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REVIEW ARTICLES

Antiproliferative Activities of Some Biologically Important Scaffolds

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Antiproliferative Activities of Some Biologically Important Scaffolds

SUMMARY

Cancer remains as one of the leading causes of death in the world and as a result there is a pressing need for the development of novel and effective treatments. The goal in cancer chemotherapy is to provide cytotoxic effect to prevent tumor growth. Nowadays, drugs that cause damage to DNA or inhibit DNA synthesis by antimetabolite characteristics that may prevent cell division through microtubule inhibition, inhibition of topoisomerase and inhibition of the key tyrosine kinases are used in cancer treatment. Among other factors, inherent and acquired resistance to treatment and the dose-limiting toxicity caused by the narrow therapeutic window of many cancer drugs are major obstacles in effective cancer therapy. Since clinically useful drugs have problems with toxicity, drug resistance and bioavailability, there is an ongoing effort to find new compounds that may be safer or more effective. A literature survey revealed that a number of biologically active scaffolds have important affects in the development of antiproliferative agents. This article summarizes the antiproliferative agents containing indole, pyrazole or isoxazole backbones and combretastatin A-4 analogues with various mechanism of actions that have published in recent years.

Key Words: *Cancer, CA-4, Indole, Pyrazole, Isoxazole, Antiproliferative Agent.*

Bazı Biyolojik Olarak Önemli Yapıların Antiproliferatif Aktiviteleri

ÖZET

Kanser dünyanın önde gelen ölüm nedenlerinden biri olmaya devam etmektedir ve yeni ve etkili tedavilerin geliştirilmesi için acil bir ihtiyaç bulunmaktadır. Kanser kemoterapisinde amaç, tümör gelişimini önlemek için sitotoksik etki sağlamaktır. Günümüzde, DNA'da hasara neden olan veya mikrotübül inhibisyonu, topoizomeraz inhibisyonu ve önemli tirozin kinazların inhibisyonu yoluyla hücre bölünmesini engelleyebilecek antimetabolit özellikleri ile DNA sentezini inhibe eden ilaçlar kanser tedavisinde kullanılmaktadır. Diğer faktörlerin yanı sıra, birçok kanser ilacının dar terapötik indeksi nedeniyle yol açtığı doz sınırlayıcı toksisite ve tedaviye karşı doğal ve kazanılmış direnç, etkin kanser tedavisi için önemli engellerdir. Klinik olarak faydalı ilaçların toksisite, ilaç direnci ve biyoyararlanım ile ilgili sorunları olması nedeniyle, daha güvenli veya etkili olabilecek yeni bileşikler bulmak için sürekli bir çaba vardır. Bir literatür araştırması, bazı biyolojik olarak aktif yapıların antiproliferatif ajan geliştirme çalışmalarında önemli bir yere sahip olduğunu ortaya koymuştur. Bu makalede, son yıllarda yayınlanan çeşitli etki mekanizmalarına sahip, kombretastatin A-4 (CA-4) analogları ve indol, pirazol veya izoksazol halkaları içeren antiproliferatif ajanlar özetlenmektedir.

Anahtar kelimeler: *Kanser, CA-4, İndol, Pirazol, İzoksazol, Antiproliferative Ajan,*

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INTRODUCTION

Cancer is the second most common cause of death in developed countries after cardiovascular diseases. Cells divide uncontrollably and form malignant tumors and spread to other parts of the body (Panathur et al., 2013). According to the WHO (in 2014 report), there were about 14 million new cases of cancer in the world in 2012 and about 8 million deaths due to cancer. Approximately 60% of the cancer cases in the world are found in Asia, Central and South America (Stewart et al., 2014). Approximately 20 million new cancer cases and 13 million cancer-related deaths are expected in 2030 (Boyle et al., 2008; Stewart et al., 2014). While 85-90% of the cancer are caused by DNA damage, exposure to mutagen, progressive changes in cell DNA and replication errors, the remaining 10-15% are hereditary (Yokus et al., 2012) .

The definitive treatment of cancer is very difficult. Surgical intervention and radiation therapy or chemotherapy alone are intended to remove cancer cells. Single drug or combination therapies have been developed that are effective for some highly lethal cancer types such as Hodgkin's lymphoma, testicular cancer and some leukemia cancers (Alberts et al., 2015). Immunotherapy, gene therapy and the use of angiogenesis inhibitors are accepted as new cancer treatment methods (Dellabona et al., 1999; Torrero et al., 2004). Nowadays, these methods are applied as combination therapies for cancer treatment (Feng et al., 2003). Depending on the type of the cancer, it is possible to prolong the life span or to reduce the possibility of recurrence of the disease as a result of treatment with chemotherapy (Bozza et al., 2016).

Ideal treatment is the elimination of cancer cells without harming normal cells (Alberts et al., 2015). Chemotherapy is an important treatment method in the treatment of cancer (Kim et al., 2010; Inceler et al., 2012; Baytas et al., 2014; Bozza et al., 2016). Due to the several side effects, drug resistance and failure of antitumor drugs to exert their effects in certain cases of cancer, the design and discovery of non-traditional, efficient and safe chemical classes of agents are prime targets in contemporary medicinal chemistry.

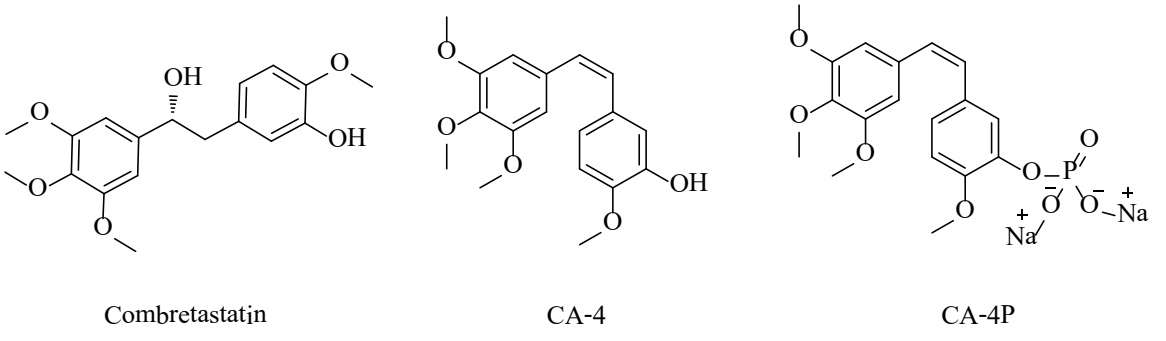
A literature survey revealed that a number of biologically active heterocyclic scaffolds have important roles in the development of antiproliferative agents. This article summarizes the antiproliferative agents containing CA-4 analogues and indole, pyrazole or isoxazole scaffolds with various mechanism of actions that have been published in recent years. It may help in the investigation of new antiproliferative agents and development of novel candidate anticancer drugs in the future.

NEW ANTICANCER AGENT DEVELOPMENT STUDIES

CA-4 Analogues

Microtubules (MT) have a basic role in cellular functions such as cell motility, intracellular transport and cell division. Cellular MT is in the state of continuous polymerization to depolymerization (La Regina et al., 2007; La Regina et al., 2011; La Regina et al., 2013; Li et al., 2015). Microtubules also play an important role in the phases of the mitotic division and provide cell division. New anticancer drugs have an important goal in developing drugs. Medicines targeting microtubules are medicaments that impair the balance between the polymerized and depolymerized forms of microtubules, which bind to the colchicine binding site on the tubule and inhibit the polymerization of microtubules (Bhattacharyya et al., 2008) Inceler, 2012 #41. The vinca alkaloids and taxoids currently used clinically target the tubulin protein (Konieczny et al., 2015).

Combretastatin, a natural product (Figure 1), has been isolated from the *Combretum caffrum* grown in South Africa (Ley et al., 2007; Gutiérrez et al., 2013; Baytas et al., 2014). Numerous studies have been conducted on the combretastatin analogues and their anticancer effects have been demonstrated (Ohsumi et al., 1998; Yang et al., 2012). CA-4 (Figure 1) and its analogs are compounds with potent biological activity and are important in medicinal chemistry.



Combretastatin CA-4 CA-4P

Figure 1. Combretastatin, CA-4 and CA-4P structures

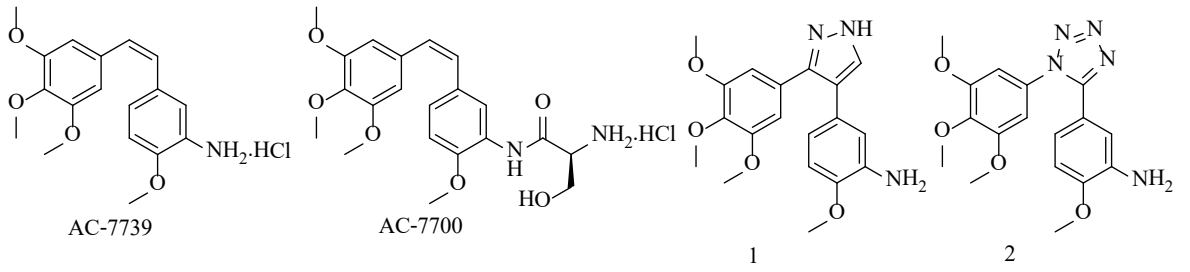


Figure 2. CA-4 analogues: AC-7739, AC-7700, compound **1** and **2**

CA-4 has a low water solubility construction and its water soluble derivatives have been synthesized to improve its bioavailability. In 2012, phase II clinical trials for the use of combretastatin A-4 phosphate (CA-4P) (Figure 1) in ovarian, lung and thyroid cancers were performed (Nathan et al., 2012). Recent studies have reported that CA-4P destroys 90-99% of the vasculature in controlled doses and causes tumor necrosis. Phase III clinical trials are continuing on the detrimental effect of CA-4P on vascularity (Gao et al., 2016). CA-4P (fosbretabulin) has been approved by the FDA, for use in thyroid cancer (Abma et al., 2017).

A number of studies have been carried out with the CA-4 structure, and these analogues have been shown to have antimitotic activity. It has been reported that the *Z* (*cis*) isomer of the CA-4 compound was active, but this derivative was readily converted to the *E* (*trans*) isomer. Therefore, various heterocyclic rings instead of the double bond in the linker were introduced to stabilize the active configuration (*Z*) (Ohsumi et al., 1998; Blanch et al., 2012). Starting from AC-7739 and AC-7700 (Figure 2), a series of their analogues were synthesized. By introducing pyrazole and tetrazole rings instead of the double bond in the linker, compound **1** and **2** (Figure 2) were synthesized. Compound 1, a pyrazole derivative, showed antiproliferative activity with IC_{50} value of 8.4 nM against colon-26 adenocarcinoma cell line and this compound inhibited tubulin polymerization at IC_{50} value of 3 μM. The cytotoxicity IC_{50} value of the compound **2** bearing the tetrazole ring was found to be 7.2 nM and the compound **2** inhibited the tubulin polymerization at IC_{50} of 2 μM concentration (Ohsumi et al., 1998; Blanch et al., 2012).

Nakamura and colleagues synthesized new series of CA-4 analogues by placing double bond with a silicon atom that allowed the same distance between two phenyl rings (Nakamura et al., 2013). The average length of a C-C bond was measured to be 1.53 Å, while the average length of a Si-C bond was measured as 1.89 Å (Cicak 2010). The distance between two phenyl rings in CA-4 was 3.0 Å, and the distance between two phenyls in compound **3** (Figure 3)with silicon is calculated as 3.3 Å. Compound **3** inhibited tubulin polymerization by 51% at a concentration of 30 **μM** and showed cytotoxicity with an IC_{50} value of 7 nM in the human breast cancer (MCF7) cell line. Furthermore, according to the physicochemical studies, it was found that compound 3 is more stable than CA-4 (Nakamura et al., 2013).

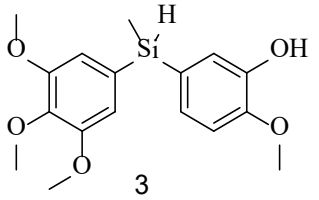


Figure 3. CA-4 analog containing silicone: compound **3**

Indoles

Indole is an important heterocyclic system, which is an important part of most alkaloids (Sharma et al., 2010). There are indole rings in many structures, from naturally occurring compounds to synthetic compounds (Figure 4) (Zhang et al., 2015). In addition, it is known that indole derivatives have many biological activities such as anticancer (Liu et al., 2014), antifungal, antioxidant (Sharma et al., 2010), antiviral (Zhang et al., 2015; Cihan-Ustundag et al., 2016), anti-inflammatory (Abdellatif et al., 2016), antituberculosis (Desai et al., 2016), anti-HIV (Brigg et al., 2016).

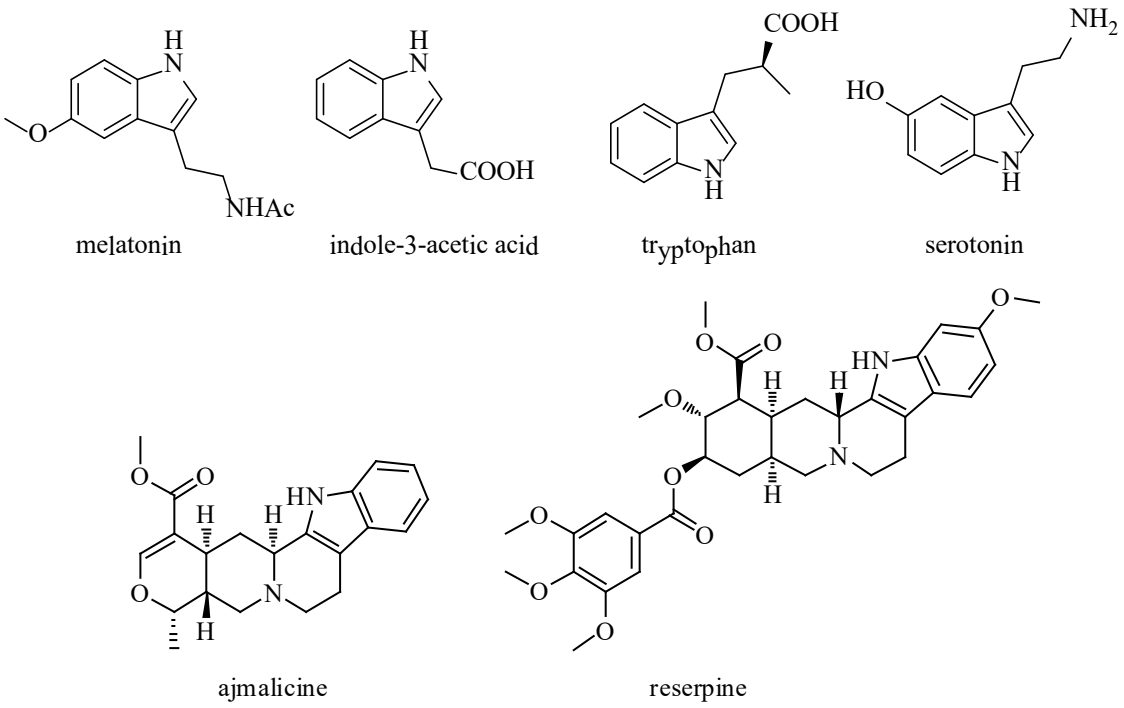


Figure 4. Some natural compounds bearing indole-structures

Indole skeleton-bearing compounds have been reported to have anticancer and tubulin polymerization inhibitory effects (De Martino et al., 2004; Liu et al., 2014; Kamath et al. 2015; Tantak et al., 2015; Das Mukherjee et al., 2016).

Three pharmacophore structures have been identified for CA-4 derivatives: (1) the double bond with the *cis*-configuration, (2) the A-ring containing 3,4,5-trimethoxyphenyl, (3) the B-ring (Figure 5). According to docking studies, the A-ring containing 3,4,5-trimethoxyphenyl plays an important role in the inhibition of tubulin polymerization. For this reason, modifications have been made to the new analogue in the double bond and on the B-ring. In a study in 2016, new CA-4 analogues were synthesized by Duan et al and the activities on tubulin were examined (Duan et al., 2016). In this work, compounds with different groups such as acyl hydrazones (compound **4**), chalcones (compound **5**), or amide (compound **6**) instead of double bond, bearing indole ring instead of ring B of CA-4, were synthesized (Figure 5). IC_{50} values for compound **4** were found between 0.08-35.6 μM on various cancer cell lines; human liver cancer (HepG2 and Hela), MCF7, human lung cancer (A549), human colon adenocarcinoma (HT-29) and squamous carcinoma cell (SCC-4) cell lines; and the IC_{50} value for antitubulin effect was determined as 4.46 μM (Duan et al., 2016).

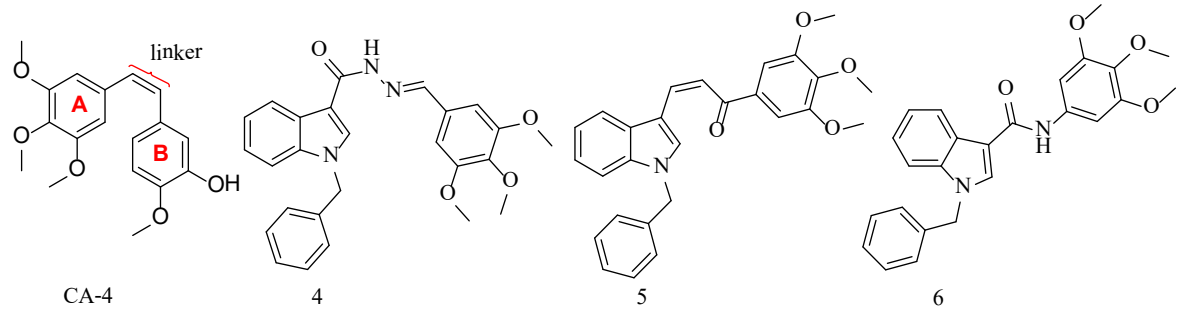


Figure 5. Indole based CA-4 analogs: acyl hydrazone, chalcone or amide instead of a double bond

In a study by De Martino et al., compound **7** (Figure 6) which designed by the similarity of the CA-4 and arylthioindole (ATI), was found as the most active derivative. It was reported that compound **7** inhibited tubulin polymerization (IC_{50}= 2.0 μM) and the IC_{50} value on the MCF7 cell line was reported as 0.013 μM (De Martino et al., 2004).

La Regina et al. published four different studies on ATI in 2007, 2009, 2011 and in 2013. Three pharmacophore groups (Figure 6) were identified with structure-activity studies (SAR) for the ATI structure: (A) The substituent at positions 2 and 5 of the indole, (B) 3,4,5-trimethoxyphenyl ring and (C) sulfur or carbonyl in the linker. It was determined that compound **8** inhibited tubulin polymerization (IC_{50}= 1.6 μM), prevented proliferation in MCF7 cells (IC_{50}= 0.043 μM), and caused cell cycle arrest in the HeLa cancer cell line at the G2/M phase (La Regina et al., 2007). Continuing this study, they synthesized compounds **9** and **10** which were bearing sulfur atom and carbonyl group in the linker, respectively , and obtained that compound **9** and **10** inhibited tubulin polymerization (IC_{50}= 0.99 and 0.67 μM, respectively) (Figure 6) (La Regina et al., 2009). After that, they synthesized new indole compounds having heterocyclic rings at 2nd position of indole such as furan, thiophene or pyrrole. Especially compound **11** which bore thiophene at 2nd position of indole showed very potent tubulin polymerization inhibitory activity with IC_{50} value of 0.74 μM (Figure 6) (La Regina et al., 2011). Compound **12** was found to be less active than compound **11** (IC_{50}= 1.3 μM) (Figure 6) (La Regina et al., 2013).

In a study conducted by Wen et al., the new indole analogues were synthesized by introducing selenium atom instead of the unsaturated linker of CA-4, and antitumoral effects were reported. Among the synthesized compounds, the IC_{50} values of compound **13** (Figure 6) were 12.3, 13.5 and 25.1 nM, against the human gastrointestinal cancer (SGC790), epidermal carcinoma (NW) and fibrosarcoma (HT1080) cell lines, respectively. Furthermore, according to the results of docking studies, it was shown that compound **13** binded to the colchicine binding site in the same orientation as CA-4 (Wen et al., 2015).

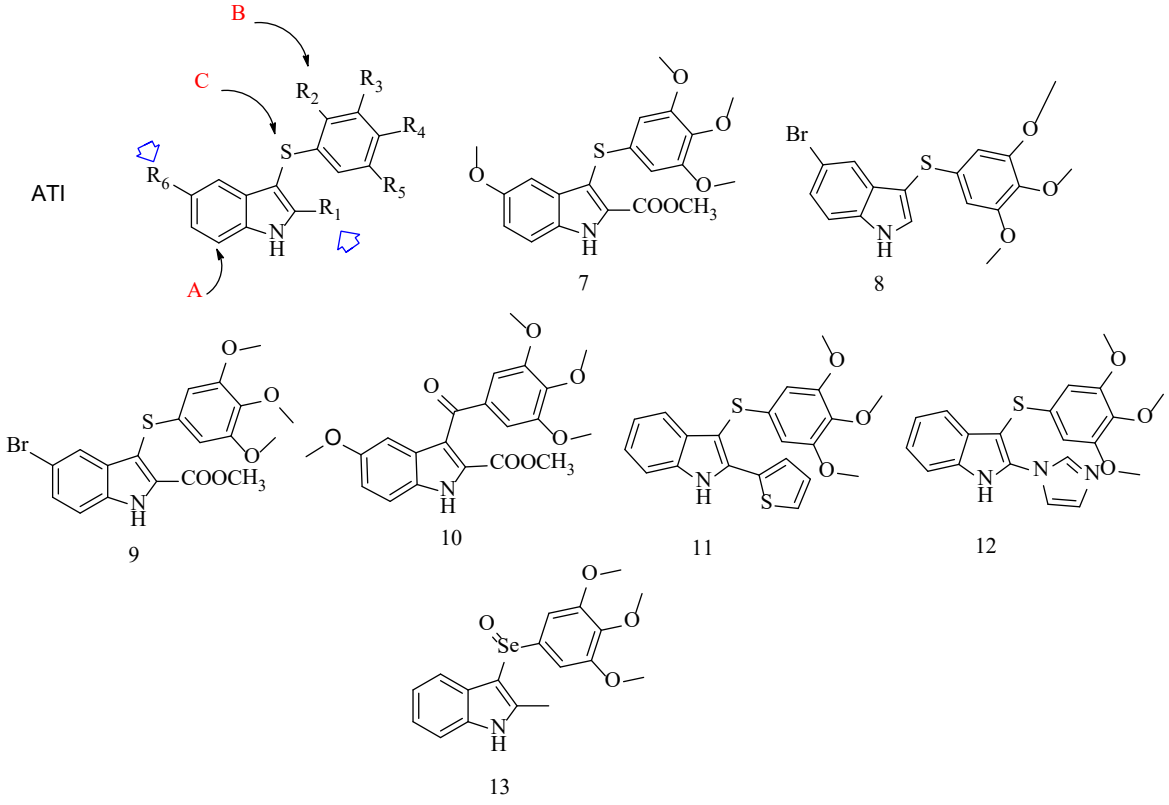
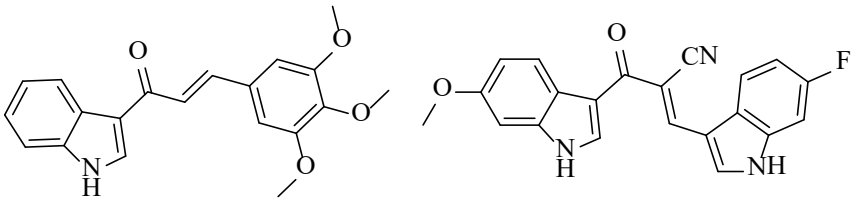


Figure 6. ATI structure and its potent analogues

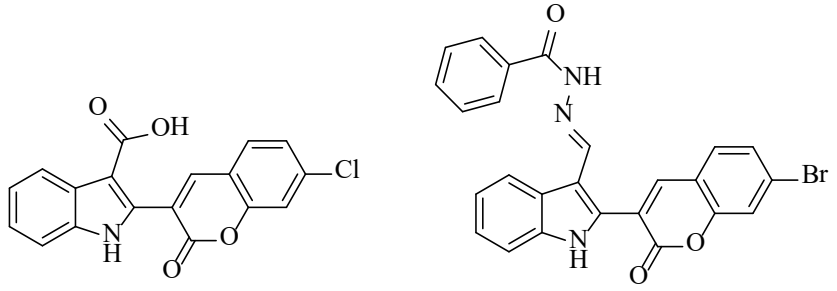
Kumar et al. examined the cytotoxic effects of indolyl chalcone analogues in two separate studies in 2010 and in 2014. The IC_{50} value of compound **14** was 0.03 μM in human pancreatic carcinoma (PaCa-2) cell line, meanwhile it was determined to be 0.10 μM against both A549 and human prostate cancer (PC-3) cell lines. The antiproliferative IC_{50} value of compound **15** (Figure 7) in cell line A-549 was 0.8 μM (Kumar et al., 2010; Kumar et al., 2014).



14 15

Figure 7. Indolylchalcone analogues

Indole-coumarin hybrid compounds were synthesized by Kamath et al. (Kamath et al., 2015; Kamath et al., 2016). Introducing coumarin ring at 2nd position and carboxylic acid at 3rd position of indole gave compound **16** (Figure 8), and its IC_{50} value in MCF7 cells was found as 5.5 μM (Kamath et al., 2015). In a study conducted in 2016, while keeping the coumarin ring in the second position of the indole, the methylene-benzohydrazide group was introduced to the third position of the indole. The IC_{50} value against the human MCF7 line for the most active compound **17** was determined as 9.1 μM. Induction of apoptosis was shown using Annexin-V staining (Kamath et al., 2016).



16 17

Figure 8. Indole-coumarin hybrid compounds

In two different studies conducted in 2011 and in 2016, compounds bearing the bis-indole structure were synthesized. Within these derivatives, IC_{50} values of compound **18** (Figure 9) were found to be 0.044, 0.062 and 0.162 μM in human prostate carcinoma (LNCaP), HT-29 and MCF7 cell lines, respectively. Cell apoptosis was also observed on two different prostate cancer cell lines (LNCaP and PC-3) for this compound (Ahn et al., 2011). In a study by Das Mukherjee et al in 2016, the bis-indole structure was preserved and the carbohydrazide moiety was added between these two indole rings and it was found that this structure in the linker was the pharmacophore group. This series demonstrated both the inhibition effect on tubulin polymerization and cytotoxic activity against A549. Among the compounds synthesized, the IC_{50} value for the inhibition of tubulin polymerization was found to be 7.5 μM for compound **19** (Figure 9) and the IC_{50} value on A549 was found to be 2 μM (Das Mukherjee et al., 2016).

18 19

Figure 9. Bis-indole derivatives

In 2012, Lai et al. synthesized compounds bearing indole-acrylamide core structure similar to SAHA (suber anilo hydroxamic acid) (vorinostat) (Figure 10). SAHA is approved as a drug for use in the cutaneous T-cell lymphoma by FDA. N-hydroxyacrylamide and an aromatic ring found in the SAHA structure have

been reported as pharmacophores. The compounds synthesized in this work have both indole ring as aromatic structure and N-hydroxyacrylamide pharmacophores, and these compounds have been reported to exhibit potent activity on different human cancer cell lines. Compound **20** (Figure 10) showed stronger antiproliferative effect than SAHA against human hepatoma (Hep3B), human breast (MDA-MB-231), PC-3 and A549 cancer cell lines (GI_{50}= 0.41, 0.48, 0.62 and 1.02 μM, respectively), as well as inhibitor activity for histone deacetylase (HDAC) 1,2 and 6 isoenzymes (IC_{50}= 12.3, 4.0 and 1.0 nM respectively) (Lai et al., 2012).

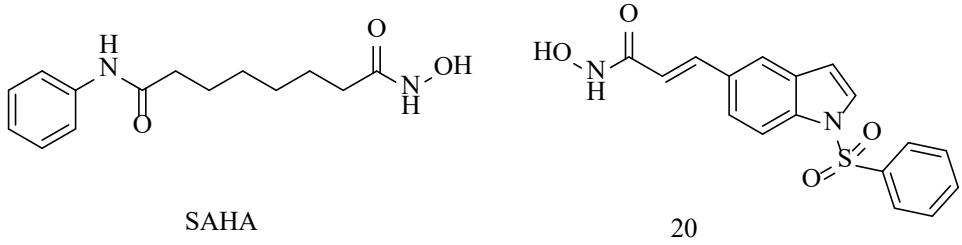


Figure 10. SAHA and its Indole-acrylamide analogues

Cinnamon amides having an α,β-unsaturated ketone moiety (phenylcinnamides in Figure 11) were shown to bind to tubulin, thereby causing an inhibition of its polymerization and alteration in the tubulin microtubule equilibrium (Leslie et al., 2010). Inspiring from phenylcinnamide structure a series of novel indolylacrylamide derivatives were synthesized and their anticancer activities against five human cancer cell lines were evaluated (HeLa, MCF7, MDA-MB-231, Burkitt lymphoma (Raji) and human promyelocytic leukemia (HL-60)). The synthesized compounds structurally resemble the indolyl chalcone structure as well. Compound **21** showed significant antiproliferative activity against both the Raji and HL-60 cell lines with IC_{50} values of 9.5 and 5.1 μM, respectively. The most active compound **21** also exhibited moderate inhibitory activity on tubulin polymerization. Flow cytometric analysis of cultured cells treated with **21** also demonstrated that the compound caused cell cycle arrest at the G2/M phase in HL-60 and HeLa cells. Moreover, **21** caused an apoptotic cell death through the activation of caspase-3. Docking simulations suggested that **21** binded to the colchicine site of tubulin (Baytas et al., 2014).

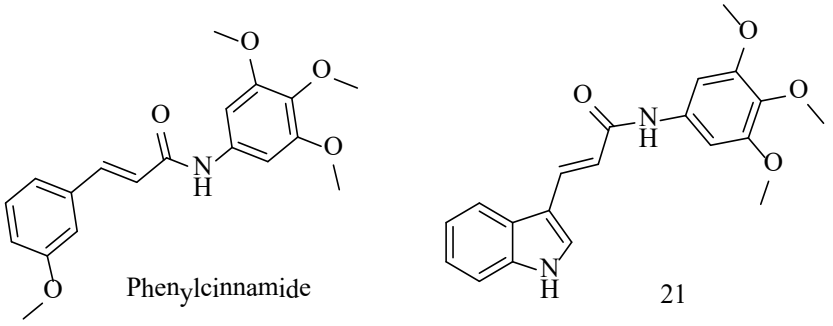


Figure 11. Penylcinnamide and compound **21**

Pyrimidine was found as a core structure in a large variety of compounds that exhibited important biological activities. 2,4,5-Substituted pyrimidine derivatives were synthesized and evaluated in vitro for inhibition against human hepatocellular carcinoma (BEL-7402) cancer cell proliferation. Compound **22** (Figure 12) exhibited excellent inhibitory activity with an IC_{50} of 0.024 μM for the BEL-7402 cancer cell line. 6-Methyl cyclopenta fused pyrimidines especially compound **23** (Figure 12) demonstrated potent tubulin depolymerization activity along with potent in vitro and in vivo antitumor activity (Xie et al., 2009; Zhang et al., 2014). In a following work, compound **24** (Figure 12) showed potent inhibition of tumor cells expressing vascular endothelial growth factor receptor 2 (VEGFR-2) and platelet-derived growth factor receptor β (PDFGR-β), comparable to sunitinib. Compound **24** also caused cell cycle arrest in the G2/M phase and apoptosis. Microtubule depolymerization through binding at the colchicine site was determined to be the primary mechanism of antitubulin action for **24**. In 2015, Hu et al. synthesized piperazine containing indole-pyrimidine derivatives, and the aminopyrimidine structure was

reported to be an important pharmacophore group in these group compounds (Hu et al., 2015). The most promising compound **25** (Figure 12) showed more potent and broad-spectrum cytotoxic activities with the IC_{50} values ranging from 5.01 to 14.36 μM against A549, MDA-MB-231 and MCF7 cell lines. Meanwhile, **25** also displayed the most potent tubulin polymerization inhibitory activity with IC_{50} value of 11.2 μM. Furthermore, molecular docking analysis demonstrated **25** interacts and binds efficiently with the tubulin protein at the colchicine-binding site (Hu et al., 2015).

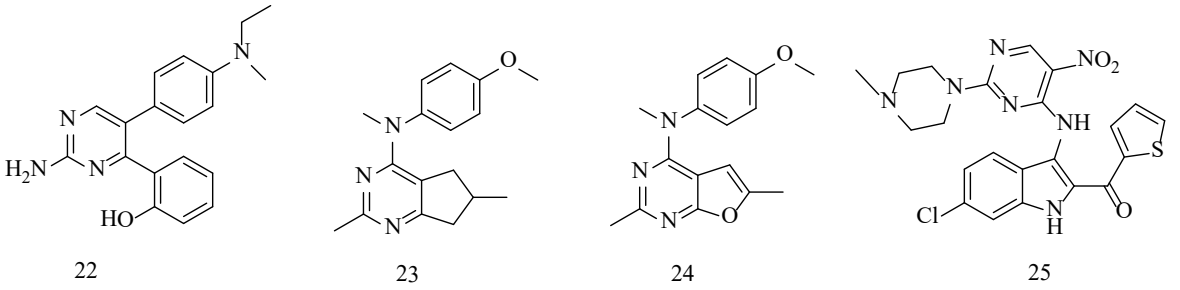


Figure 12. Aminopyrimidines and 2,3-disubstituted indole analogues

Tantak et al. synthesized derivatives of 2-(3'-indolyl)-*N*-arylthiazole-4-carboxamides and evaluated both antibacterial and anticancer activities of these compounds. In studies on cancer cells, compound **26** showed activity against the human embryonic kidney (HEK 293T) (IC_{50}= 8.60 μM). Meanwhile, compound **27** was found cytotoxic at IC_{50} value of 3.41 μM against the HeLa cancer cell line. Preliminary mechanism of action studies further indicated that thiazole carboxamide derivative **26** induced apoptosis in HeLa cells (Tantak et al., 2015).

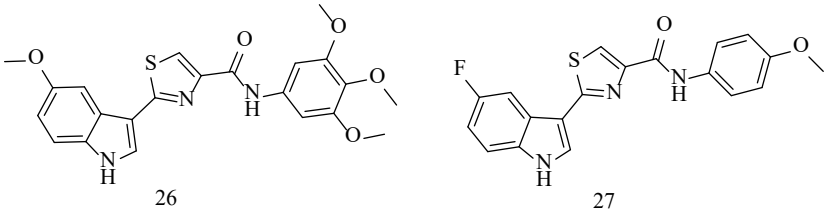


Figure 13. 2-(3'-Indolyl)-*N*-arylthiazole-4-carboxamide analogues

In a study by Zhang et al., antiproliferative activities of indole-3-pyrazole-5-carbohydrazide derivatives were evaluated in vitro. Using the MTT method antitumor activities of compounds in HepG2, A549, human stomach cancer (BGC823) and human breast cancer (BT474) cell lines were evaluated. In this study, it was found that a large number of compounds showed cytotoxicity against HepG2, BGC823 and BT474 cells at below 5 μM concentrations and that the compounds **28** and **29** (Figure 14) caused cell cycle arrest in S phase (Zhang et al., 2011).

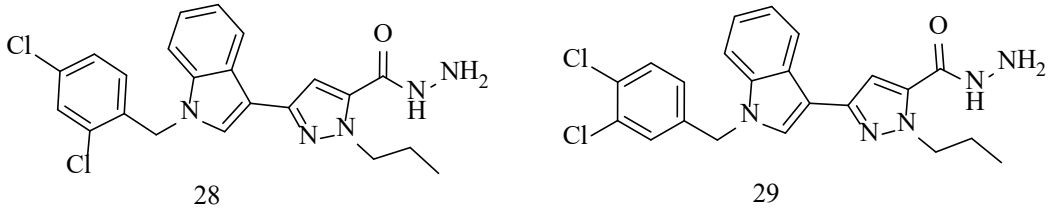


Figure 14. Indole-3-pyrazole-5-carbohydrazide analogues

Pyrazoles

The pyrazole ring is a very important ring system for pharmacological activities (Mohamed et al., 2012; Kumar et al., 2013; Li et al., 2015). Various leading clinical and commercial drugs contain pyrazole include Celecoxib, Deracoxib and Lonazolac (Figure 15). Pyrazole derivatives are known to possess many biological activities such as antiinflammatory, antipyretic, analgesic, antimicrobial, anticancer, sodium channel blocker, antiviral, antihypertensive, antioxidant and antidiabetic (Kumar et al., 2013). In recent years, it has been reported that many compounds carrying the pyrazole structure exhibited anticancer activity (Mohamed et al., 2012; Kumar et al., 2013; Shamroukh et al., 2014; Shi et al., 2015).

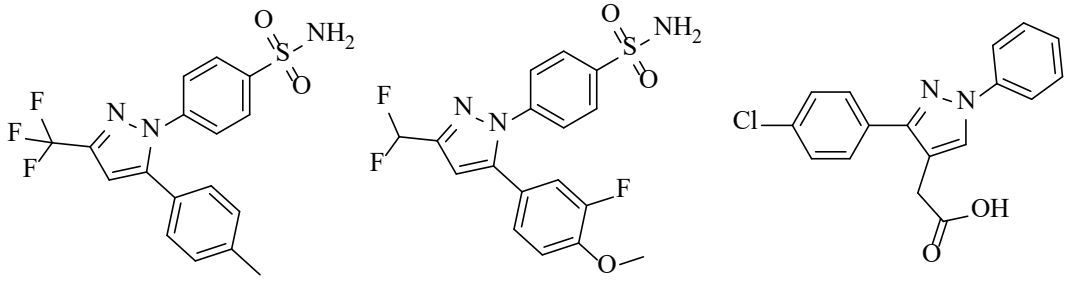


Figure 15. Pyrazole bearing pharmacological agents

In a study by Shamroukh et al. in 2014, a series of derivatives bearing pyrazole and pyrazolo[3,4-d] pyrimidine were synthesized and their inhibitory effect on the expression of the urokinase plasminogen activator (uPA) was investigated. Urokinase has been associated with malignancy in breast, lung, cervix, kidney and brain cancers, and the synthesized compounds have been shown to inhibit the expression of uPA and have been tested against human cancer cell lines such as MCF7, HepG2 and A549. The synthesized serine compounds **30** and **31** (Figure 16) were reported as the most effective compounds against MCF7 and HepG2 cell lines (IC_{50}= compound **30**, MCF7 2.60 and HepG2 3.75 μg/ml, compound **31** MCF7 3.65 and HepG2 4.20 g/ml) (Shamroukh et al., 2014).

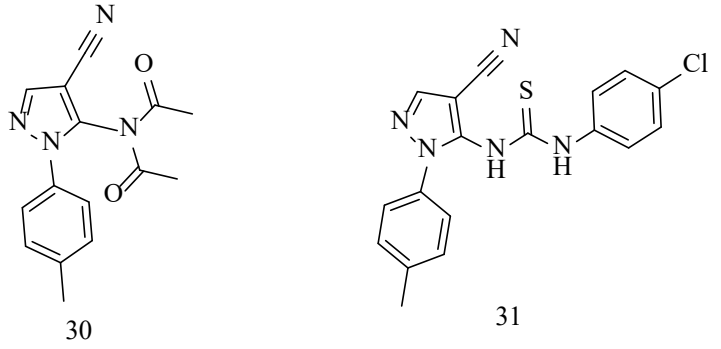


Figure 16. Pyrazole analogs

Pyrazoline derivatives structurally related to CA-4 were synthesized by Elmeligie et al. (Elmeligie et al., 2016). The cytotoxic activities of all new compounds were investigated in vitro against MCF7 and human colorectal carcinoma (HCT-116) cell lines. The cytotoxicity of **32** (Figure 17) was correlated with induction of apoptosis and caspase-3 activation in vitro thus indicating the apoptotic pathway of anticancer effect of these compounds. The inhibition of tubulin polymerization by the most active compounds **32** was evaluated (IC_{50}= 6.85 μM).

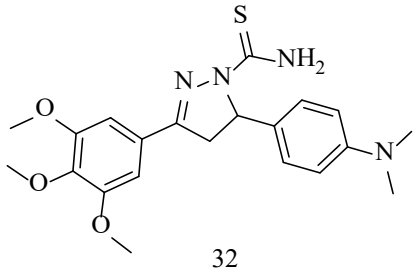


Figure 17. Pyrazole derivative **32** as a CA-4 analogue

In 2011, 36 new pyrazole derivatives were synthesized inspired from ionone structures by Balbi et al. and tested against human ovarian adenocarcinoma (A2780), A549 and leukemia (P388) cells. Compound **33** (Figure 18) showed cytotoxicity as the IC_{50} value 2.89 μM (A2780), 5.38 μM (P388). Compound **34** gave IC_{50} value of 1.22 μM against the A2780 cell line and 1.56 μM against the P388 cells. It was also determined by the western blot analysis that compound **34** stopped cell cycle through expression of P53 and p21 and caused cell death. Furthermore, compound **34** was able to significantly bind dimers of α- and β-tubulin, probably causing a molecular distortion resulting in the disassembly of microtubules (Balbi et al., 2011).

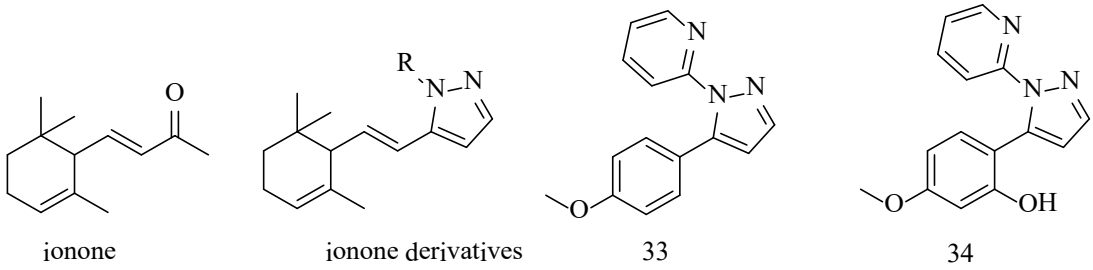


Figure 18. Ionone structure, ionone and pyrazole analogues

The YC-1 structure has a wide range of pharmacological activities such as antiplatelet, vascular relaxation and neuroprotection. A number of studies have been conducted on this compound and it has been found that it had strong cytotoxic activity. In the study by Chou et al., a series of analogues of 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1) (Figure 19) have been synthesized. It has been reported that the compounds **35** and **36** (Figure 19) synthesized by saving the pyrazole on the YC-1 structure were the derivatives with the highest anticancer activity in the lung cancer (NCI-H226) and kidney cancer (A-498) cells. Compound **35** showed cytotoxic activity against NCI-H226 and A-498 cell lines with IC_{50} values of 2.0 and 0.4 μM, respectively. Compound **36** was reported to have IC_{50} values of 1.4 and 0.4 μM in these cell lines (Chou et al., 2010).

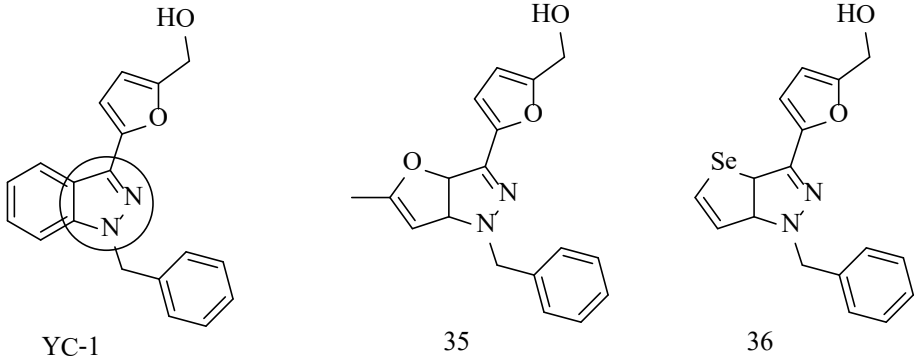
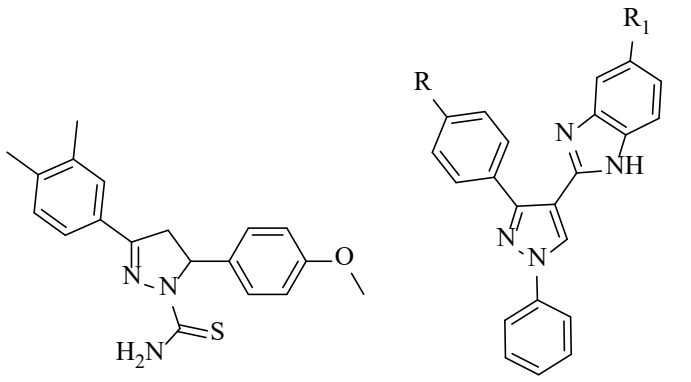


Figure 19. YC-1 structure and YC-1 analogues

By Lv et al., 3,5-diaryl-4,5-dihydro-1*H*-pyrazolethanone and carbothioamide derivatives were synthesized. The inhibitor activities on the epidermal growth factor receptor (EGFR) kinase and proliferation of MCF7 cell line were evaluated. Compound **37** (Figure 20) inhibited EGFR kinase IC_{50} at a concentration of 0.07 μM and inhibited proliferation in the MCF7 cell line (IC_{50}= 0.08 μM) (Lv et al., 2010).



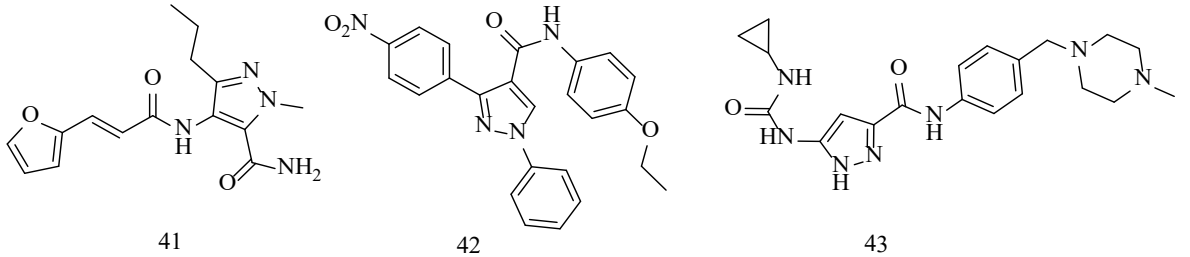
37 38, 39 and 40

Figure 20. Poli-substituted pyrazole analogues

In the same year, a series of 1,3-diphenylpyrazole-benzimidazole derivatives were synthesized by Reddy et al. Synthesized compounds showed potent antiproliferative activity against A549, MCF7 and HeLa cell lines. Compounds **38**, **39**, and **40** (Figure 20) (R = H, F, and Cl and R1 = Br, F, Cl) were identified as potent compounds, with IC_{50} values ranging from 0.83-1.81 μM. According to the studies for mechanism of action such as flow cytometry, fluorescence staining and DNA fragmentation in MCF7 cell lines, it was determined that the compounds stopped the cell cycle in G1 phase and the cells were shown to induce apoptosis (Reddy et al., 2015).

A series of pyrazole-5-carboxamide derivatives have been synthesized by Shi et al., and their antiproliferative activities against human stomach (MGC-803 and SGC-7901) and cervical adenocarcinoma (Bcap-37) cell lines has been determined and compared to fluorouracil. Compound **41** (Figure 21) exhibited strong inhibitory activity against MGC-803 cells, and showed the most potent telomerase inhibitory activity with IC_{50} value at 1.02 ± 0.08 μM. Flow cytometric analysis indicated that the cells were arrested in S phase by compound **41** (Shi et al., 2015).

In a study by Li et al., *N*,1,3-triphenylpyrazolecarboxamide derivatives showed an Aurora-A kinase inhibitor activity that played an important role in mitosis and cell division. Compound **42** (Figure 21) showed cytotoxic activity against HCT116 and MCF7 cancer cell lines with IC_{50} values of 0.39 and 0.46 μM, respectively, and inhibited Aurora-A kinase at an IC_{50} concentration of 0.16 μM in HCT116 cells (Li et al., 2012).



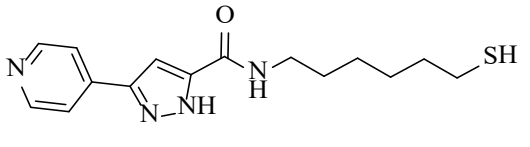
41 42 43

Figure 21. Pyrazole carboxamide analogs

In 2014, 1*H*-pyrazole-3-carboxamide derivatives were synthesized by Lu et al. Antiproliferative activities on HepG2 and HCT116 cell lines and inhibitory activities on kinase enzymes (CDK2, EGFR and ABL2) were evaluated. Among these derivatives, compound **43** (Figure 21) showed moderate activity against HepG2 and HCT116 cell lines and IC_{50} values against CDK2, EGFR and ABL2 were 7.23, 3.31 and 2.05 μM, respectively (Lu et al., 2014).

Wen et al. studied *N*-(6-mercaptohexyl)-3-substituted-1*H*-pyrazole-5-carboxamide derivatives. Compounds designed as SAHA (vorinostat) analogues

(Figure 10) were evaluated for their antitumor activity in vitro and in vivo, and inhibition effects on HDAC isoenzymes were demonstrated. Among the compounds synthesized, compound **44** (Figure 22), the most active derivative, had an antiproliferative effect IC_{50} value of 0.008 μM against HeLa. Isoenzyme assays revealed that **44** had a preference for HDAC1-3 (class I) and HDAC6 (class IIb) isoforms. In addition, the antiproliferative IC_{50} value of the compound **45** bearing the indole structure was 0.070 μM against HeLa cells (Wen et al., 2016).



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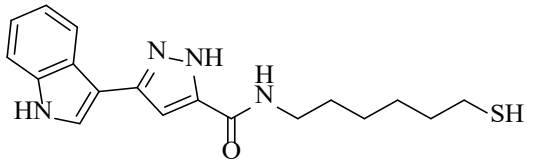
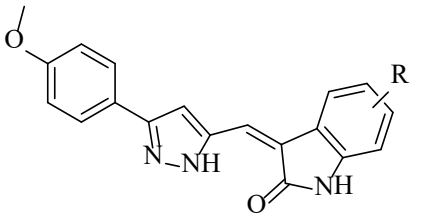


45

Figure 22. 1*H*-pyrazole-5-carboxamide derivatives as vorinostat analogs

In a study by Kamal et al. in 2015, pyrazole-oxyindole derivatives were synthesized. Inhibitory effects of the synthesized compounds on tubulin polymerization were examined and their cytotoxic activities were evaluated on four different cancer cell lines (Hela, A549, MCF7 and human prostate cancer (DU145)). The synthesized compounds were found to exhibit significant cytotoxic activity against cancer cells. Cytotoxic IC_{50} values of compounds **46**, **47** and **48** (Figure 23) (R = 5-Cl, 6-Cl and 5-OCH_3, respectively) were between 2.4-9.3 μM and IC_{50} values for tubulin inhibition were 5.90-9.20 μM (Kamal et al., 2015).

In another study by the same group, arylamino-pyrazolyl-2-propenone derivatives were synthesized. It was determined that among the compounds synthesized, the compounds **49** and **50** (Figure 23) (respectively Ar= 5-indolyl and 6-indolyl) inhibited tubulin polymerization with IC_{50} values of 1.28 and 0.28 μM, respectively. These compounds were found to inhibit cell proliferation in cancer cell lines (Hela, A549, MCF7 and HCT116) at IC_{50} values of 0.36-1.05 μM. These compounds were shown to stop cell cycle in G2/M phase in A549 cancer cell lines. Compound **50** activated the cyclin B1 protein levels and disrupted the microtubule system (Kamal et al., 2015).



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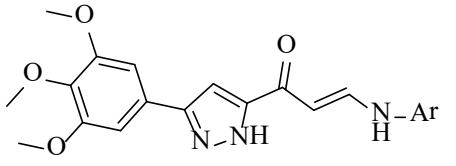
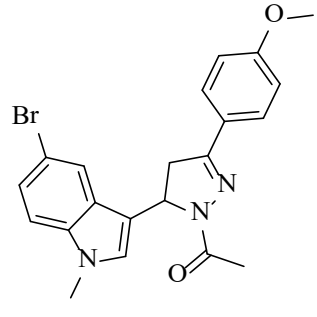


49 ve 50

Figure 23. Pyrazole-oxyindole arylamino-pyrazolyl-2-propenone derivatives

In a study by Yang et al. indolylpyrazoline derivatives were synthesized and their effects on both the tubulin polymerization and the cytotoxicity on cancer cells (A549, MCF7 and HepG2) were investigated. Compound **51** showed the most potent tubulin polymerization inhibitory activity (IC50 1.98 μM) (Figure 24) Cytotoxicity of the compound against A549, MCF7 and HepG2 cell lines was found within the range of 0.15-0.25 μM. It was also determined that this compound induced anticancer activity by inducing apoptosis in A549 cells and causes accumulation of cells in the G2/M phase of the cell cycle. Molecular docking and 3D-QSAR studies were performed and inhibitor–tubulin interactions, several interactions were observed with the protein residues in the colchicine binding site (Yang et al., 2016).



51

Figure 24. Indolylpyrazoline derivative **51**

Chalcone type 1,3-diarylpyrazole compounds **52**, **53** and **54** (Figure 25) were synthesized, and were found to be more cytotoxic than doxorubicin (IC_{50} values 3.3-5.0 **μM**) against MCF7, HepG2 and HCT116 (Mohamed et al., 2012).

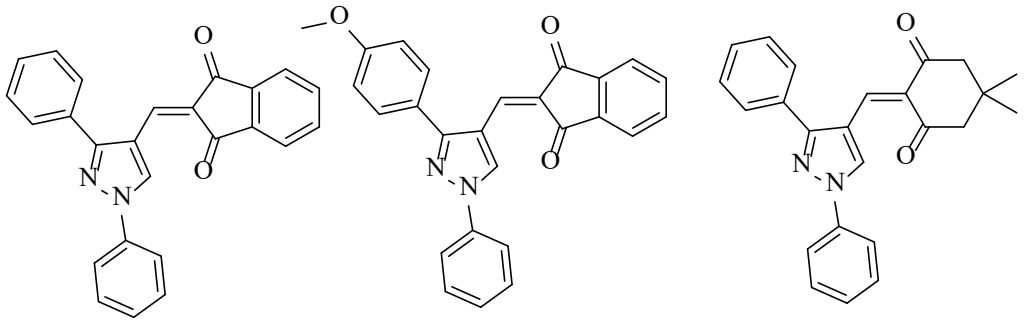


Figure 25. Chalcone type 1,3-diarylpyrazole derivatives

In 2012, 1 - phenyl - 3 - thiophenylpyrazole derivatives were synthesized by Baytas et al. and their anticancer activities were evaluated in MCF7, MDA-MB-231, HeLa, Raji, and HL60 cell lines. In this series, compound **55** (Figure 26) showed cell growth inhibition with the highest activity on Raji and HL60 cells (Inceler et al., 2012). In their ongoing work, 1,3-diarylpyrazole-4-carboxamide derivatives were synthesized and their antiproliferative activities were evaluated in the same cell lines. IC_{50} values of compounds **56** and **57** (Figure 26) against Raji cell line were found to be 8.12 and 9.63 μM, respectively (Baytas et al., 2013).

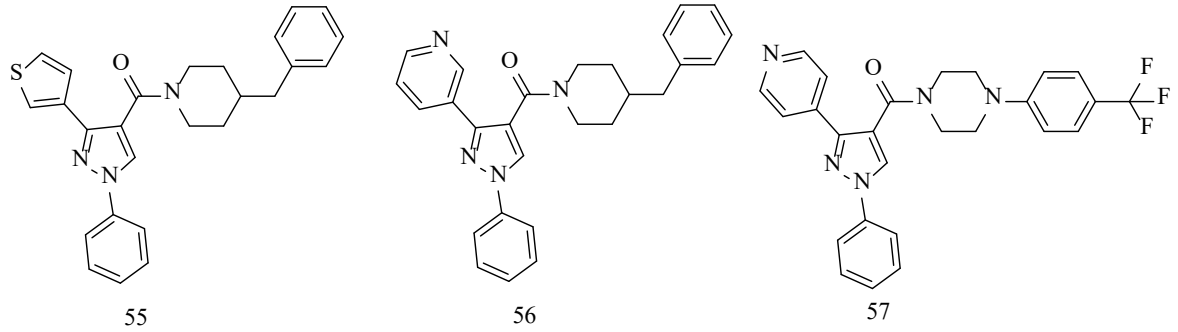
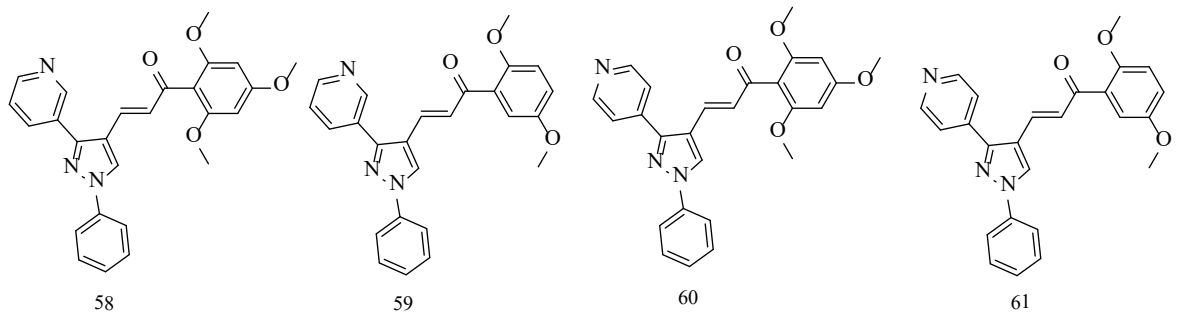


Figure 26. 1,3-Diarylpyrazole-3-carboxamide derivatives

In a study by the same group published in 2017, the activities of 42 pyrazolic chalcone derivatives were synthesized and examined against Huh7, MCF7 and HCT116 cell lines. The IC_{50} values of 14 compounds were found to be between 0.3-4.0 μM against Huh7, MCF7 and HCT116 cell lines. These compounds were also evaluated in the liver cell panel consisting of Huh7, HepG2, Mahlavu, and SNU-475 cell lines and IC_{50} values for compounds **58**, **59**, **60** and **61** (Figure 27) were determined to be in the range of 0.5-4.8 μM. Flow cytometric analysis of hepatocellular carcinoma (HCC) cells treated with compounds **58**, **59**, **60** and **61** demonstrated that these compounds caused cell cycle arrest at G2/M phase followed by the apoptotic cell death and impaired cell growth as shown by real-time cell growth surveillance. Consistent with these results, western blotting of HCC cells treated with the compounds resulted in molecular changes for cell cycle proteins, where p21 levels were increased independent of p53 and the levels of the key initiators of mitosis Cyclin B1 and CDK1 were shown to decrease upon treatment (Hawash et al., 2017).



58 59 60 61

Figure 27. Pyrazolic-chalcone derivatives

Isoxazoles

Isoxazole derivatives are compounds which are of great importance in terms of pharmacological activities. There are a large number of drugs that carry the isoxazole structure, found to have many biological activities such as antituberculous, analgesic, antipyretic, anti-inflammatory, antiplatelet, anti-HIV, antifungal, antibacterial, antioxidant and anticancer (Rajput et al., 2015).

In a study conducted in 2011, 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids with different linker architectures were designed and synthesized and their cytotoxic activities against MCF7, A549, oral (KB), PC-3 and A2780 cancer cell lines were evaluated using SRB method in vitro. GI_{50} values against MCF7 and A549 cells of a large number of compounds were found to be below 0.30 μM. Furthermore, compound **62** (Figure 28) was found to be below 1 μM of GI_{50} value against 18 different cancer cell lines. Flow cytometric analysis of these compounds showed increased cells in G2/M phase, which suggested G2/M cell cycle arrest. Further, compound **62** showed disruption of microtubules as well as fragmentation of nuclei and compound **62** was also identified as an effective cyclin B1 inhibitor as well as the CDK1 inhibitor (Kamal et al., 2011).

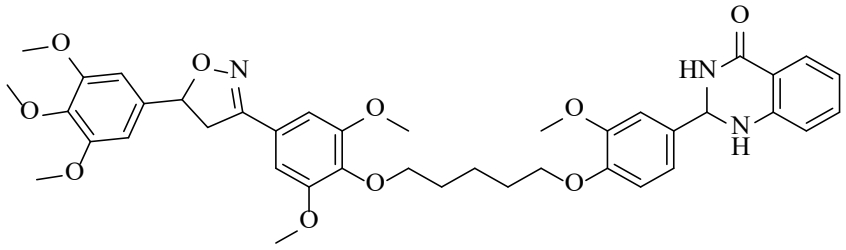


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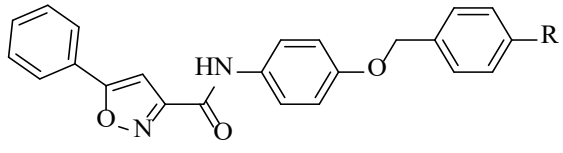
Figure 28. 2,3-Dihydroquinazolinone-isoxazoline hybrid derivatives

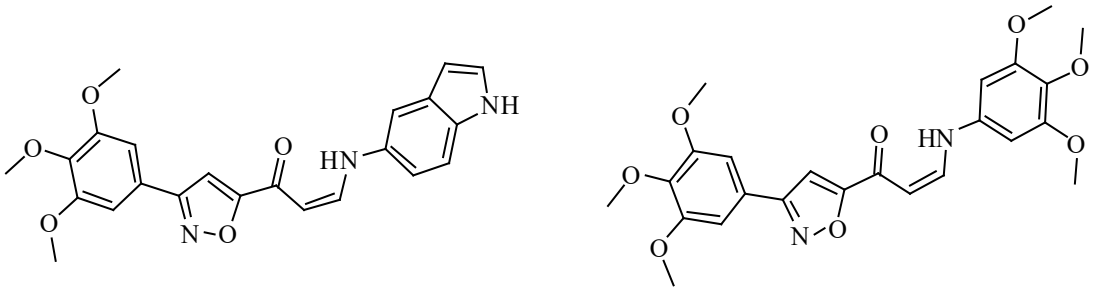
In a study by Veeraswamy et al. in 2012, 5-substituted isoxazole-3-carboxamide derivatives **64** were studied and the cytotoxic effects of these analogues on the A549 cell line were examined. Some of the synthesized compounds (Figure 29) were found to exhibit moderately potent proliferative activity (50% compared to control at 0.1 μM) and low cytotoxic activity (20% of cytotoxicity/growth inhibition at 10 μM). Thus, these compounds showed biphasic response including pro- or anti-cancer effect (Veeraswamy et al., 2012).



64

Figure 29. 5-Substituted isoxazole-3-carboxamide derivatives (R= H, 3-F, 4-F, 3-Cl and 4-Cl)

In a study by Kamal et al. in 2015, arylamino-isoxazolyl-2-propenone derivatives were synthesized and their cytotoxic activity against Hela, A549, MCF7 and HCT116 cells was demonstrated. Among the compounds synthesized, the IC_{50} values of Compound **65** and **66** (Figure 30) were found to be below 4.0 μM (Kamal et al., 2015).



65 66

Figure 30. Arylamino-isoxazolyl-2-propenone derivatives

CONCLUSION

Over the past decades, continuing efforts have been reported on the discovery and development of anticancer compounds. There has been an escalating interest in the development of bioactive molecules, CA-4 analogs as well as bearing the heterocycles such as indole, pyrazole and isoxazole. This review aims at highlighting the status of these moieties in design and development of novel candidate drugs as anticancer agents.

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