eli-Lilly'. Oral unit dosage forms of strength 20–700 mg are reported to recommend depending upon the bioavailability of the therapeutic (Figure 13.6). Kohmoto et al. [38] investigated cytotoxic bisalkaoid 'dragmacidine' from deep water marine sponge Dragmacidine sp. The bis-indole molecule present in dragmacidine enables it as a vital pharmacophore owing to its specific potent efficacy compare to mono-indole derivatives against various carcinoma cells.

Approximately three decades ago, a research team of Kohmoto investigated its potential efficacy against human lung cancer (A549 cell lines) in a minimum dose range of 1–10 μg/ml [38]. Further, Wright and coworkers [39] studied various bioactive bis-indole alkaloids (dragmacidins, hamacanthins, topsentins, and nortopsentins) isolated from marine sources *Spongosorites* that are adorned with a number of pharmacological activities such as cytotoxic, antibacterial, antiviral, and antiplasmodial effects. Among these compounds dragmacidins G comprised one pyrazine ring adorned with two indole groups were reported to possess cytotoxic action against NIH3T3 mouse fibroblast cell line with minimum IC 50 (7.8 μM). *In vivo* study performed in several human pancreatic carcinoma cells (PANC-1, MIA PaCa-2, ASPC-1, and BxPC-3) for 72 h depicted potential action of Dragmacidins G with IC 50 value in the range of 14–27 μM. Dragamacidin exhibited inhibition of serine/threonine phosphoprotein phosphatase and ultimately mitotic arrest at metaphase [39].

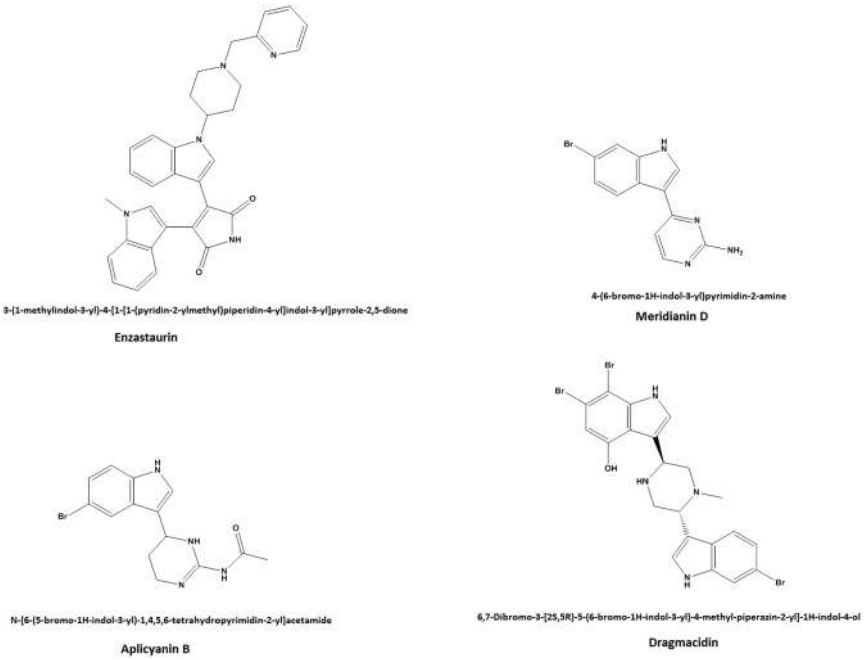

Figure 13.6: Indole-derived miscellaneous protein kinase and cyclin-dependent kinase inhibitors.

13.3 Tubulin inhibitors

Numerous heterocyclic scaffold-based pharmacophores have been proven to mediate anticancer activity owing to tubulin arrest. Myriad researches and patents are notified to discuss tubulin polymerization through indole heterocyclic compounds. Conformational alteration of microtubules affects cell shape, cell division, cell motility, and intracellular transportation. Modification of tubular activity and microtubule dynamics are targeted to alleviate several ailments and disorders including malignancies [40]. Several indole derivatives such as arylthioindoles, indole-3-oxy-glyoxylamides, 2-phenylindoles, oxindoles, carbazoles, indole-3-acrylamides, etc. are reported for their potent tubulin inhibition and anticancer activity [41].

13.3.1 Vinca alkaloids

Madagascar rosy or Vinca (*C. roseus*, Apocynaceae) is a popular perennial tropical plant bearing enormous ethnomedical properties. Vinca is been utilized in folk and Ayurvedic medicine for the alleviation of versatile ailments since ancient times. European medical practitioners used *Vinca* extract for the treatment of headaches and

diabetes. Indian folk and Ayurvedic medicines have a record on extensive application of *Vinca* as the hypoglycemic, hypolipidemic, and vasodilating agent. Its flower is been used to brew tea to treat skin disorders (eczema, acne, and dermatitis), and leaves juice is applied to relieve wasp-sting [42]. Chinese herbal preparations also include *Vinca* extract for the management of malaria, sores, hypertension, diabetes, and Hodgkin lymphoma [43].

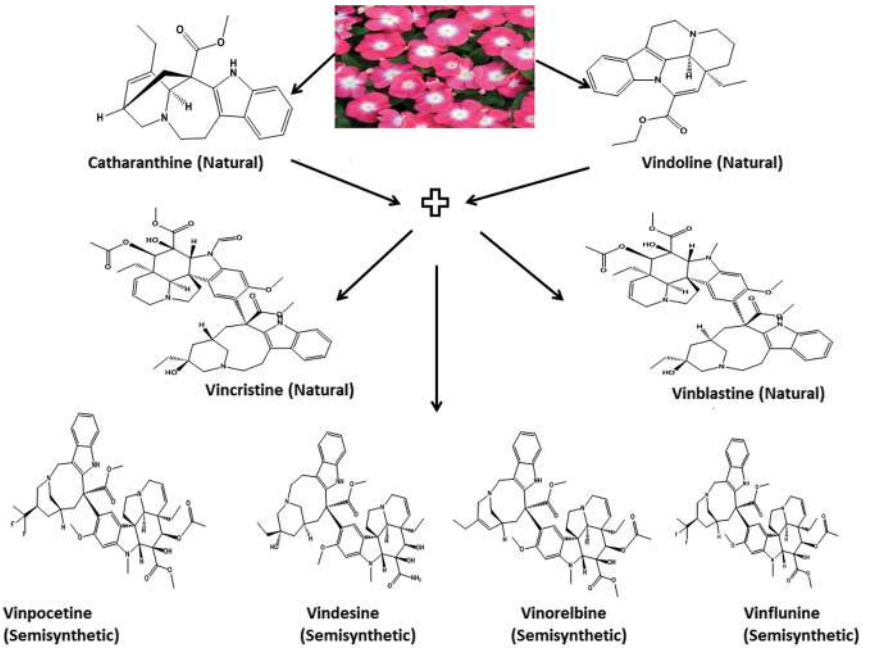

Figure 13.7: Natural and synthetic Vinca alkaloids.

Terpenoids indole Vinca alkaloids are generally collected from different species of tropical catharanthus such as *Catharanthus lanceus, C. roseus, Catharanthus longifolius, Catharanthus ovalis,* and *Catharanthus* pulsillus. Natural cytotoxic Vinca alkaloids are formed by the amalgamation of inherent catharanthine and vindoline (Figure 13.7). Vinca alkaloids were very first highlighted by R. Nobel and C. Beer (1950) during the treatment of hyperglycemic and blood pressure disorders [44]. The united States and Europe Cancer authorities have approved cancer fighter vinca alkaloids i.e. vincristine (approved in 1963), vinblastine (1961), and vinorelbine (1994) vindesine (2008) vinfluine (2008) for their compelling cytotoxic effects [45, 46]. Figure 13.7 compiles several natural and derived indole core-based anticancerous *Vinca* alkaloids.

Cytotoxic *Vinca* alkaloids have a great affinity for tubulin dimers that disrupt microtubule dynamics. At the molecular level, two vinca alkaloids get bind over one building block protein 'tubulin' to inhibit polymerization that leads to an interruption in

microtubule aggregation. High restriction on spindle formation checks cell division and replication that consecutively causes metaphase arrest. Figure 13.8 summarizes the typical anticancer action of vinca alkaloids. The FDA approved vincristine for the treatment of acute leukemia, Wilm's tumor, rhabdomyosarcoma, and Hodgkin's disease.

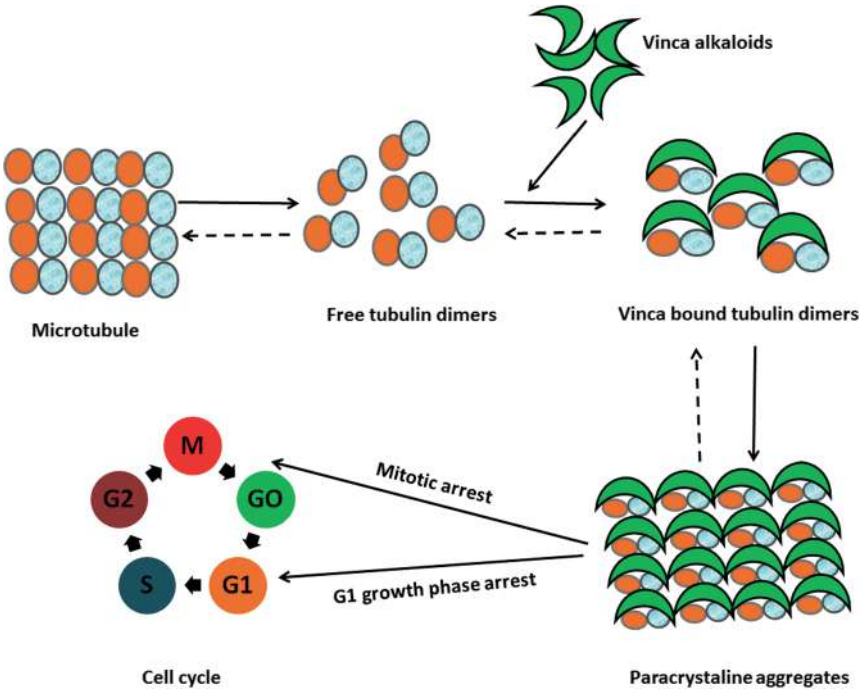

Figure 13.8: Schematic representation of microtubule dynamics disturbance through vinca alkaloids.

Vinblastine is reported for the management of germ cell tumors, Hodgkin and non-Hodgkin disorders, and breast cancer. Vinorelbine exhibited the same activity as vinblastine and was extensively applied for breast cancer and bone tumor cells owing to its potent antiproliferation effects. Vindesine has a short serum half-life (24 h) and is intended to utilize for alleviation of melanoma, uterine, and lung cancer. Clinical application of Vinca alkaloids was restricted due to several adverse effects such as vomiting, nausea, peripheral neuropathy, ataxia, urinary retention, diarrhea, and multidrug resistance [47]. Novel drug delivery strategies as nanoparticles-based carrier, liposomes, polymeric micelles, and chemically modified approaches are frequently applied that minimize toxicity and improve therapeutic efficiency [48]. Maia and coworkers have successfully developed biocompatible and nonimmunogenic vincristine-loaded hydroxyapatite nanoparticles (285 ± 10.29 nm) for the mitigation of osteosarcoma, a bone cancer. Chorioallantoic membrane assay exhibited antiangiogenic and antiproliferative efficiency of developed vincristine-loaded nanoparticulate system [49].

Zhang et al. developed vincristine – oleic acid-loaded submicron emulsion through classical high-pressure homogenization technique. The designed system encompassed regular size (157.6 ± 12.6 nm), uniform spherical-shaped globules with optimum zeta potential (−26.5 ± 5.0 mV). High encapsulation efficiency (78.64 ± 3.4%) and enhanced mean residence time (from 187 to 227 min) suggested the potential application of vincristine embedded submicron emulsion for the alleviation of cancers. Pharmacokinetic analysis on MCF-7 cells of mice revealed a greater area under the curve (~17,164 mcg/L/min), higher cytotoxicity ($p < 0.05$) and augmented apoptosis ($p < 0.05$) of the developed system compared to vincristine solution [50]. The frequent usages of vinca alkaloids are limited due to their number of undesirable side effects and poor solubility. To overcome adverse effects and improve solubility, Zu and co-workers have proposed the design and development of vinblastine sulfate nanoparticles embedded in folate conjugated bovine serum albumin (VBLS-FA-BSANP) for the purpose of tumor-targeting. The system was optimized via central composite design considering four independent variables (ethanol rate, ethanol amount, degree of crosslinking, and bovine serum albumin) and two response variables (mean particle size and residual amino groups). The developed nanoparticles were nanodimensional (~156 nm) with optimum residual amino groups (~669 nM/mg). The outcomes from Response Surface Methodology revealed that an increased ratio of VBLS to BSA amplified both, entrapment efficiency and drug loading. Prepared VBLS-FA-BSANP system exhibited passive cancer cell targeting owing to the presence of folate that has a strong binding affinity and increased cellular uptake of anticancer therapeutics [51].

Vinorelbine, a semisynthetic indole derivative vinca alkaloid has been explored for the management of advanced cancer diseases namely breast cancer, metastatic NSCLC. Few marketed preparations of vinorelbine have reported serious adverse effects such as granulocytopenia, venous discoloration, phlebitis, and urticaria that suggested the need for system modification for efficient antineoplastic drug delivery. In this context, Bahadori et al. developed and characterized vinorelbine-loaded sterically stabilized phospholipid nanomaterials (approximately 15 nm). The developed biocompatible and biodegradable system was 6.7 times more potent than bare vinorelbine against MCF-7 breast cancer cells [52]. The results depicted a safe and effective passive targeted drug delivery system for advanced breast cancer. Nowadays, vinorelbine is recommended along with other cytotoxic agents (cetuximab and cisplatin) to alleviate NSCLC. Similarly, another effective vinca alkaloid 'vinflunine' has been acclaimed in lung cancer owing to chemopreventive potential with platinum-based therapeutics [53].

13.3.2 Indole chalcone derivatives

Naturally occurring chalcones have been reported for their prominent pharmacological and therapeutic actions. Numerous antioxidative, anticancer, antimutagenic, and antiinflammatory actions of chalcones have been emphasized. Reports emphasize

augmented cytotoxic actions in cancer cells on an amalgamation of indole and chalcones by the virtues of their tubulin polymerization and antiproliferation activities in diverse cells (PC3, PAN02 cells, A549, and CLR2119). Cong and coworkers demonstrated anticancer activity of one of the indole-chalcone derivative compounds (FC77) that exhibited cancer cell growth arrest (NCI 60 human cancer cells) through binding with tubulin dimer. FC77 had potential activity against multi-drug-resistant cancer cell lines with minimum action on healthy peripheral blood cells. The outcomes suggested the novel application of indole-chalcones for safe and effective antineoplastic therapeutics. The potent action on tubulin dimer changes cell cycle dynamics more effectively than other popular therapeutics such as paclitaxel and vincristine. Moreover, significantly low IC 50 of FC77 (3 nM) than vincristine (37 nM) has attracted researchers to design precise microtubule targeting agents for alleviating multi-drug resistant lung cancers [54].

Zhao et al. [55] have developed novel indol-chalcone derivatives that dramatically restricted tumor growth both, *in vitro* and *in vivo* as well. The compound had the potential to activate the Nrf-2/HO-1 pathway and to manage apoptosis (A549 cells of lung cancers). Nrf-2 (nuclear factor-erythroid2-related factor) is expressed in various tissues that maintain cellular redox balance. Developed indol-chalcone derivatives were Nrf-2 activators and exhibited chemopreventive activity in a time-dependent manner. Hoechst 33258 staining assay was performed to compare anticancer activity via induced apoptosis among synthesized compounds [55]. In this series, Badria et al. synthesized five different *N*-ethyl-3-acetylindole derived crystalline chalcones (3a–3e) that showed antiproliferative activity, as illustrated in Figure 13.9. Their anticancer activity was evaluated against various cancer cell lines such as liver (HepG2 and HuH-7), prostate (PC-3), breast (triple-negative MDA-MB-231 and ER-positive MCF-7), oral (SAS), and colon (HCT-116) carcinoma.

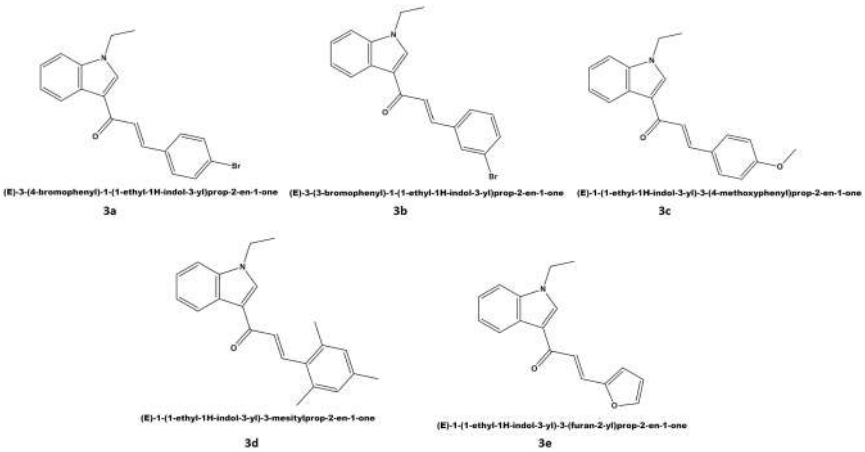

Figure 13.9: *N*-ethyl-3-acetylindole-derived chemopreventive chalcones.

The (3-4,5-dimethylthiazole- 2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay revealed great sensitivity for triple-negative breast cancer (MDA-MB-231). All the molecules (3a–3e) exhibited susceptibility toward breast cancer. Whereas, the molecules 3b, 3d, and 3e were found to be little effective against Hep G2, compared to hepatocellular carcinoma cells (HuH-7). However, molecules 3c and 3d did not present cytotoxic action for prostate, oral, and colon cancers [36].

13.3.3 Bisindolyl hydrazide-hydrazone derivatives

Similar to indole, pharmacophore hydrazide-hydrazone was also identified to be efficacious for the treatment of several ailments including cancer, microbial infections, tuberculosis, convulsions, and inflammation owing to the presence of nitrogen-containing heterocyclic ring in their chemical configuration [56]. Sundaree et al. [57] synthesized indole-3-carboxaldehyde derived antiproliferative hydrazide-hydrazones that are represented in Figure 13.10. The developed series of indole-based hydrazide-hydrazones were evaluated for their anticancer activity against breast cancer cell lines (MDA-MB-231 and MCF-7), prostate (PC3, LnCaP, and DU145), and pancreatic cancer cell lines (PaCa2). The anticancer assay revealed antiproliferative action of developed molecules in a concentration ranging from 100 to 1 nM. The cytotoxic activity was dependent both, on the type of substitution of the indole nitrogen atom and hydrazide moiety [57].

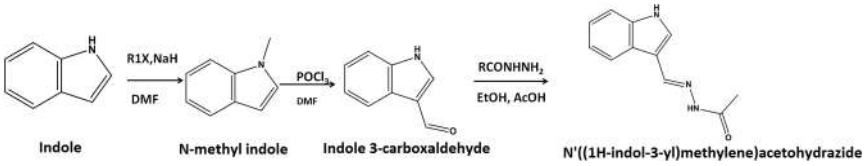

Figure 13.10: Common pathway for synthesis of bioactive indole-3-carboxaldehyde-derived hydraxide-hydrazone.

Mukherjee and coworkers [58] utilized an indole scaffold for the development of Bis indolyl hydrazide-hydrazone (NMK-BH3) to describe its potency against lung A549 cancer cell lines. The chemical structure of FC77 and NMK-BH3 are depicted in Figure 13.11. The synthesized compound NMK-BH3 depicted antiproliferative efficacy for human lung adenocarcinoma, peripheral blood mononuclear cells, and normal human lung fibroblast cells with IC50 of 2, 62, and 49 Mm, respectively. The outcomes suggested the potential therapeutic use of NMK-BH3 for cancerous cells with minimum damage to normal healthy cells. Studies such as immunofluorescence, western blotting, and flow cytometry identified cell apoptosis through depolarization of mitochondrial and cellular interphase at the G2/M phase of the cell cycle.

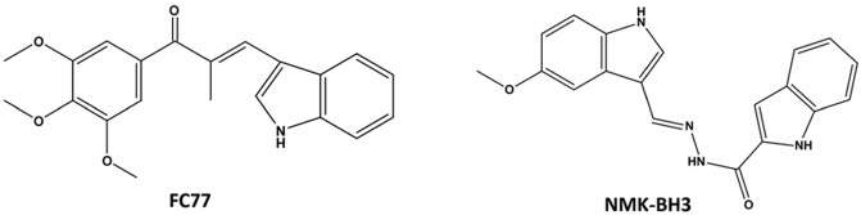

Figure 13.11: Chemical structures of synthetic indole-based tubulin inhibitors.

Hence, it can be affirmed that NMK-BH3 exhibited strong and specific tubulin binding and changed tubulin dynamics [58].

13.3.4 Arylthioindoles and indole-3-glyoxylamides

These indole derivatives (arylthioindoles and indole-3-glyoxylamides) are potent inhibitors of tubulin polymerization, disturbed tubulin assembly at colchicine sites, and hence display restriction in cancer cell growth. Martino et al. [59] described the potent antitumor activity of arylthioindoles owing to the inhibition of tubulin polymerization. They synthesized novel compound 'methyl 3-[(3,4,5-trimethoxyphenyl) thio]-5-methoxy-1H-indole-2-carboxylate' illustrated in Figure 13.12. These compounds possessed 1.6 times more potency against breast cancer cells (MCF-7 cells) compared to colchicine [59]. Synthetic indolylglyoxyl amides are investigated as novel tubulin inhibitors for the management of cancers. Buvana et al. discussed more active indole-based lead compound, Indibulin, D-24851, and reviewed its potency against ovarian cancer cells (SKOV3), glioblastoma (U87), and pancreatic cancer cells (ASPC-1) [60]. Chemically, Indibulin is *N*-(pyridine-4-yl)-[1-(4-chlorobenzyl) indol-3-yl] glyoxymide that possessed anticancer, anti-HIV, and antiprion activities.

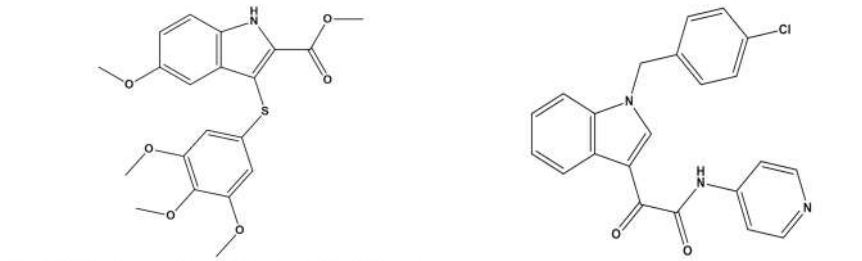

Figure 13.12: Synthetic indole-based tubulin polymerization inhibitors.

13.4 Histone deacetylase inhibitors (HDAC inhibitors)

Recently introduced histone deacetylase inhibitors are a new class of anticancer agents that are involved in epigenetic and nonepigenetic cell regulation governed by sequential apoptosis and cell cycle arrest. Normal cell physiology comprises balanced histone acetylation and deacetylation through histone acetyltrasferases (HAT) and HDAC enzymes, respectively. Acetylation of histones mediates gene transcription through opening chromatin structure. Deacetylation of histone tightens their interaction with DNA that closes chromatin structure and checks gene transcription. The imbalance between the functions of HAT and HDAC enzymes is sighted in cancers [61].

13.4.1 Dacinostat

Dacinostat (NVP-LAQ824), a potent HDAC inhibitor is widely used in cancer research and induction of apoptosis in myeloid leukemia cell lines owing to its low IC50 of 32 nM. It is a cinnamic acid hydroxamate that also arrests the cell cycle and causes both acute/chronic programmed cell deaths. NVP-LAQ824 showed a synergic effect with imatinib, a tyrosine kinase inhibitor to override drug resistance during the treatment [62].

13.4.2 SAHA

Kelly et al. [63] focused on discovery, development, and biological activity of indole-containing cap group 'SAHA'. FDA approved, orally bioavailable SAHA is widely recommended to treat cutaneous T-cell lymphoma. The molecule presents a new class of targeted anticancer activity by altering the transcription of genes in a broad spectrum of neoplasm. Its minimum dose is well tolerated by cancer patients suffering from hematologic and solid tumors. SAHA was capable of post-translational modifications of histones/chromatins which are accountable for epigenetic gene regulation [63]. Komatsu and coworkers extended the above study and demonstrated the potential significance of SAHA (later 'varinostat') for the management of NSCLCs. They found that SAHA suppressed cell growth in dose-dependent manner (minimum IC50 2 μM) in the NSCLC patients. Fluorescence-activated cell sorting analysis revealed cell cycle arrest by SAHA at the G0-G1 phase [64].

13.4.3 SL142 and SL325

Two new indole-derived HDAC inhibitors, SL142 and SL325 were identified for their potential to arrest cell growth hence cause apoptosis with minimum toxicity for normal cells. Chemically both are novel cyclic amide bearing hydroxamic acid derivatives that

induce cell growth inhibition. Their combination treatment is widely accepted with retinoic drugs for enhanced apoptosis and suppression of tumor growth (H441 and A549 cell lines). Both exhibited prominent caspase-3 activity and G0/G1 cell cycle arrest at minimum concentration. Moreover, both were found to be significantly active in the treatment of prostate cancer by the virtue of being p21 promoters and their cytostatic actions [65]. Figure 13.13 illustrates the chemical structure of different indole-based HDAC inhibitors.

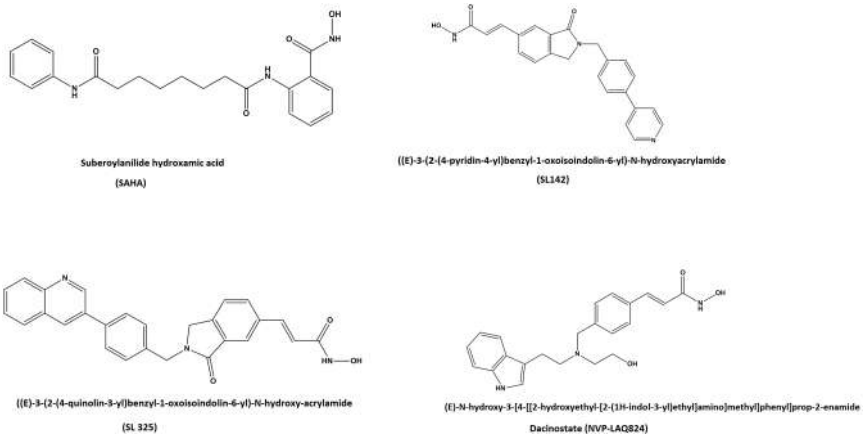

Figure 13.13: Miscellaneous HDAC inhibitors comprising indole core molecule.

13.4.4 1-Arylsulfonyl-5-(*N*-hydroxyacrylamide) indoles

Lai et al. [66] described *in vivo* antiproliferative activity of a series of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide) indoles, potent HDAC inhibitors. The substitution of *N*-hydroxyacrylamide at position 5 of indole heterocycle exhibited maximum biological action relative to C3, C4, C6, and C7 positions. The IC 50 value of the developed compound (MPT0E014) against various HDACs (HDAC1, 2, and 6 isoenzymes) ranged between 1 and 12.3 Nm. The *in vivo* study performed on a human xenografted model bearing A549 cell displayed a 47% of delay in tumor growth [66]. The Western blotting analysis of cell lysate revealed dose-dependent upregulation of histone and tubulin acetylation against PC-3 cell lines (prostate cancer).

13.5 DNA topoisomerase inhibitors

Topoisomerase is a class of nuclear enzymes required for cell proliferation mediated through DNA replication. Topoisomerase I involves breaking and rejoining one of a single strand of DNA whereas Topoisomerase II relaxes topological tension between

DNA double helices. Indole-based compounds bind to the DNA topoisomerase resulting prevention of coiling and resealing of DNA. Aggregation of DNA inhibits its replication and causes cell death [67]. Various indole-based compounds such as Bis (hydroxymethyl) indolizino-indoles, makaluvamine, hybrid BO-1978, and ursolic acid-derived *N*-aminoalkyl have been reported for their chemopreventive actions.

13.5.1 Bis(hydroxymethyl)indolizino-indoles

Numerous bis(hydroxymethyl)indolizino-indoles have been synthesized to investigate their anticancer activity. Their alkylcarbamates derivatives such as β-carboline and bis (hydroxy-methyl) pyrrole are considered as DNA topoisomerase inhibitors and DNA cross-linking molecules, respectively. These indole-derived synthetic molecules were investigated for the management of human breast carcinoma, colon cancer, and lung adenocarcinoma owing to their significant cytotoxicity. The treatment exhibited more than 99% of tumor remission as these molecules induced DNA cross-linking; inhibited topoisomerase I and II and cell cycle disturbance at the S phase [68]. The cytotoxic activity of bis (hydroxymethyl) indolizino-indoles derived molecules was extended by Chang and coworkers. They investigated anticancer activity against small cell lung cancer cells (A549) and claimed topoisomerase II inhibition by synthesized pharmacophore. A sequential mechanism of action initiated by inhibition of topoisomerase II, followed by cell cycle arrest at the G2/M phase, and tumor cells apoptosis were reported. Additionally, their research claimed that the synthesized indole-based molecules (indolizino[8,7-b] indole hybrids) are more potent than other anticancer agents such as cisplatin and etoposide [69].

13.5.2 Makaluvamine

The natural marine sponge 'Zyzzya' has been explored for the isolation of chemopreventive indole-based alkaloid makaluvamine. The compound was investigated against human colon carcinoma cells (HCT 116) for its chemopreventive action [70]. Makaluvamine enhanced cell toxicity toward a topoisomerase II-cleavable complex-sensitive cell culture by the virtue of inhibition of topoisomerase II decatenation of kinetoplast DNA. Additionally, it increased the life span of nude mice bearing solid tumors of human ovarian cancer cells. Reports displayed its prominent cytotoxic action owing to DNA topoisomerase II inhibition against human lung cancer cell lines. A series of biologically active makaluvamine analogs were synthesized which are effective against human adenocarcinoma (A549 and H1299) at very low IC values of 0.3 and 0.58 μM, respectively, by the process of apoptosis and S1 phase cell arrest [71]. Wang et al. [72] synthesized four novel makaluvamines analogs (FBA-TPQ, PEA-TPQ, MPA-TPQ, and DPA-TPQ) and determined their biological structure-activity

relationship. Figure 13.14 compiles synthetic makaluvamine analogs that check DNA Topoisomerase II. The cytotoxic activity was assessed against breast cancer cell lines (MCF-7 and MDA-MB-468). The *in vitro* cytotoxic activity was compared and amidst all, FBA-TPQ exhibited the strong potential to cause apoptosis in xenograft tumors in nude mice in dose-dependent manner [72].

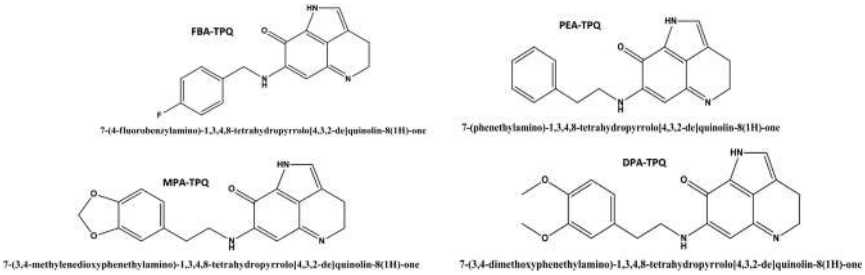

Figure 13.14: Synthetic Makaluvamine analogs derived for inhibition of DNA topoisomerase II.

13.5.3 Bisindolylalkanes (Hybrid BO-1978)

Chen et al. [73] synthesized a potent derivative of indolizino [6,7-b]indole comprising a β-carboline group that inhibits DNA topoisomerase II for the management of NSCLC. The synthesized [3-ethyl-6-methyl-6,11-dihydro-5H-indolizino[6,7-b]indole-1,2-diyl] dimethanol (BO-1978) displayed desirable cytotoxicity against xenograft and orthotopic lung models on mice. Figure 13.15 illustrates the chemical structure of natural makaluvamine and synthetic BO1978 The pharmacokinetic study of BO-1978 revealed a long half-life (~23 h), medium clearance rate, and moderate distribution that suggested safe and effective administration daily [73].

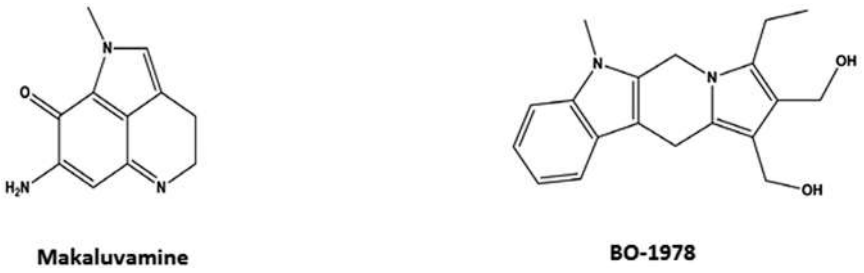

Figure 13.15: Indole-derived natural Makaluvamine and synthetic BO-1978.

13.5.4 Indole-based Ursolic acid bearing *N*-aminoalkyl side chains

Naturally occurring pharmacologically active ursolic acid is a pentacyclic triterpenoid and widely explored for its diverse medicinal values including anticancer activity. Ursolic acid modulates cellular transcription factor that regulates metastasis, angiogenesis, apoptosis, and autophagy of cancerous cells [74]. Li et al. [75] designed and evaluated novel indole derivatives comprised ursolic acid-*N*-(aminoalkyl) carboxamide for their cytotoxic activities against hepatocarcinoma cell lines namely SMMC-7721 and HepG2. Enzyme inhibition assay and molecular docking analysis defined decreased mitochondrial membrane potential and cell apoptosis of SMMC-7721 cells owing to an/the inhibition of topoisomerase II. The presence of *N*-(3-(4-methylpiperazine-1-yl) propyl) carboxamide side-chain provided anticancer potency against cancerous cells [75].

13.6 Conclusion

Indole-based derivatives have been vigorously explored by researchers as a powerful probe for the management of life-threatening ailments including cancers. Myriad compounds such as camalexi (apoptosis inducer), labradrins (kinase inhibitors), indibuli (tubulin assembly inhibitor), 3-aroylindole (modulation of tubulin polymerization) also possess indole as the parent molecule and are reported to be anticancerous. Although, significant advancements in designing and clinical study of newer indole derivatives are evident challenges associated with drug resistance and their appropriate management in multi-drug therapy in cancer patients are to be monitored to resolve clinical intricacies.

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14 Indole based prostate cancer agents

Abstract: Cancer weakens the immune system which fails to fight against the rapidly growing cells. Among the various types of cancers, prostate cancer (PCa) is causing greater number of deaths in men after lung cancer, demanding advancement to prevent, detect and treat PCa. Several small molecule heterocycles and few peptides are being used as oncological drugs targeting PCa. Heterocycles are playing crucial role in the development of novel cancer chemotherapeutics as well as immunotherapeutics. Indole skeleton, being a privileged structure has been extensively used for the discovery of novel anticancer agents and the application of indole derivatives against breast cancer is well documented. The present article highlights the usefulness of indole linked heterocyclic compounds as well as the fused indole derivatives against prostate cancer.

Keywords: DU-145; indole derivatives; LNCaP; oncological drugs; PC-3; prostate cancer.

14.1 Introduction

Cancer is a group of diseases in which abnormal cells divide in an uncontrolled manner and invade or spread to other locations in the body. Cancer weakens the immune system, thereby failing it to fight against the rapidly growing cells and results into severe damage to the body. Prostate cancer (PCa) is known to cause large number of deaths in men after lung cancer and is first among new cancer cases occurring in USA from 2011 to 15 [1]. Studies have shown that risk for PCa is the combination of several factors. Established risk factors for PCa include family history of the disease, race, advanced age and certain genetic polymorphisms [2, 3].

Based on 2012–2016 cases and deaths reports by PCa, about three million people are suffering with PCa in the United States and nearly 12% men are on risk of being diagnosed with PCa in their lifetime [4]. Since 1992, over the period of about 25 years, about 55% reduction in the new cases was reported where the death rate was reduced to

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about 19 in 2016 compare to 39 in 1992 per 100,000 persons investigated. No much improvement was observed from 2014 to 2016.

The diagnosis for PCa relies on the specific detection of high levels of prostate cancer gene 3 (PCA3) associated with serum prostate-specific antigen (PSA). The most useful *in vitro* model for the PCa research is cell culture. Several cell lines are available for the exhaustive and comprehensive study of anticancer activity and to figure out the molecular mechanism of PCa, several cell models have been developed from patient biopsies (Table 14.1) [5]. Among all of these, mostly three lines PC-3, DU-145 and LNCaP are used in research on PCa.

Several murine models are also widely utilized in PCa research wherein patient derived xenografts are prepared by implanting PCa tissue or cells in genetically engineered humanized mice. These xenografts are prepared by direct grafting of PCa tissues at subcutaneous, orthotopic and subrenal capsule (SRC) sites of immunodeficient mice [6–8].

Several small molecule heterocycles and few peptides are being used as oncological drugs [9] targeting PCa (Figure 14.1). Oncovin and Velban are the two-indole based oncological drugs being approved for multiple myeloma and Hodgkin's lymphoma, respectively. Indole represents one of the most important heterocyclic rings which is a privileged scaffold in drug discovery [10]. A number of natural products as well as medicinally important synthetic small molecules contain indole nucleus which is also built into proteins in the form of essential amino acid L-tryptophan [11–13]. The immunomodulatory role of indoleamine-2,3-dioxygenase (IDO) to self-regulate immune responses play a crucial role in enabling cancers to evade the immune system highlighting IDO as a therapeutic target in cancer [14, 15].

Among the several small molecule IDO inhibitors, 1-methyl-D-tryptophan (D-1MT, Indoximod) is in clinical trial for the treatment of metastatic PCa and over 50 phase III clinical trials using 1-MT alone or in combination with other therapeutics are under process. Various FDA approved PCa drugs, along with their year of approval are shown

Table 14.1: Research cell lines and murine models for prostate cancer.

PCa cell lines		Mouse model for PCa	References
pRNS-1-1	LNCaP	Pten	[38]
BPH 1	22Rv1	TRAMP	[34]
LAPC-9	ARCaP	Hi Myc	[5]
MDA	PC-3	Lo Myc	[42]
RWPE-1	DU-145	LADY	[43]
LAPC-4	PIN	MPAKT	[6]
VCaP	PIN cells	Xenograft	[7, 8]
PCa2a/2b	C4-2	Allograft	[20]
ARCaP	C4-2B	Metastatic models	[5]

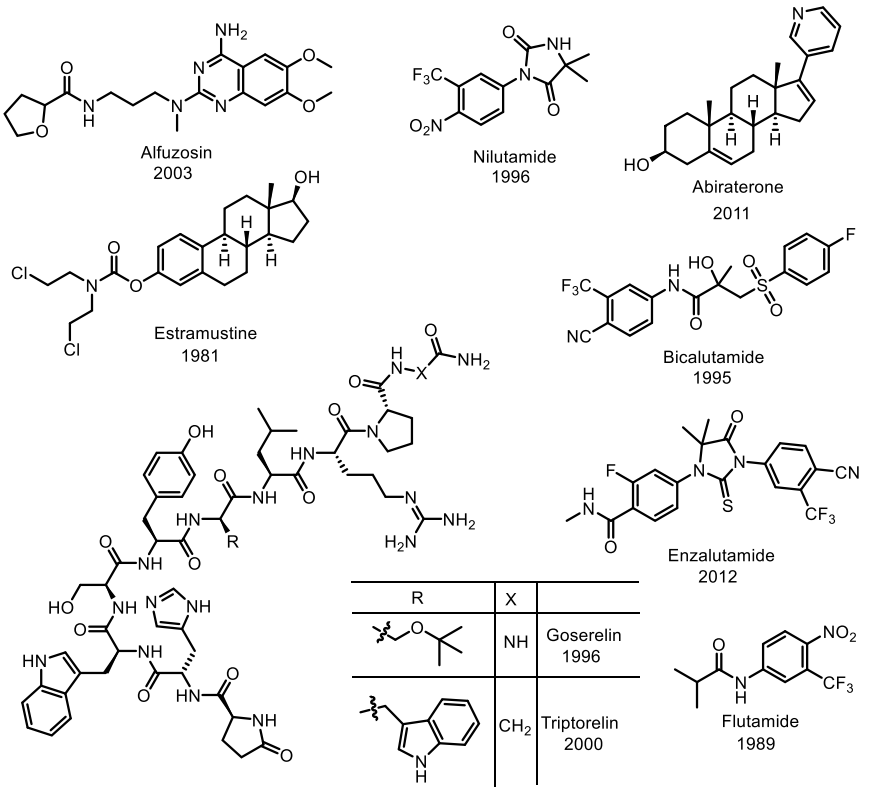


Figure 14.1: Oncological FDA approved drugs targeting prostate cancer.

in Figure 14.1. The only two tryptophan containing PCa therapeutics include decapeptides goserelin and Triptorelin which were approved in 1996 and 2000, respectively.

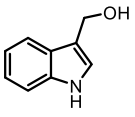
Recently, Sidhu and coworkers have described the role of indole derivatives against breast cancer [16]. In order to highlight the role of indole derivatives towards the development of PCa treatment, a database of indole derivatives active against PCa is compiled in the present article.

The indole derivatives active against PCa are classified into two categories:

i) Indole linked heterocyclic PCa agents
ii) Fused indole derivatives as PCa agents

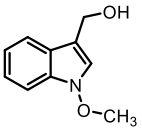
14.2 Indole linked heterocyclic prostate cancer agents

Epidemiological studies have shown that environmental factors, particularly dietary supplements play a crucial role in lowering the rate of PCa. Several classes of compounds obtained from fruits and vegetables have shown protective effect against PCa.

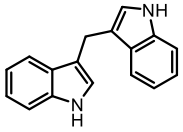


1 (I3C)

IC_{50}=150 μM (LNCaP)
160 μM (DU145)
285 μM (PC-3)

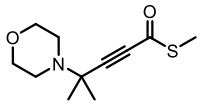


2 (NI3C)



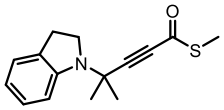
3 (DIM)

IC_{50}= 40 μM (LNCaP)
20 μM (DU145)
40 μM (PC-3)



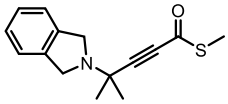
MATE

IC_{50} = 9.3 μM (DU145)



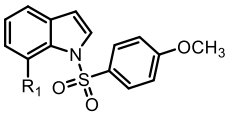
4

IC_{50} = 7 μM (DU145)



5

IC_{50} = 6.2 μM (DU145)



6

IC_{50} = 4-14 μM (DU145)

Compd.	R_1	IC_{50} (DU145) (μM)
6a	4-pyridyl	7.06
6b	2-furanyl	4.44
6c	1-methyl-1-imidazol-1-yl	6.01
6d	3,4-dichlorophenyl	6.21
6e	4-methylsulfonyl phenyl	7.25
6f	4-cyanophenyl	6.16
6g	4-chlorophenyl	6.25
6h	4-cyano-3-fluorophenyl	8.75
6i	-NH-CO-4-CN-C_6H_4	8.19

Figure 14.2: Inhibitory concentrations of indole derivatives against PCa cell lines.

In particular, indole-3-carbinol (I3C, **1**, Figure 14.2) present in different plant food substances such as cauliflower, radishes, cabbage, mustard greens, arugula etc. (the family members of the genus *Brassica*) [17] are known to induce G1 block of cell division and down regulate the growth of LNCaP prostate carcinoma cells. I3C is known to function as inhibitor of Akt activation and suppressed the production of NF-*k*B, and also induced apoptosis. In addition to I3C (**1**), *N*-methoxyindole-3-carbinol (NI3C, **2**), found in few cruciferous vegetables (Figure 14.2), was a more potent inhibitor inducing cell cycle arrest in human colon cancer cell lines (DLD-1 and HCT-116) [18].

3,3′-Diindolylmethane (DIM, **3**), obtained from Cruciferae family and I3C, both exerted anticancer effects through the modulation of serine/threonine protein kinase (PKB/Akt), nuclear factor kappa B (NF-*k*B), mitogen-activated protein kinases (MAPK) and B-cell lymphoma-2 (Bcl-2) signaling pathways. *In vitro* studies by Yannai and coworkers have shown that DIM and I3C selectively inhibited the growth of PCa cell lines by inducing p53 independent apoptosis [19].

Quash and coworkers synthesized indole containing α,β-acetylenic amino-thiolesters (**4** and **5**) with several other heterocycles and investigated their anti-cancer

potency using PCa epithelial cells (DU145) as well as prostate epithelial normal cells (HPENC) [20]. The inhibitory activity of 2,3-dihydroindole **4** and 1,3-dihydroisoindole **5** was similar to that of *S*-methyl 4-methyl-4-morpholinopent-2-ynethioate (MATE), an irreversible inhibitor of aldehyde dehydrogenase 1 (ALDH1).

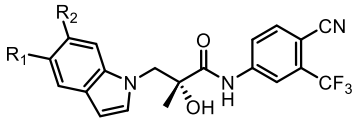
The indole derivatives **4** and **5** were found to be reversible inhibitors of normal cells and irreversible inhibitors of PCa cell lines (Figure 14.2). The inhibitors induced early apoptosis in DU145 and PCa cell lines by increasing the levels of mitochondrial superoxide and decrease in the redox potential.

A series of *N*-arylsulfonyl indole derivatives (**6**) synthesized by Zhou et al. were observed to be a dual inhibitor of STAT3 and tubulin using MTT assay [21]. Several of the synthesized compounds (Figure 14.2) demonstrated *in vitro* anti-proliferative activity at less than 10 μM concentration and inhibited STAT3 phosphorylation at Tyr705. Overall nine compounds (**6a**–**6i**, Figure 14.2) inhibited the cell proliferation for DU145 and PCa cell lines comparable to that of standard reference inhibitor (**E28**, IC_{50} = 1.03 μM). The lead compound also blocked the cell cycle in A549 (lung cancer cell) at G2/M phase and inhibited tubulin polymerization.

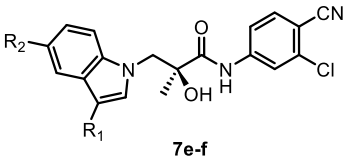
The selective androgen receptor degraders (SARDs) activities in indole (**7**) and indoline (**8**) derivatives were reported by Hwang and coworkers [22]. Both the classes have been shown to exhibit excellent activity against PCa cell lines (Figure 14.3). During the detailed SAR investigation, a small advantage was observed for indolines, whereas no much-pronounced effect of chlorine or trifluoromethyl group at position-3 of phenyl ring was observed. Indole moiety having electron-withdrawing group at C6 position (**7d**) and fluorine at C3- and C5-position (**7e** and **7f**) showed pronounced activity. The nitro substituted indole derivatives, **7c** (5-NO_2, IC_{50}: 88 nM) and **7d** (6-NO_2, IC_{50}: 58 nM), demonstrated strong splice variant SARD activity and potent *in vitro* antagonism. Corresponding indoline derivatives (**8a–d**) were found to exhibit better activities in comparison to their indole analogues.

Goswami and coworkers reported the synthesis of 2-aryl/pyridin-2-yl-1*H*-indoles (**9**–**12**) starting from substituted salicylaldehydes, using a structure-guided approach [23]. Compound **9** showed most potent and selective inhibition against Hepsin. Key interactions of amidine group, the cyclohexyl ring and the 3°-hydroxyl functionality at the binding site pocket were revealed by X-ray crystallography (PDB ID: 1P57). Compounds **9** and **10** (Ki of 0.1 mM for Hepsin) displayed good inhibition of invasion and migration of LNCaPC42B4 and PC-3 (Figure 14.3) without signs of any cytotoxicity. The related indole analogues, **11** and **12** also showed potential activity against PC-3 and LNCaP cell lines.

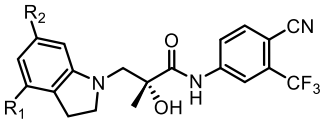
Cherkasov and coworkers described the synthesis of 1*H*-indole-2-carboxamide derivatives (Figure 14.4) showing binding function 3 (BF3) inhibitory and anti-proliferative activity against LNCaP and enzalutamide-resistant PCa cell lines [24, 25]. The lead benzimidazole analogue **13** (IC_{50} = 4.2 μM) as well as its indole derivative **14** (IC_{50} = 5.4 μM) showed the comparable inhibitory activities. In view of these results, a novel series of indole derivatives was developed (scaffold **15**; Figure 14.4) as anti-



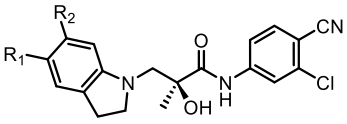
7a-d



7e-f

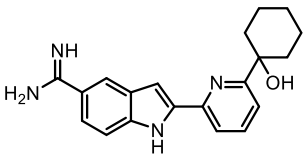


8a-b



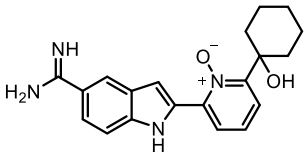
8c-d

Compound	R_1	R_2	IC_{50} (μM)
7a	H	H	0.064
7b	H	F	0.085
7c	NO_2	H	0.088
7d	H	NO_2	0.058
7e	F	H	0.045
7f	H	F	0.094
8a	F	H	0.059
8b	H	F	0.039
8c	H	F	0.037
8d	F	F	0.032



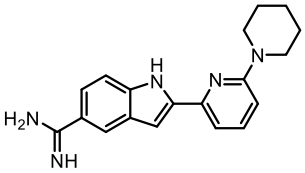
9

EC_{50} = 2.6 μM (PC-3)
0.9 μM (LNCaPC42B4)



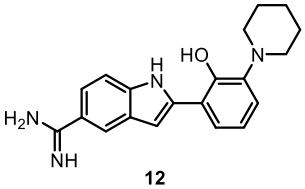
10

EC_{50} = 1.8 μM (PC-3)
0.6 μM (LNCaPC42B4)



11

EC_{50} = 5.2 μM (PC-3)
2.6 μM (LNCaPC42B4)



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EC_{50} = 4.9 μM (PC-3)
4.2 μM (LNCaPC42B4)

Figure 14.3: Structure and activity of indole derivatives against PCa cell lines.

androgenic heterocycles targeting the BF3 site. The IC_{50} values were determined using cell based enhanced green fluorescent protein (eGFP) androgen receptor transcriptional reporter assay and was further quantified by studying effect of compounds on PSA (prostate specific antigen). Molecular docking experiments suggested better activity for the indole derivatives with methyl group at R_4 and R_5 positions of the proposed scaffold **15**. In particular, the R_4 methyl substituted analogue **15a** was slightly more active than **15b**. Whereas, the fluoro substitution at R_4 (**15c**) as well as R_5 (**15d**) drastically enhanced the activity. Compound **15e** without the methyl group on the furan

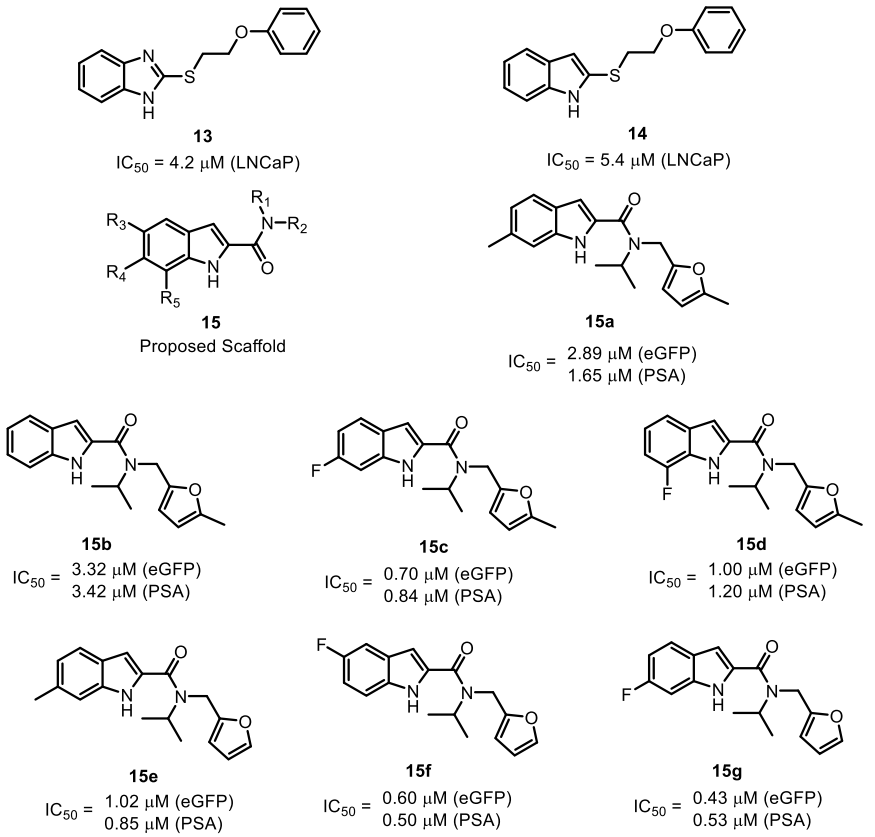
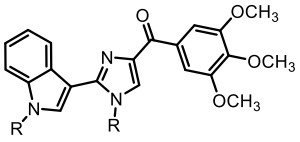


Figure 14.4: Antiproliferative activity against LNCaP and enzalutamide-resistant PCa cell lines.

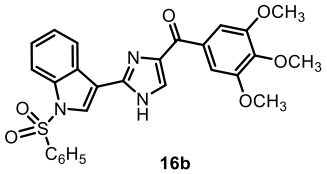
ring was found to be more active than the parent compound **15a**. As expected, the F-substitution in the indole ring (**15f** and **15g**) also improved the activity (Figure 14.4).

Chen et al. synthesized 2-aryl-4-benzoylimidazole analogues (**16**) starting from indole-3-carboxaldehyde and evaluated their antiproliferative activities against three human PCa cell lines (LNCaP, PC-3, and Du 145) using Colchicine as positive control [26] (Figure 14.5). Few compounds showed potent activity against PCa cell lines *in vitro*. Compound **16a** showed the maximum potency against all the three PCa cell lines exerting this effect by inhibiting tubulin polymerization. A bulky phenylsulfonyl group on indole ring (**16b**) lowered the activity by 50 times. Whereas, a small methyl group in compound **16c** showed moderate potency. The benzofuran as well as benzothiophene derivatives **16d** and **16e**, respectively showed relatively better activities.

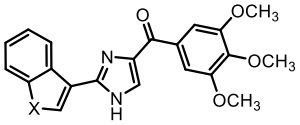
The 5α-reductase inhibitors are the potential chemopreventive agents associated with several controversies with their use [27]. Finasteride (MK-906), a steroidal inhibitor and ONO-3805, a nonsteroidal inhibitor are the promising 5α-reductase



16a (R=H)
16c (R=CH_3)

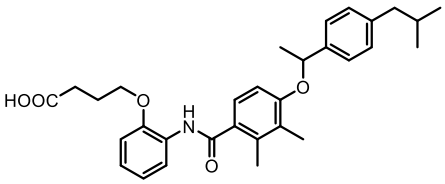


16b

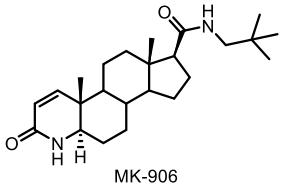


16d (X=O)
16e (X=S)

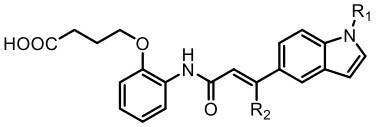
Compd.	IC_{50} (nM) PC-3	LNCaP	DU145
16a	3.7	2.8	3.9
16b	105	107	189
16c	40	35	76
16d	33	14	45
16e	20	29	51
Colchicine	11.5	16.3	11.2



(±)-ONO-3805



MK-906



17

5α-reductase inhibitory activity of active compounds

Compound	R_1	R_2	IC_{50} (nM)
17a	benzhydryl	Me	5.6
17b	benzhydryl	Et	2.5
17c	4,4'-difluorobenzhydryl	Me	3.3
17d	4,4'-difluorobenzhydryl	Et	0.48
17e	1-propylbutyl	Me	2.3
17f	1-propylbutyl	Et	2.8
MK-906			10
(±)-ONO-3805			2.5

Figure 14.5: Anti-proliferative activity of ABI-III analogues **16** against PCa cell lines and 5α-reductase inhibitory activity of indole derivatives **17a–f**.

inhibitors [28]. The researchers from Kyowa Hakko Kogyo Co. Ltd., Japan reported a detailed SAR studies around the indole-based structure **17** derived from the lead ONO-3805, in which steroidal skeleton is excluded [29]. The synthesized compounds

were evaluated against rat prostatic 5α-reductase (IC_{50} values are shown in Figure 14.5) where many compounds exhibited excellent inhibitory activities in nanomolar range. In the *in vivo* study, the inhibitory effects of few indole derivatives, MK-906 and ONO-3805 on DHT production was studied. Their inhibitory effect on rat and human 5α-reductase isozymes was also investigated. The indole derivatives were found to be active *in vitro* and inhibited DHT production *in vivo*. In particular, compound **17d** was found to be 20-fold more potent than MK-906 *in vitro*, and significantly inhibited DHT production at 3 mg/kg. Compounds **17c**, **17d** and **17f** exhibited extremely potent inhibitory activity on rat type 2 isozyme from epididymis. The assay results showed that the indole derived nonsteroidal 5α-reductase inhibitors exerted an opposite selectivity for rats and human isozymes as compared to MK-906.

Liu et al. synthesized several indole-3-carboxamide derivatives (**17**) and evaluated their binding affinity for B-cell lymphoma-2 (Bcl-2) and Mantle cell lymphoma (Mcl-1) protein based on a lead (WL-276, anti-apoptotic Bcl-2 protein inhibitor) from their previous work. WL-276 exhibited selective and highly potent inhibitory activity against Bcl-X_L protein warranting an extensive SAR to develop antagonist against hormone-refractory PCa (HRPC) [30]. During this investigation, compound **20** was found to be more effective than WL-276 (**18**) against Bcl-2 protein. Whereas, compound **19** was found to be a dual inhibitor of Bcl-2 and Mcl-1 protein and did not show any affinity to the Bcl-X_L protein, suggesting a specific role of additional indole ring towards the development of dual inhibitors targeting Bcl-2 and Mcl-1 protein (Figure 14.6).

Xu et al. from the Key Laboratory of Chemical Biology at Shandong University designed and synthesized several 1-phenyl-1*H*-indole derivatives showing promising Bcl-2/Mcl-1 inhibitory activity. As compare to WL-276, compounds **21** and **22** were found to be better anti-proliferative agents showing selective inhibitory activity against Bcl-2/Mcl-1 proteins without showing any affinity towards Bcl-X_L [31].

Yang and coworkers from the same laboratory described 1,2,4,7-*tetra*-substituted indoles as Akt inhibitors [32]. The ester derivative **23a** and its bioisostere with

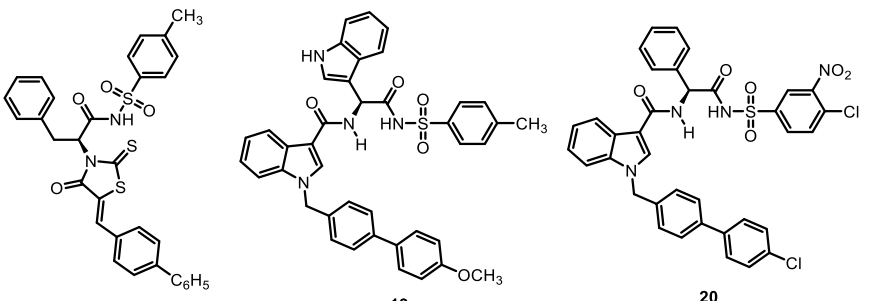


Figure 14.6: Binding affinity of indole-3-carboxylic acid derivatives for Bcl-2 and Mcl-1 protein.

1,2,4-oxadiazole ring (**23c**) exhibited the most potent inhibitory activities against Akt1 at nano molar concentration. These lead structures inhibited the phosphorylation of the GSK3 protein and showed anti-proliferative activities in PC-3 cell line. In given series of compounds, **23b** and **23d** showed the maximum activity against PC-3 cell line (Figure 14.7).

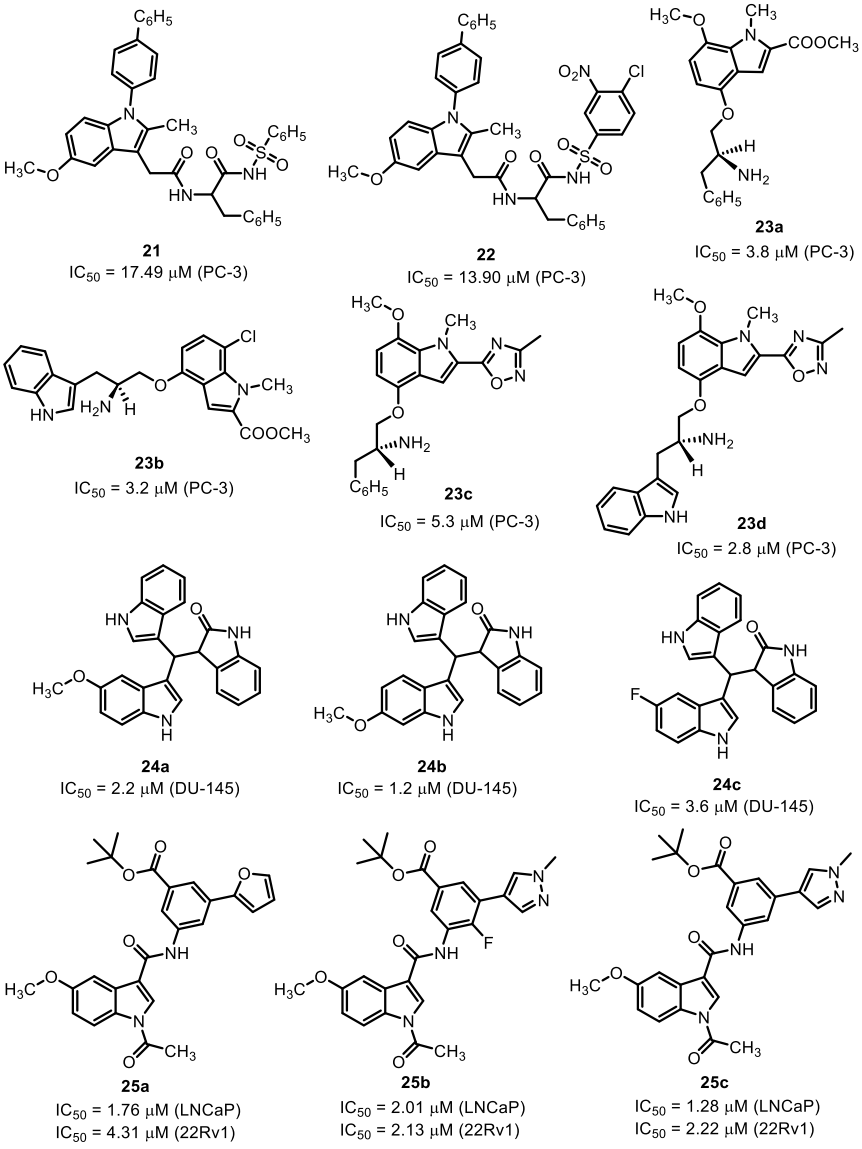


Figure 14.7: Anti-proliferation effects of indole derivatives against PCa cell lines.

Kamal and coworkers synthesized 16 3,3′-diindolyloxyindoles and evaluated against five human cancer cell lines using MTT assay [33]. The propeller shaped bisindolyl-indolinones **24a–24c** were the most active against DU-145 with IC_{50} of 2.2, 1.2 and 3.6 μM, respectively (Figure 14.7).

Xiang et al. from Guangzhou Medical University designed *N*-acetyl-3-carboxamide derivatives of 5-methoxy-indoles using structure-based virtual screening as CBP/EP300 bromodomain inhibitors for the treatment of castration resistant PCa (CRPC) [34]. The synthetic optimization and bioevaluation resulted in ester derivatives **25a–25c** showing promising activity against LNCaP and 22Rv1 cell lines (Figure 14.7). Compound **25c** potently inhibited cell growth of both the cell lines and also lowered the expression of androgen receptor (AR), c-Myc and AR target genes.

Shaveta and Singh synthesized 27 indole derivatives and evaluated them against 60 different human tumor cell lines [35]. Several compounds exhibited significant anti-cancer properties. Few potent compounds with their growth inhibitory concentration (GI_{50}) against PCa cell lines are shown in Figure 14.8. The anti-cancer activity of the lead compounds is related to its ability to inhibit dihydrofolate reductase (DHFR). The indole derivatives **28** and **29** exhibited the most potent activity against PC-3 cell lines. In the molecular docking studies, compound **26** was docked in the active site of DHFR. The indole part aligned parallel to one β-sheet indicating the favorable hydrophobic interactions, whereas another sheet was overlapped by the polar interactions of *p*-chlorobenzoyl moiety. In addition, a favorable H-bond interaction was observed between pyrazole nitrogen and Gln35 residue of the enzyme.

Purushottamachar and coworkers studied the small modifications of drug candidate Galeterone, **30** to modulate the androgen receptor activity [36]. Compound **32** was more potent with respect to antiproliferative and androgen receptor degrading activities, in comparison to **30** (Figure 14.8). Compounds **30** and **32** degraded both full-length and truncated androgen receptors in CWR22rv1 human PCa cells. In contrast, the indole derivative **31**, caused significant up-regulation of androgen receptor. Compound **30** was observed to be the most potent CYP17 inhibitor whereas compounds **31** and **32** were found to be the weak inhibitors of CYP17.

Jain and coworkers evaluated antitumor activity and carried out a detailed SAR studies around tryprostatin A (**33**) [37]. The analysis revealed the importance of 2-isoprenyl moiety on the indole scaffold towards the inhibition of tsFT210 cell proliferation. In addition, indole N-H substitution with alkyl or aryl groups retained the activity. The replacement of proline with other amino acids such as L-valine and L-phenylalanine as well as 6-methoxy indole derived scaffolds were also found to be active. Among the synthesized analogues, compound **34** (IC_{50} = 11.1 μM) was equipotent with etoposide (a standard drug) towards its activity against PC-3 cell line. Another tryprostatin A isomer **35**, synthesized using D-proline showed enhanced activity suggesting a critical role of specific stereochemistry for the observed activity (Figure 14.8).

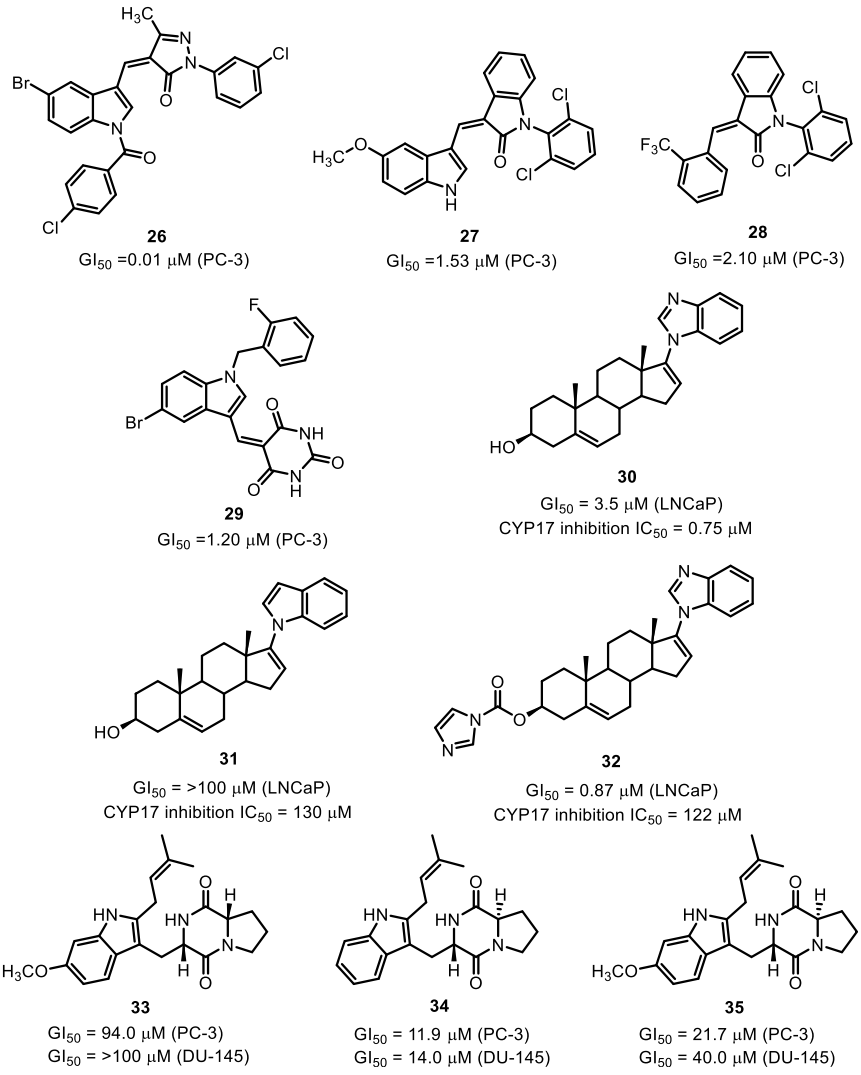
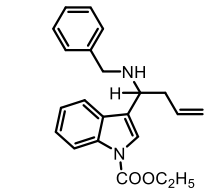


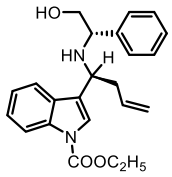
Figure 14.8: Growth inhibitory concentrations of indole derivatives and lead molecules.

Singh and coworkers synthesized a library of 2-[1-(indol-3-yl)-but-3-enylamino]-2-phenylethanols (**36**) and screened against NCI-60 cancer cell line panel where few of the related analogues **36a–e** showed appreciable activities [38]. The presence of C3 phenylglycinol moiety on the indole ring was found to be crucial for exhibiting anti-cancer activity (Figure 14.9).

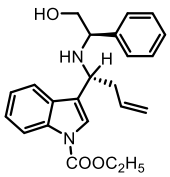
Panathur and coworkers synthesized a library of C2 linked 1,2,4-triazolyl-indoles (29 compounds) and evaluated their cytotoxicity against three different human cancer cell lines including PCa cell lines [39]. The compounds **37a, 37b, 38a** and **38c** showed



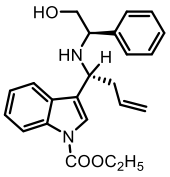
36a
Log GI_{50} = -4.64 μM (PC-3)
Log GI_{50} = -4.68 μM (DU-145)



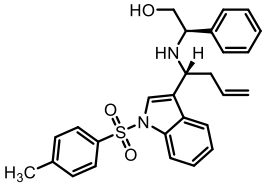
36b
Log GI_{50} = -4.65 μM (PC-3)
Log GI_{50} = -4.56 μM (DU-145)



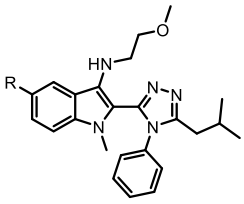
36c
Log GI_{50} = -4.63 μM (PC-3)
Log GI_{50} = -4.71 μM (DU-145)



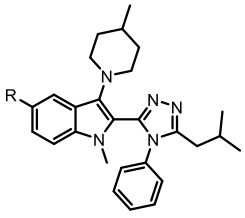
36d
Log GI_{50} = -4.80 μM (PC-3)
Log GI_{50} = -4.52 μM (DU-145)



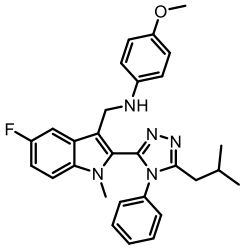
36e
Log GI_{50} = -4.75 μM (PC-3)
Log GI_{50} = -4.57 μM (DU-145)



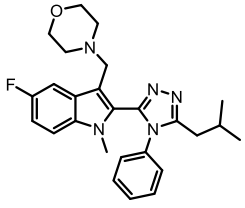
37a (R=F) (50% LNCaP)
37b (R=H) (48% LNCaP)



38a (R=F) (51% LNCaP)
38b (R=H) (88% K562)



38c (58% LNCaP)



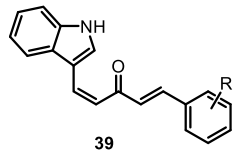
38d (88% K562)

Figure 14.9: Concentrations of compounds (**36a–e**) resulting in growth inhibitions of 50% (log GI_{50}) *in vitro* of PCa cell lines and cell growth inhibition (%) of the compounds (**37–38**) at 10 M against LNCaP and K562 cells.

good inhibitory activity against LNCaP cells (Figure 14.9). The 4-methyl piperidine and morpholine substituents at C3 (**38b** and **38d**, respectively) demonstrated potent inhibition of cell growth (~88% against K562 cells).

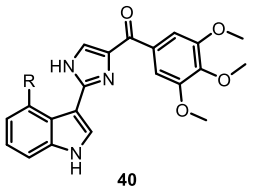
Silent mating type information regulation 2 homolog 1 (SIRT1), a deacetylase protein is being targeted to control several metabolic disorders [40, 41]. SIRT1 inhibitors regulate the benign hyperplasia of PCa [42, 43]. The *in vitro* active compounds **37a, 37b, 38a** and **38c** were screened for deacetylation inhibitory activity using recombinant human SIRT1 enzyme. Compound **38c** exhibited 70% deacetylation inhibitory activity and demonstrated promising effect in the prostate hyperplasia animal models (Figure 14.9).

Ramya and coworkers synthesized 1,5-diaryl-1,4-pentadien-3-one linked indole moieties (**39a–d**) and evaluated for their cytotoxic activity against eight cancer cell lines [44]. Compounds **39a** and **39d** showed potent activity against PC-3 and DU145 cell



39

Compound	R	PC-3	DU145
39a	4-OCH_3	3.51 μM	13.62 μM
39b	2,5-di-OCH_3	6.34 μM	7.71 μM
39c	3,4-di-OCH_3	5.52 μM	13.32 μM
39d	3,4,5-tri-OCH_3	3.15 μM	8.92 μM
40a	H	89.9 nM	169.1 nM
40b	CH_3	5.20 nM	13.8 nM



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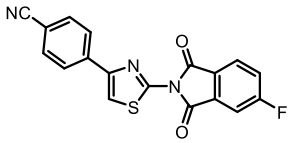
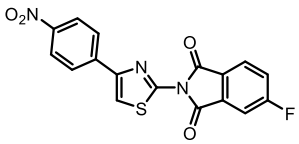


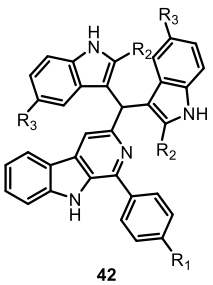
41a

5.96 μM (LNCaP); 43.12 μM (PC-3); 42.3 μM (3T3)



41b

7.78 μM (LNCaP); 39.88 μM (PC-3); 36.5 μM (3T3)



42

Compound	R_1	R_2	R_3	DU145 (μM)
42a	CH_3	H	Br	1.86
42b	OCH_3	Br	H	2.63
42c	F	H	H	1.80
42d	F	H	NO_2	1.92
42e	F	H	NO_2	2.34
42f	CH_3	H	F	5.81

Figure 14.10: Inhibitory concentration of indole derivatives against prostate cancer cell lines.

lines (Figure 14.10), where compounds **39b** and **39d** induced apoptosis in PC-3 cells. Compound **39b** arrested PC-3 cells in G2/M phase of the cell cycle while compound **39d** treatment resulted in increase in the G2/M population.

Very recently, Wang et al. synthesized 24 imidazole substituted indole derivatives (**40a–b**) for the SAR evaluation as ABI-231 (a tubulin inhibitor under clinical trials for PCa) analogues and evaluated their biological activity against NCI-60 cell line panel [45]. Compound **40b** having 4-methyl substituent was found to be the most potent analog which was 3-fold more potent than ABI-231. Compound **40b** was found to be highly active in the tumor growth inhibition of PC-3/TxR prostate xenograft.

Saravanan and coworkers synthesized 2-(4-phenylthiazol-2-yl)isoindoline-1,3-dione derivatives (**41**) and evaluated their anti-prostate cancer activity against LNCaP and PC-3 cell lines. Compounds **41a** and **41b** were found to be the most active against LNCaP cell lines, whereas showed moderate activity against cancerous PC-3 and non-cancerous 3T3 cell lines [46].

Kovvuri et al. synthesized a series of β-carboline linked bisindole compounds and evaluated them for their anti-proliferative activity against PCa cell line (DU145) [47]. The bromine and the fluorine substituted compounds **42a**, **42c** and **42d** exhibited excellent activities in the range of 1.8–1.9 mM against DU145 cell lines. From the spectroscopic studies it was predicted that compounds exhibit unique DNA binding which is comparable to combilexin. Compounds **42a**, **42e** and **42f** also exhibited good activities against PCa cell lines (Figure 14.10).

14.3 Fused indole derivatives as prostate cancer agents

Balasubramanian et al. from Bristol-Myers Squibb, New Jersey prepared a library of indolocarbazoles and explored their topoisomerase-I activity as well as cytotoxicity against HCT116, A2780, HCT29, M5076 and PC3 cell lines [48]. The lead compound BMS-251873 (**43**), showed potent activity against PCa and also displayed a broad-spectrum antitumor activity in the *in vivo* tumor model against colon as well as ovarian cancer. No major off-target liabilities were observed during their investigation. Compound **43** also exhibited antitumor efficacy against slow-growing human PC3 xenograft. The more selective bis-indolocarbazole **44** because of its poor plasma and tumor exposure resulted in lack of distal site activity. The tetrafluoro derivative **45** was poorly selective as well as less active.

Barraja et al. synthesized 1,2,3,5-tetrazino[5,4-*a*]indol-4-one by the reaction of isocyanates with 2-diazoindoles [49]. All the synthesized compounds were evaluated for antitumor activity against NCI-60 cell line panel. Some compounds exhibited excellent anti-proliferative activity (GI_{50}) in the micromolar range. Compound **46** showed most prominent activity against PC-3 and DU145 cell lines (Figure 14.11).

Zhao and coworkers from Shenyang Pharmaceutical University, China, isolated over 30 alkaloids from the fruits of *Euodia rutaecarpa* [50]. The cytotoxicities of the isolated compounds were evaluated against leukemia (HL-60) and PCa cell lines (PC-3). Most of the tested compounds showed cytotoxic activity against HL-60 cell lines, wherein the indole-based alkaloid **47** exhibited the highest activity in comparison to the positive control 5-fluorouracil. The phenolic indolopyridoquinazoline alkaloid (**49**) exhibited higher cytotoxicity against HL-60 and was moderately active against PC3 cell line. The *N*-methylated analogue **47** showed many-fold improvement in activity as compare to compound **49** (Figure 14.11).

Liew et al. reported the isolation of subditine (**50**), an indole alkaloid and related analogues from the bark of *Nauclea subdita* [51]. All the isolated alkaloids were screened for their cytotoxicities against LNCaP and PC-3 cell-lines. Among these, compound **50** exhibited effective cell growth inhibition of LNCaP. The structural analogue angustoline (**51**) showed much less activity compared to subditine (Figure 14.12). The increased cell permeability as well as nuclear fragmentation and disruption of cytoskeletal structures resulted in apoptosis of PCa cell lines. The

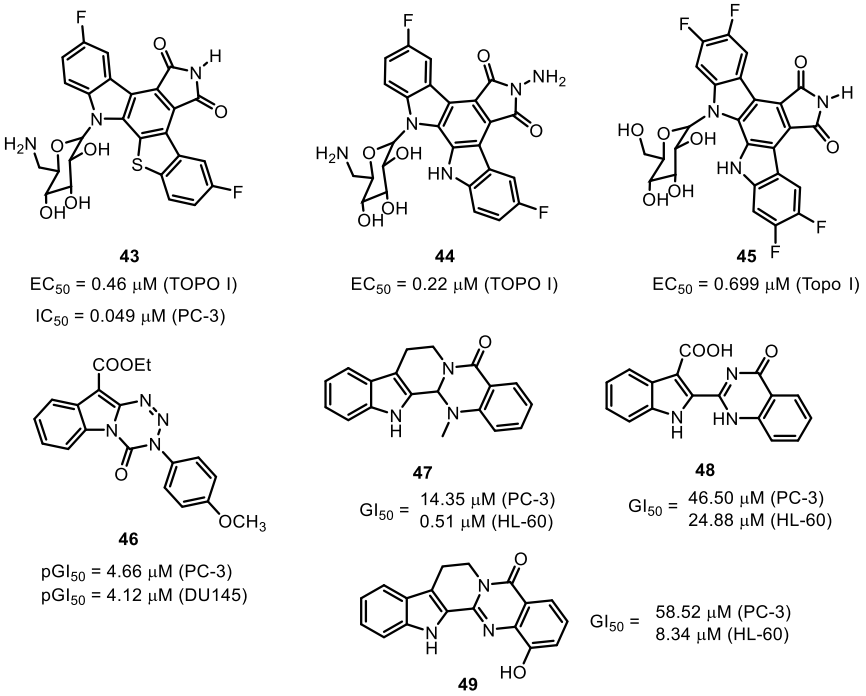


Figure 14.11: Topoisomerase I activity of fluoroindolocarbazoles and activity of fused indole derivatives for PCa cell lines.

enhanced ROS production in both LNCaP and PC-3 was also observed. Subditine selectively induced p53 upregulation in LNCaP cell line whereas, Bcl-2 and Bcl-xl expressions were down regulated. PC-3 and LNCaP cell lines were also utilized by Lobo et al. to screen a series of tetracyclic indenoindoles synthesized by the reaction of ninhydrin with several enaminones [52]. The C7 dimethyl and dichlorophenyl at position-5 were the key requirements for the activity as observed in indeno[1,2-b]indole derivative **52.** The demethylated analogue **53** exhibited much less activity as compare to compound **52** (Figure 14.12). The observed invasion of the PCa cells was related to the MMP-9 inhibition. The molecular docking studies suggested that the lead compound **52** binds to the S1′ subsite of the matrix metallopeptidase-9.

Liu et al. reported the synthesis of a series of indole fused imidazopyridines (**54**) and evaluated *in vitro* activity against PCa cell lines [53]. The efficacy of the lead compound **54a** at 0.1 mg/kg was equal to 1.0 mg/kg dose of standard reference drug doxorubicin and was safe up to a dose of 500 mg/kg. Automated flexible ligand docking and fluorescence quenching experiments confirmed the interaction of **54a** with DNA.

Chaniyara and co-workers from Academia Sinica, Taipei, synthesized a series of indolizino[6,7-*b*]indoles (**55**) and their carbamate derivatives (**56**) for antitumor studies [54]. Many of the synthesized analogues exhibited considerable *in vitro* cytotoxic

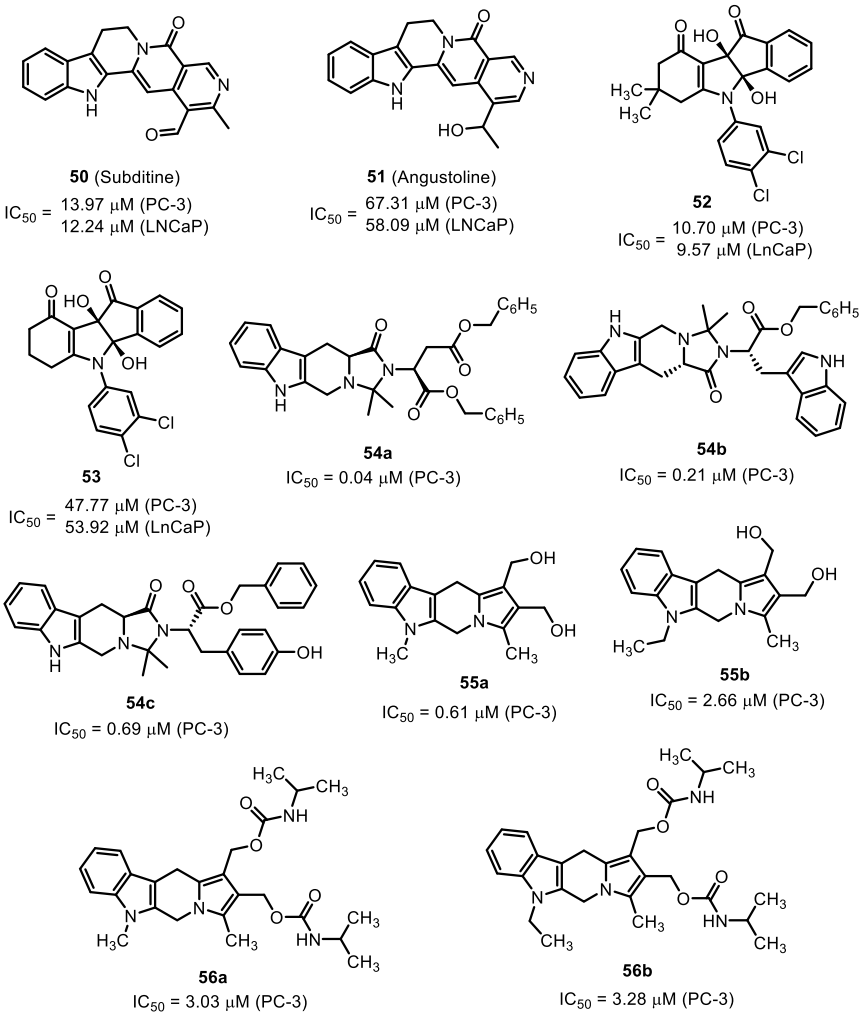
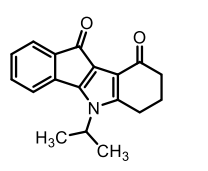


Figure 14.12: IC_{50} values of polycyclic fused indole derivatives against PCa cell lines.

activity against PC-3 cell lines. The mechanistic studies suggested multiple modes of action, such as inhibition of topoisomerase I and II, induction of DNA cross-linking, and cell-cycle arrest at the S-phase.

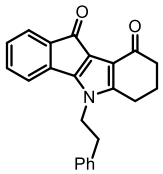
Synthesis of indeno[1,2-*b*]indole derivatives (**57a–57c**) with human protein kinase CK2 inhibitory activity was reported by Hundsdörfer and co-workers [55]. The selective CK2 activity of one of the library compound, 5-isopropyl-7,8-dihydroindeno[1,2-*b*] indole-9,10(5*H*,6*H*)-dione (**57**) was confirmed by screening the compound against 22 other human protein kinases. Compound **57** competitively inhibited ATP with Ki of 0.06 μM. Compounds **57a** and **57b** with small structural variations at position-5 also showed promising activity against CK2.

Yan et al. synthesized cyclic indole derivatives and evaluated their anti-proliferative activity against various cancer cell lines [56]. Among the synthesized derivatives, compounds **58a–58f** displayed most potent activity (IC_{50} = 31–49 nM against PC-3) when compared with combretastain A-4. The tubulin polymerization inhibitory effect of **58f** was elucidated using immunofluorescence assay which predicted the disruption of intracellular microtubule network and interference of cell mitosis. Cellular mechanism suggested that compound **58f** arrested the cell cycle at G2/M phase and induced apoptosis of cancer cells in a time and dose dependent manner. The lead compound **58f** exhibited excellent *in vitro* tubulin polymerization inhibitory activity and low cytotoxicity (Figure 14.13).



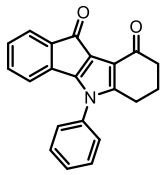
57a

IC_{50} = 0.11 μM



57b

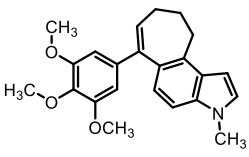
IC_{50} = 0.82 μM



57c

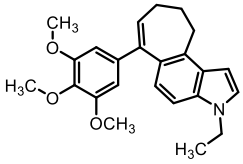
IC_{50} = 1.44 μM

Compdound	57a	57b	57c
CK2 Inhibition (%)	93	66	60



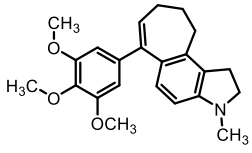
58a

IC_{50} = 0.093 μM



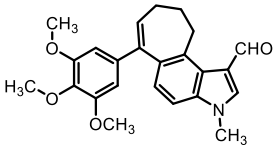
58b

IC_{50} = 0.085 μM



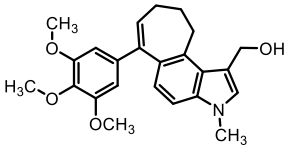
58c

IC_{50} = 0.077 μM



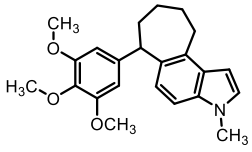
58d

IC_{50} = 0.031 μM



58e

IC_{50} = 0.049 μM



58f

IC_{50} = 0.047 μM

Compd.	58d	58e	58f	CA-4
IC_{50} (μM)	0.47	0.41	0.15	1.12

Effects of more active compounds on tubulin polymerization inhibition

Figure 14.13: Inhibitory activity of indeno[1,2-b]indole derivatives (**57**) against CK2 and antitumor activity of cyclic indoles (**58**) against PC-3 cell lines.

Indole skeleton, being a privileged structure has been extensively used for the discovery of novel anticancer agents (Figure 14.14). The majority of active analogues showed the presence of various bulky substituents at N1, C2 or C3 positions. Whereas, the C5, C6 and C7 positions were mostly occupied by small electron donating or withdrawing substituents. It was also observed that the multicyclic indole derivatives having PCa related activity are mostly fused at C2 and C3 position of the indole scaffold.

Overall, the prevalence of PCa is an important health issue. Higher incidences of PCa occur in men from Western countries than Asia and North Africa due to environmental (diet and lifestyle) and genetic variations (family history) between these populations. In an effort to understand the molecular pathogenesis of this disease, numerous cell models have been developed along with the murine models which resulted in the discovery of several small molecule chemotherapeutic agents to treat this deadly condition. Among the several oncological drugs currently approved for human use, Oncovin and Velban are the two-indole based oncological drugs being approved for multiple myeloma and Hodgkin's lymphoma, respectively. Among the various synthetic small molecules, the indolyl/indolinyl propanamides (**7**/**8**) discovered as selective androgen receptor degraders can potentially be developed for the treatment of prostate cancer. The BF3 inhibitory potential of 1H-indole-2-carboxamides (**15**) with antiproliferative activity against LNCaP and enzalutamide-resistant PCa cell lines as well as the 2-indolyl-4-benzoyl-imidazoles (**16**/**40**) targeting tubulin polymerization also hold promise for further pharmacodynamic studies. Few of the complex polycyclic heterocycles having indole moiety such as BMS-251873 and related fluoroindolocarbazoles (**43**/**44**) as well as N-methylated indolopyridoquinazoline alkaloids (**49**) and indole fused imidazopyridines (**54**) showed promising results and may need further formulation studies.

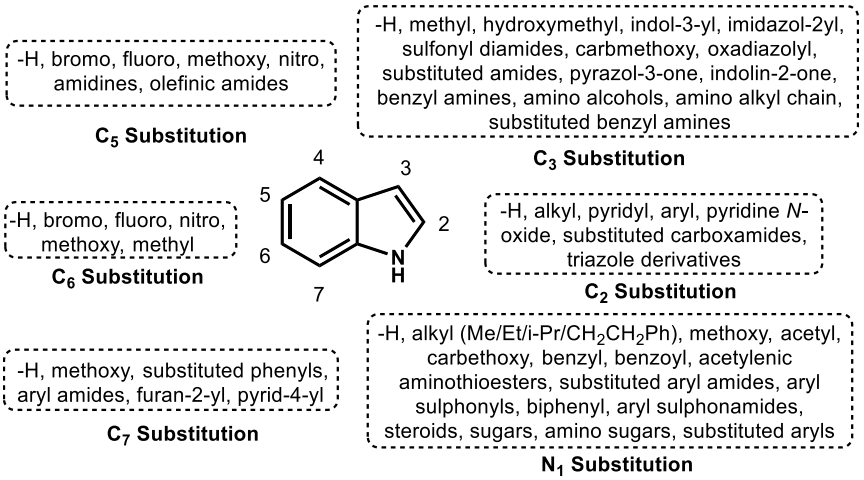


Figure 14.14: Indole based PCa agents: a chemical space.

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Index

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