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Review; Recent approaches on Tubulin Polymerization inhibitors (2018-2022)

Azhar O. El-Abd[1 *], Basem Mansour[1], Naglaa I. Abdel-Aziz[1,2] , Magda A. El-Sherbeny[1,2]
[1]Department of Pharmaceutical chemistry, Faculty of Pharmacy, Delta University for Science and Technology, Gamasa 11152, Egypt.
[2]Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

*Correspondence: Azhar O. El-Abd; Tel.+201006105121; Email:azhaarosama89@gmail.com.

ABSTRACT
Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer treatment usually involves the use of single or combined strategies including surgery, radiotherapy and chemotherapy. Chemotherapy is most often used to treat cancer, since cancer cells grow and multiply much more quickly than most cells in the body. The dynamic equilibrium of tubulin-microtubule is an essential aspect of cell survival and tubulin is a significant target for cancer drug development. Tubulin exists in the α-β dimer form which polymerizes to form microtubule and further depolymerizes back to tubulin dimer. The microtubule plays an essential role in mitosis and cell multiplication. Disruption of microtubules can induce cell cycle arrest in G2-M phase and formation of abnormal mitotic spindles. A number of naturally occurring compounds such as combretastatin, colchicines, paclitaxel, epothilones and vinblastine affect cancer cells by changing dynamics of tubulin such as polymerization and depolymerization. In this review, we briefly introduce an overview of tubulin polymerization inhibitors.

Keywords: *Cancer, Chemotherapy, Tubulin, Microtubules, Polymerization, Inhibition.*

1. Introduction

Cancer is a large group of diseases in which abnormal cells divide without control and can invade nearby tissues. It is considered one of the primary causes of mortality and one of the greatest serious illnesses worldwide.(Matiadis & Sagnou, 2020) It accounts for nearly 10 million deaths in 2020, with most deaths due to lung cancer, followed by colon, liver, stomach, and breast cancer. The World Health Organization (WHO) has projected that by 2030, more than 13 million annual deaths are expected to occur from cancer globally. (Alam et al., 2022)

The main treatments include surgery, chemotherapy, and radiotherapy. Traditional chemotherapeutics target DNA or processes vital for cell division leading to cellular death, while nontraditional therapies target vulnerabilities specific to cancer cells, such as mutations in gene and proteins.(Aisner, 2007) Eventually, chemotherapeutics are classified according to their mechanism of action and include alkylating agents, antimetabolites, microtubule targeting agents, topoisomerases, and antibiotics.(Shewach & Kuchta, 2009) The existing anticancer agents formulated from natural and synthetic products exert toxicity due to the low site-specificity. Hence, new selective potent and effective anticancer drugs are needed for the treatment of cancer.(Oskuei et al., 2021)

Microtubules are dynamic polymers of α- and β-tubulin dimers that have an essential role in maintenance of the cell structure, cellular processes, and cell division. It accomplishes its physiologic function by the dynamic equilibrium of the polymerization or depolymerization of α,β-heterodimer (**figure 1**). (Borys, Joachimiak, Krawczyk, & Fabczak, 2020) .

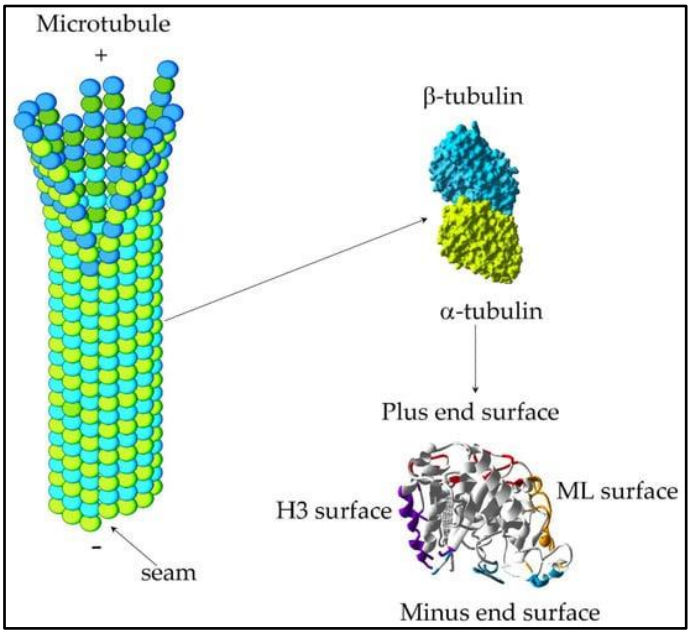


Figure 1. Structure of microtubules, tubulin heterodimers, and functional surfaces of tubulin

Microtubule disruption can induce cell cycle arrest in G_2/M phase and abnormal mitotic spindle formation .Several natural products such as colchicine, paclitaxel, and the vinca alkaloids inhibit tubulin polymerization by binding to tubulin at their respective binding sites. Thus these natural products have been widely used in clinical for the treatment various cancer including breast cancer, leukemia, lymphoma, ovarian cancer, colon cancer and small cell lung cancer.(Kode et al., 2020)

Tubulin targeting drugs are one of the most important tools for drug discovery. They have attracted medicinal chemists attention in the past two decades. Several natural compounds such as paclitaxel(Mosca, Ilari, Fazi, Assaraf, & Colotti, 2021), docetaxel(Khosravi & Asadi, 2021), vincristine(Škubník, Pavlíčková, Ruml, & Rimpelová, 2021), vinblastine(Chagas & Alisaraie, 2019), combretastatin (CA-4)(Siemann, Chaplin, & Walicke, 2009), colchicine(Lin, Kuo, Wu, & Chuang, 2016), podophyllotoxin(Shah et al., 2021) and resveratrol(L. Yang et al., 2019) act by altering microtubule dynamics **(figure 2).** These drugs have significantly improved survival rates and management of several cancer types among children and adults. However, toxicity and drug resistance are the main properties requiring constant improvement. Hence, the need for developing new drugs was an urge to improve treatment outcomes and decrease side effects.(Ebenezer, Shapi, & Tuszynski, 2022)

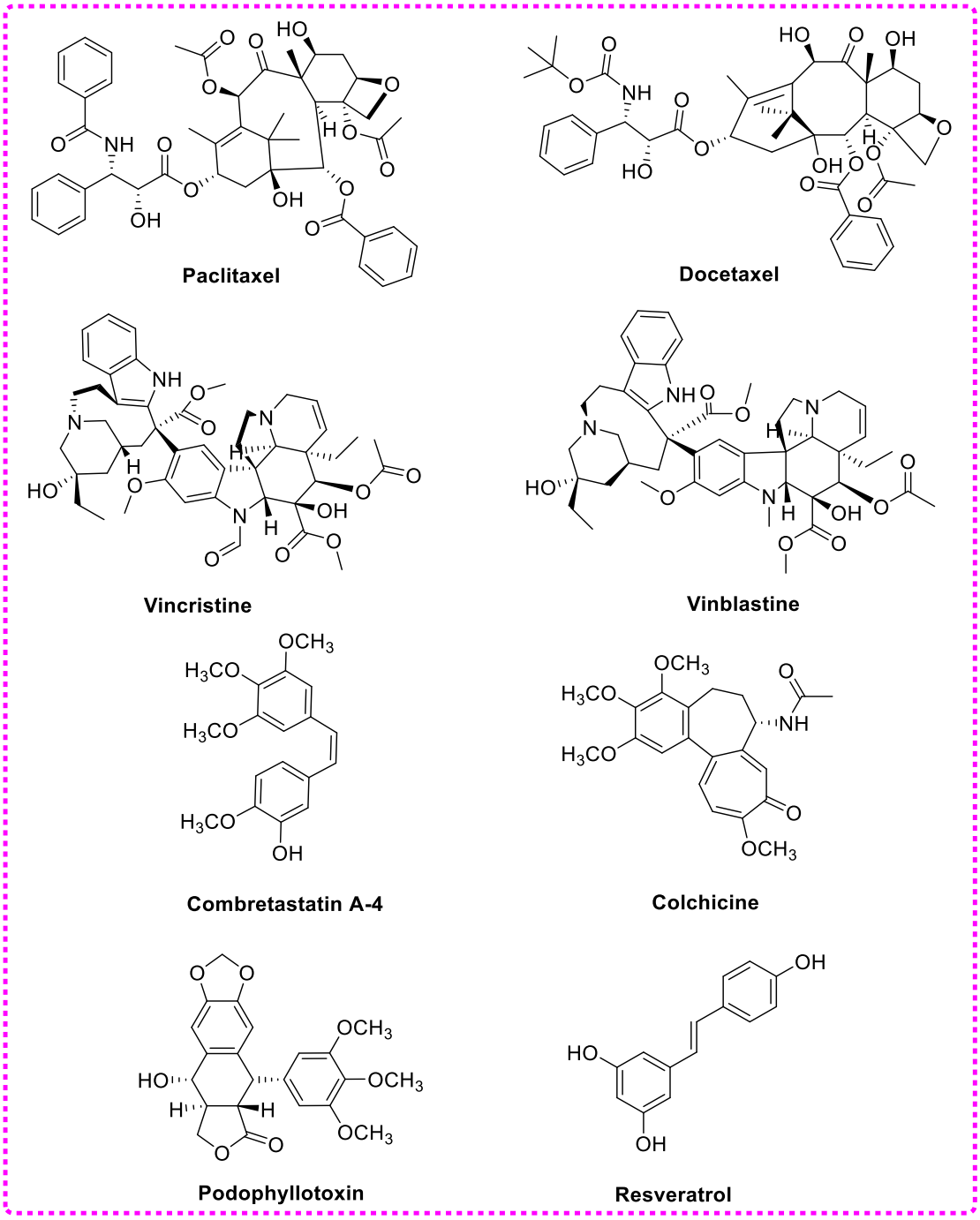


Figure 2. Chemical structure of some tubulin polymerization inhibitors

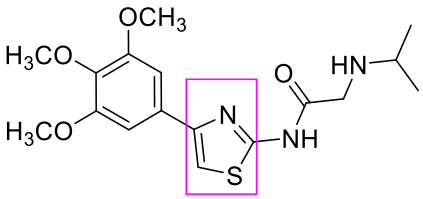
Discussion

2. Tubulin polymerization inhibitors:

2.1. *Thiazole derivatives*

In our previous work, a new series of 2,4-disubstituted thiazole derivatives containing 4-(3,4,5-trimethoxyphenyl) moiety was synthesized and evaluated for their potential anticancer activity as tubulin polymerization inhibitors. All designed compounds were screened for cytotoxic activity against four human cancer cell lines, namely HepG2 (human hepatocellular carcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), HCT-116 (human colorectal carcinoma cell line) and HeLa (epitheloid cervix carcinoma cell line), using MTT assay and CA-4 as a

reference drug. Some compounds showed superior activity against the tested cell lines, Further investigation for the most active cytotoxic agents as tubulin polymerization inhibitors was also performed in order to explore the mechanism of their antiproliferative activity. The obtained results suggested that compounds **1**, **2**, and **3** remarkably inhibit tubulin polymerization, with IC_{50} values of 2.95 ± 0.18, 2.00 ± 0.12, and 2.38 ± 0.14 µM, respectively, which exceeded that of the reference drug combretastatin A-4 (IC_{50} 2.96 ± 0.18 µM). Molecular docking studies were also done to investigate the possible binding interactions between the targeted compounds and the tubulin active site. The interpretation of the results showed clearly that compounds **2** and **3** were identified as the most potent tubulin polymerization inhibitors with promising cytotoxic activity and excellent binding mode in the docking study.(El-Abd et al., 2022)



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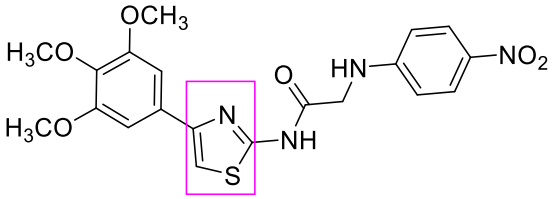

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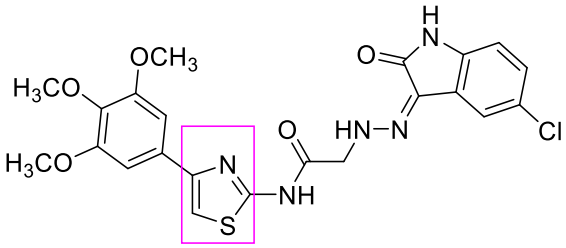
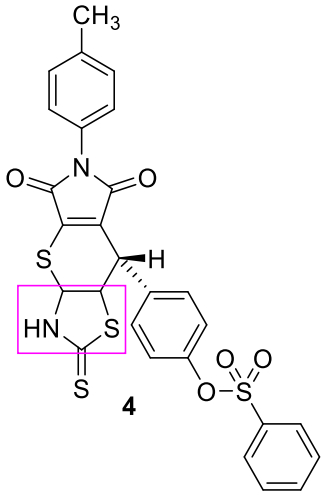


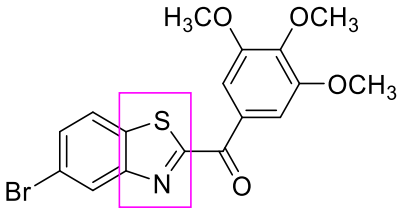
3

Metwally and co-workers (Metwally, Badawy, & Okpy, 2022) have synthesized a novel series of thiopyrano[2,3-d]1,3-thiazole derivatives incorporating arylsulfonate moiety. Four human cancer cell lines MCF7, Hela, HepG2 and human PC3 (prostate cancer) were used to test the cytotoxic activities of several of the produced chemicals. According to the findings, compound **4** had an inhibitory effect on tubulin polymerization with an IC_{50} value of 0.832± 0.031 µg/ml, which was comparable to the value for the positive control Colchicine (IC_{50} = 0.810±0.10). Additionally, compound **4** triggered apoptosis and produced cell cycle arrest at the G2/M phase.



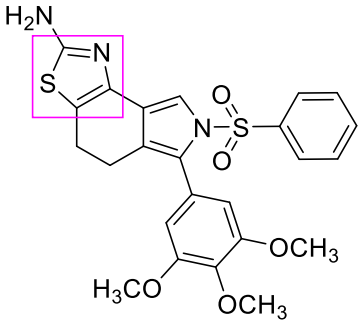
4

A number of benzothiazole derivatives were created, and a targeted SAR analysis was carried out. Among the tested compounds, compound **5** had the strongest antiproliferative effects against four cancer cell lines MCF-7, H1299 (human non-small cell lung carcinoma cell line), HeLa and B16-F10 (murine melanoma cell line). Furthermore, compound **5** demonstrated dose-dependent inhibition of tubulin polymerization in vitro.)(Komuraiah et al., 2021)(



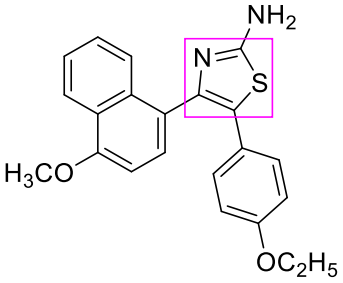
5

In 2021, a series of [1,3]thiazolo[4,5-*e*]isoindoles as tubulin polymerization inhibitors has been produced. On the whole NCI human tumor cell line panel, evaluation of the antiproliferative ability of the novel compounds revealed that numerous compounds are capable of preventing tumor cell proliferation at micromolar-submicromolar doses. The most active derivative **6** was found to cause cell cycle arrest at the G2/M phase and induce apoptosis in HeLa cells.(Spanò et al., 2021)



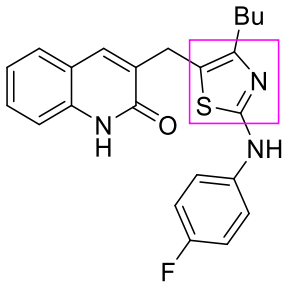
6

Wang *et al.* (G. Wang et al., 2021) have synthesized a novel series of thiazole-naphthalene derivatives as tubulin polymerisation inhibitors. The synthesized compounds were evaluated for their anti-proliferative activities. The majority of the tested compounds exhibited moderate to potent antiproliferative activity on the MCF-7 and A549 (human pulmonary adenocarcinoma) cancer cell lines. Among the tested compounds, compound **7** was identified as the most active against MCF-7 and A549 cancer cell lines with IC_{50} values of 0.48± 0.03 and 0.97±0.13 µMm, respectively. Additionally, mechanistic tests showed that **7** strongly suppressed tubulin polymerization with an IC_{50} value of 3.3 µM, in contrast to colchicine (IC_{50} = 9.1 µM). According to a molecular modeling study, compound **7** interacts efficiently to tubulin's colchicine binding site.



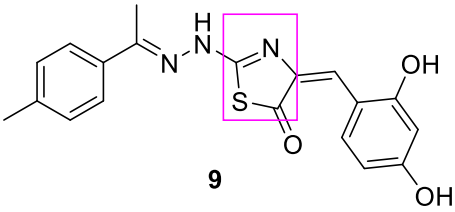
7

A new series of 2-oxoquinoline compounds incorporating arylaminothiazole was created. Using the MTT assay, the synthesized compounds were tested for their *in vitro* cytotoxicity activities against the cancer cell lines HeLa, NCI-H460 (hypotriploid human cell line), T24 (human bladder carcinoma cell line), and SKOV3 (ovarian adenocarcinoma). The test cancer cell lines were most effectively inhibited by compound **8**, with IC_{50} values ranging from 4.4 to 8.7 µM. According to the tubulin polymerization assay results, compound **8** can form hydrogen bonds with important amino acid residues in the active site of tubulin by binding to the colchicine site of the protein, according to a molecular docking study.(Fang et al., 2021)



8

A new series of thiazol-5(4*H*)-ones derivatives has been designed and synthesized in an eco-friendly manner. The anti-proliferative activity of the novel derivatives was assessed using the MTT assay on three human cancer cell lines (HCT-116, HepG-2, and MCF-7), using colchicine as a positive control. Compounds **9** and **10** were the most effective tubulin polymerization inhibitors with IC_{50} values of 9.33 and 9.52 nM, respectively.(El-Naggar, Eissa, Belal, & El-Sayed, 2020)



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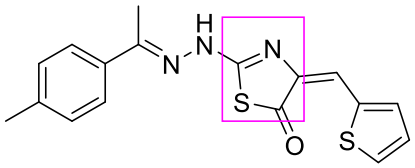
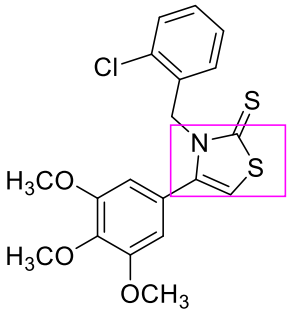


10

Ansari and co-workers(Ansari et al., 2020) have synthesized new series of thiazole-2(3*H*)-thiones containing 4-(3,4,5-trimethoxyphenyl) moiety as diaryl-heterocylic analogs of CA-4 with anticancer activity. Derivatives **11** and **12** outperformed the others in terms of their ability to inhibit MCF-7 cells, with IC_{50} values of 1.14 and 2.41 µg/mL, respectively. Additionally, they exhibited remarkable tubulin polymerization inhibitory activity and cell-death inducing properties. *In vitro* tests showed that these compounds had no discernible cytotoxicity toward normal cells. The compound's binding to the tubulin's colchicine-binding site was also confirmed by an *in vitro* binding assay and an *in silico* docking investigation.



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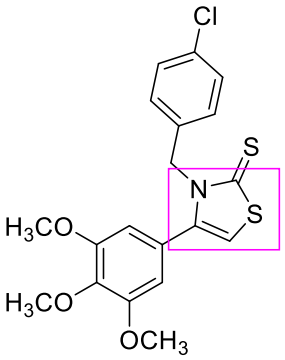
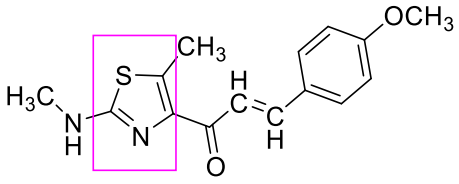


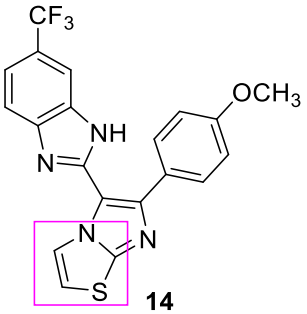
12

Farghaly and her colleagues(Farghaly, Masaret, Muhammad, & Harras, 2020) have synthesized new series of thiazole-based chalcones and 4-hetarylthiazoles (rigid chalcones) and their anti-proliferative activity was estimated against three human cancer cell lines; HepG-2, A549 and MCF-7. 3-(4-Methoxyphenyl)-1-(5-methyl-2-(methylamino)thiazol-4-yl)prop-2-en-1-one **13** had significant and broad antitumor activity that was more potent than Doxorubicin.



13

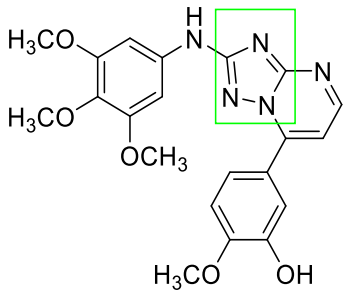
A series of imidazo[2,1-*b*]thiazole-benzimidazole conjugates was produced and tested for their antiproliferative activity against four human cancer cell lines; HeLa, A549, MCF-7 and DU-145 (human prostate cancer cell line) along with normal HEK-293 (Human Embryonic Kidney) cell line. Compound **14** displayed significant cytotoxicity against A549 with IC_{50} value 1.08 µM. Further, cell cycle analysis revealed that this compound arrested the cell cycle at G2/M phase in A549 cells. Furthermore, the tubulin polymerization assay suggest that **14** had significant inhibitory effect on the tubulin assembly with an IC_{50} value of 1.68 µM. Additionally, molecular docking studies revealed that compound **14** fitted in the colchicine binding site.(Baig et al., 2018)



14

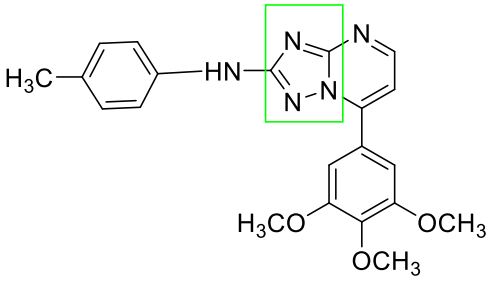
2.2. *Triazole and tetrazole derivatives*

Chen and his team(L. Chen et al., 2022) have designed and synthesized a new series of 2-(substituted amino)-[1,2,4]triazolo[1,5-*a*]pyrimidines as potential tubulin polymerization inhibitors. Compound **15** caused G2/M arrest, triggered cell death in HeLa cells, and shown strong tubulin polymerization inhibitory action with an IC_{50} value of 4.9 µM, which is equivalent to CA-4 (IC_{50} = 4.2 µM).



15

A new series of derivatives containing 7-(3′,4′,5′-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine moiety modified at its 2-position has been synthesized by Romagnoli and his coworkers.(Romagnoli et al., 2022) Compounds **16**, **17** and **18** were noticeably more active than the rest and their IC_{50} values on HeLa, A549, and HT-29 (human colorectal adenocarcinoma cell line) cancer cells were 30-43, 160-240, and 67-160 nM, respectively. Colchicine's ability to bind to tubulin was severely hindered by the p-toluidino derivative **16** (72% inhibition), which also had antiproliferative activity that was superior to CA-4 against the cancer cell lines A549 and HeLa.



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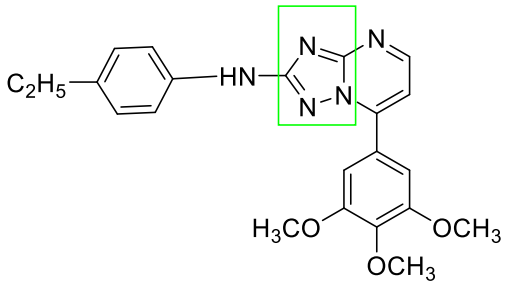

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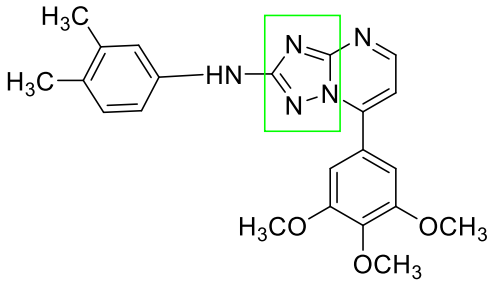
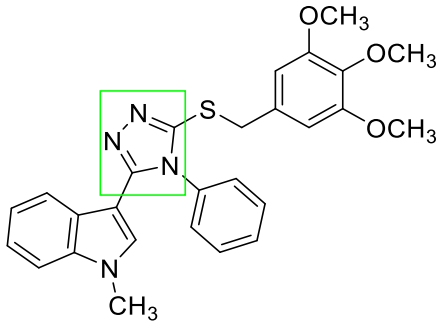


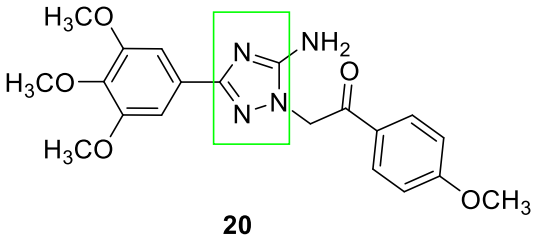
18

Novel indole-1,2,4-triazole series has been synthesized and tested for their ability to inhibit tubulin polymerization. With IC_{50} values of 0.23± 0.08 M, 0.15± 0.18 M, 0.38± 0.12 µM, and 0.30± 0.13µM, compound **19** carrying the 3,4,5-trimethoxyphenyl moiety demonstrated significant anti-proliferative activity against HepG2, HeLa, MCF-7 and A549 cells, respectively. Additionally, it prevented tubulin polymerization with an IC_{50} value that was comparable to the positive control at 2.1 ±0.12 µM. Additionally, molecular docking suggested a potential interaction mode for compound 19 with the tubulin heterodimer binding site for colchicine.(Wu, Man, Liao, Zhu, & Zhou, 2021)



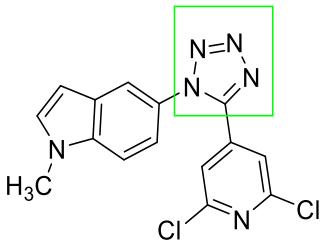
19

A new series of 5-amino-1*H*-1,2,4-triazoles possessing 3,4,5-trimethoxyphenyl moiety was synthesized, and evaluated for antiproliferative activities. Among them, analogue **20** exhibited the most potent tubulin polymerization inhibitory activity with an IC_{50} value of 9.4 µM, and molecular modeling studies revealed stable interactions of compound **20** in the colchicine-binding site of tubulin, suggesting that 5-amino-1*H*-1,2,4-triazole scaffold has the potential for further investigation to develop novel tubulin polymerization inhibitors with anticancer activity.(F. Yang et al., 2021)



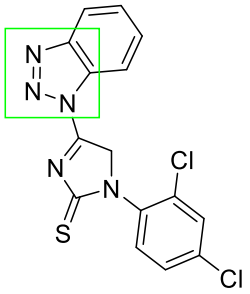
20

New tetrazole containing compounds have been synthesized by Gallego-Yerga et al.(Gallego-Yerga et al., 2021) as tubuin modulators. Compound **21** was tested *in vitro*, it showed marked activity against tubulin polymerization, as well as antiproliferative effect in HeLa cells through microtubule disruption. The mechanism of action was confirmed by cell cycle studies and immunofluorescence confocal microscopy.



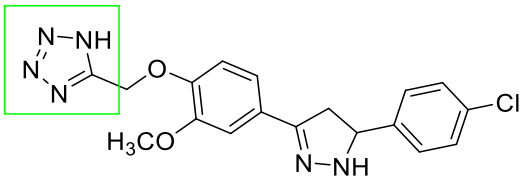
21

A new series of benzotriazole moiety bearing substituted imidazol-2-thiones at N1 has been designed, synthesized and evaluated for *in vitro* anticancer activity against the different cancer cell lines MCF-7, HL-60 (human leukemia cell line), and HCT-116. The majority of the benzotriazole analogs showed encouraging antiproliferative activity against the cancer cell lines that were put to the test. Compound **22** had the strongest activity against the cancer cell lines MCF-7, HL-60 and HCT-116 among all the synthesized compounds. Compound **22** was used for complex biological experiments, and the G2/M phase of the cell cycle was halted in HL-60 cells. The tubulin binding site of compound **22** demonstrated a considerable suppression of tubulin polymerization. Additionally, compound **22** induced apoptosis via controlling the expression of the pro- and anti-apoptotic proteins BAX and Bcl-2.(Khayyat, Mohamed, Malebari, & El-Malah, 2021)



22

New tetrazole based pyrazoline derivatives were designed and synthesized under both conventional and ultrasonic irradiation method. Several derivatives were found to be excellent cytotoxic against MCF-7, A549 and HepG2 cell lines characterized by low IC_{50} values (0.78–3.12 µg/mL). Compounds **23** and **24** demonstrated an antiproliferative effect comparable to that of CA-4.(Dofe et al., 2020)



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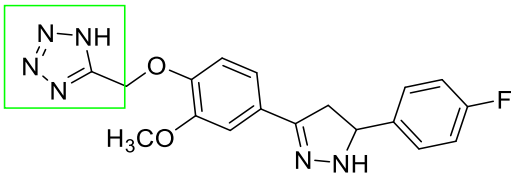
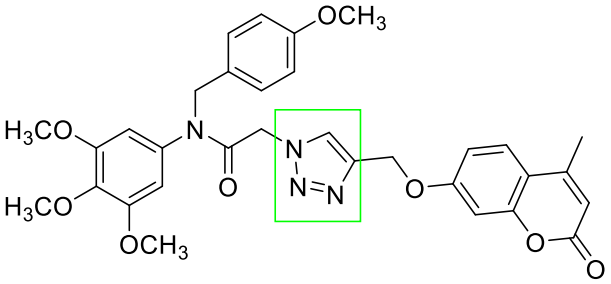


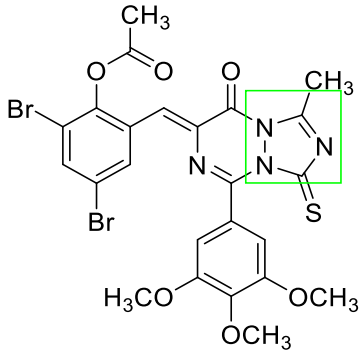
24

New trimethoxyphenyl-1,2,3-triazole hybrids were designed, synthesized and screened for their antiproliferative activity against three cancer cell lines PC3, HepG2 and MGC803 (gastric cancer cell line). Among them, compound **25** displayed better antiproliferative activity results with IC_{50} values from 0.13 μM to 1.74 μM than anticancer drug colchicine. Inhibiting MGC803 cell growth and colony formation, inducing G2/M phase arrest by down-regulating CDK1 (Cyclin-dependent kinase 1), and promoting apoptosis via controlling DR5 and the Bcl-2 family are all potential effects of compound **25**. Furthermore, through interacting with the colchicine site, **25** substantially inhibited the polymerization of tubulin.(Fu et al., 2019)



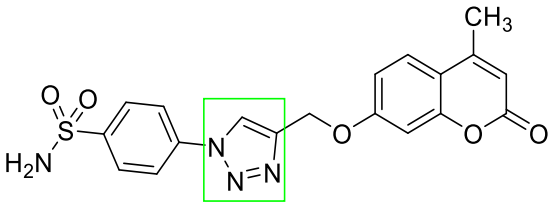
25

New triazole derivatives were synthesized as potential antitumor agents. Some derivatives revealed potent anticancer activity over MCF-7 breast cancer cells higher than podophyllotoxin by approximate 6-fold and potent inhibitory activity of cell proliferation and cell cycle arrest at G2/M phase. Compound **26** displayed excellent β-tubulin percentage of polymerization inhibition equivalent to that exhibited by podophyllotoxin.(Zaki et al., 2018)



26

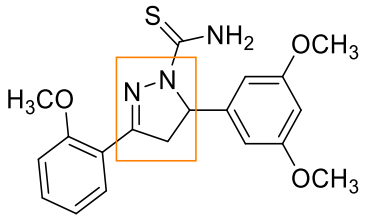
Novel sulfanilamide-1,2,3-triazole derivatives were synthesized and tested as tubulin polymerization inhibitors. Compound **27** displayed potent tubulin polymerization inhibitory activity. The inhibitory concentration that reduced the polymerized tubulin by 50% (IC_{50}) of compound **27** was 2.4 μM .(Guo, Zhen, Guo, Zhang, & Zhou, 2018)



27

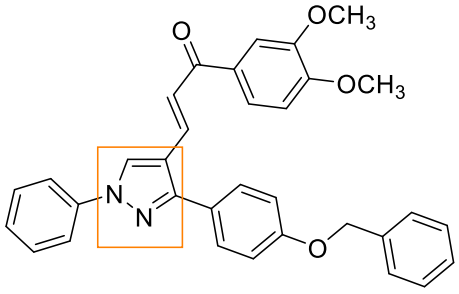
2.3. *Pyrazole derivatives*

3,5-Diphenyl-2-pyrazolines were synthesized and screened to investigate their ability to inhibit tubulin polymerization. The outcomes demonstrated that derivative **28** had the best activity against human colon cancer HCT116 cells. Cell cycle arrest in G2/M was caused by derivative **28**. Additionally, it resulted in dispersed microtubules, and inhibition of formation of mitotic spindle. In addition, the binding mode between tubulin and derivative **28** was elucidated by *in silico* molecular docking. Derivative **28** was superimposed with colchicine and fitted the colchicine-binding site well. These findings imply that derivative **28** prevents the development of the mitotic spindle during mitosis in HCT116 cells by binding to the tubulin's colchicine binding site and inhibiting tubulin polymerization.(Shin et al., 2022)



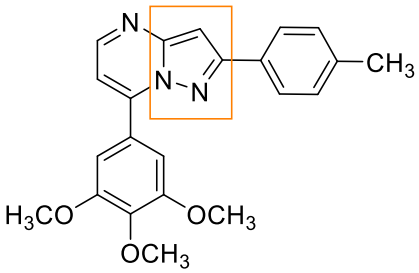
28

A new series of (*E*)-3-(3-(4-(benzyloxy)phenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenylprop-2-en-1-one conjugates was synthesized and evaluated for tubulin polymerization inhibitory activity and *in vitro* cytotoxicity against 3 cancer cell lines MCF-7, PC-3 and SiHa (human uterus carcinoma cell line), as well as a normal cell line (HEK-293T). The compounds were also tested to determine their binding modes at the colchicine-binding site of tubulin protein (PDB ID-3E22). Among all the synthesized conjugates, compound **29** exhibited excellent cytotoxicity with IC_{50} value of 2.13 ± 0.80 µM, 4.34 ± 0.98 µM, and 4.46 ± 0.53 µM against MCF-7, SiHa, and PC-3 cancer cell lines with no significant toxicity to the HEK cells. Molecular docking studies showed that the most promising compound **29** could fit well into the colchicine-binding site of the tubulin, making five hydrogen bond interactions with the most important active site residues.(Alam et al., 2022)



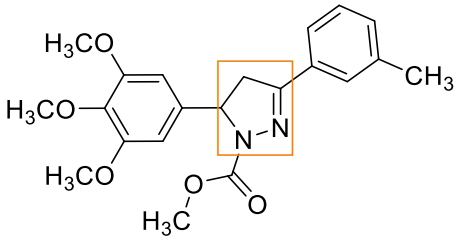
29

Li and co-workers(G. Li et al., 2020) have synthesized a new series of pyrazolo[1,5-*a*]pyrimidine analogs as novel tubulin inhibitors. Among them, with average IC_{50} values ranging from 24.8 nM to 28 nM, compound **30** had the strongest antiproliferative activity against a panel of cancer cell lines. Its direct binding to the colchicine site was confirmed by the crystal structure of **30** in complex with tubulin. Additionally, compound **30** caused cell cycle arrest in the G2/M phase, hindered cancer cell migration, and successfully inhibited tubulin polymerization *in vitro*.



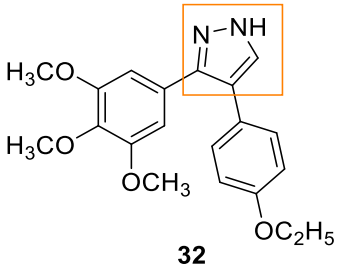
30

A series of novel 4,5-dihydro-1*H*-pyrazole-1-carboxylate derivatives was designed, synthesized and bioevaluated. Among them, the most potent compound **31** revealed comparable activity against a panel of cancer cells (GI_{50} ranging 0.05–0.98 μM) and tubulin polymerization inhibition (IC_{50} = 1.49 μM) with reference drug CA-4(P) (GI_{50} ranging 0.019–0.32 μM, IC_{50} = 2.18 μM). It was found that compound **31** disturbed microtubules, which caused G2/M arrest, leading to reactive oxygen species accumulation and apoptosis.(Zhang et al., 2019)

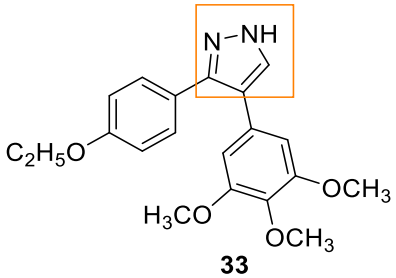


31

A series of 3-(3′,4′,5′-trimethoxyphenyl)-4-substituted 1*H*-pyrazole and their related 3-aryl-4-(3′,4′,5′-trimethoxyphenyl)-1-*H*-pyrazole derivatives, was produced and screened for their *in vitro* antiproliferative activity. The most active derivatives **32**, **33** were found to bind to the colchicine site of tubulin and inhibited tubulin polymerization at submicromolar concentrations.(Romagnoli et al., 2019)

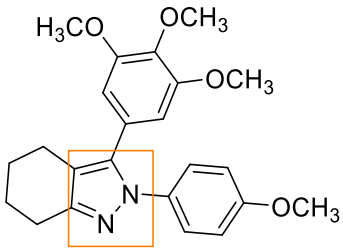


32



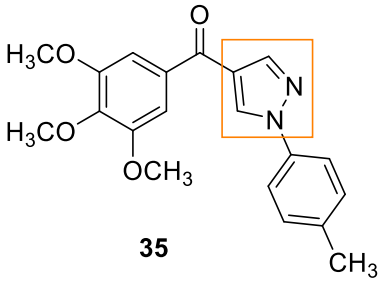
33

Several new cycloalkyl-fused 2,3-diaryl pyrazole derivatives were designed, produced, and bioevaluated as potential anti-tubulin agents. Compound **34** had the most potent antiproliferative activity against a panel of cancer cell lines (IC_{50} = 0.78–2.42 μM) and low cytotoxicity against 293T & L02 (Crocetin Protected Human Hepatocyte) (CC_{50} values of 131.74 and 174.89 μM, respectively). Moreover, **34** caused inhibition of tubulin polymerization *in vitro*, cell cycle arrest at G2/M phase, altering morphology of tubulin, accumulation of intracellular reactive oxygen species, and induction of apoptosis of HeLa cells.(Xia et al., 2019)



34

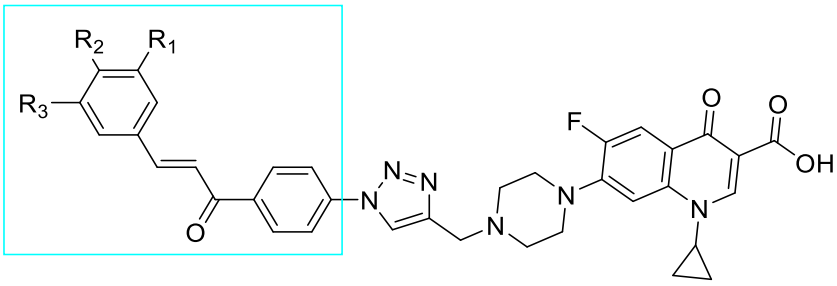
Wang *et al.* (L. Wang et al., 2018) have designed and synthesized a novel *series of (1-aryl-1*H-pyrazol-4-yl) (3,4,5-trimethoxyphenyl)methanones and ketoxime derivatives as antitubulin agents. All of the target compounds were bioevaluated *in vitro* for the anti-proliferative activities against three tumor cell lines A549, HT-1080 (fibrosarcoma cells) and SGC-7901 (human gastric cancer cell line). The most active compound was **35** which significantly inhibited tumor cells growth with IC_{50} value of 0.054–0.16 μM. Meanwhile, compound **35** could damage microtubule network and arrest SGC-7901 cell cycle at G2/M phase. Also, compound **35** showed effective inhibitory activity of tubulin polymerization. Furthermore, a molecular docking study was done to explain its binding mode at the colchicine site in the tubulin heterodimer.



35

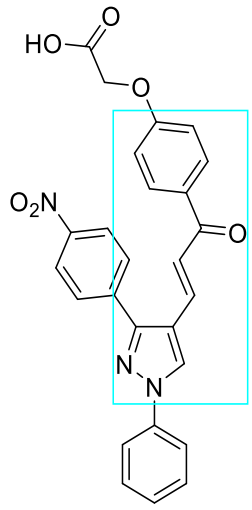
2.4. *Chalcone derivatives*

As potential anticancer drugs, a group of new 1,2,3-triazole-linked ciprofloxacin-chalcones was created. The anti-proliferative efficacy of hybrids **36-40** against colon cancer cells was remarkably strong. For HCT116, HT29, and Caco-2 cells, respectively, compounds showed IC_{50}s ranging from 2.53–8.67 μM, 8.67–62.47 μM, and 4.19–24.37 μM; doxorubicin, on the other hand, displayed IC_{50} values of 1.22–4.15 μM. In comparison to doxorubicin (IC_{50} = 1.22 μM), compounds **36-40** were the most effective against HCT116. Their respective IC_{50} values were 3.57, 4.81, 4.32, 4.87, and 2.53 μM. Also, topoisomerase I, II, and tubulin polymerization were significantly inhibited by the same hybrids.(Mohammed et al., 2022)



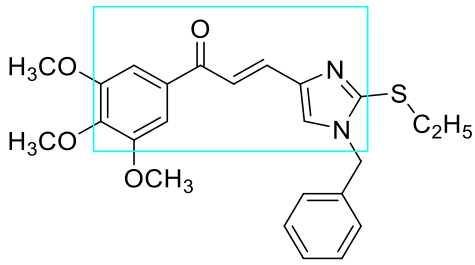
36: R_1=R_2=R_3= H
37: R_1=Cl, R_2=R_3= H
38: R_1=R_3= H, R_2= F
39: R_1=R_2= OCH_3, R_3= H
40: R_1=R_2=R_3= OCH_3

In 2022, Ahmed *et al.* (Ahmed et al., 2022) have synthesized a novel series of pyrazole-chalcone analogs of Lonazolac. The synthesized compounds were screened for their *in vitro* anticancer activity against four cancer cell lines using the MTT assay method. Among all, compound **41** was the most potent against three cancer cell lines, HeLa, HCT-116, and RPMI-822 (human myeloma cell line**)** with IC_{50} values of 2.41, 2.41, and 3.34 μM, respectively. It was also the most effective inhibitor of tubulin polymerization (IC_{50} = 4.77 μM). Molecular docking of compound **41** revealed strong connections with the observed biological results.

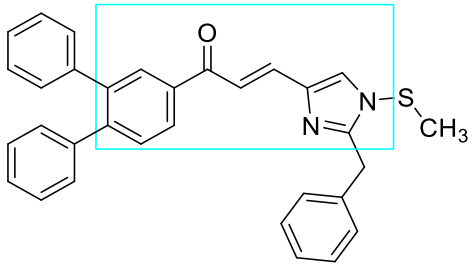


41

Oskuei *et al.*(Oskuei et al., 2021) have synthesized novel imidazole-chalcone derivatives as tubulin polymerization inhibitors and anticancer agents. A549, MCF-7, MCF-7/MX (mitoxantrone resistant breast cancer cells), and HEPG2 were used to test the antiproliferative activity of the imidazole-chalcone derivatives and they showed more cytotoxicity on A549 cancer cells compared to the other three cell lines. Among the chalcone derivatives, compounds **42** and **43** had significant cytotoxicity with IC_{50} values ranging from 7.05 to 63.43 μM against all the four human cancer cells. Further studies showed that these compounds induced cell cycle arrest at the G2/M phase at low concentrations and inuced apoptosis. Also they have inhibited tubulin polymerization similar to combretastatin A-4 (CA-4). The likely interactions of these chemicals with tubulin were illustrated by molecular docking studies of **42** into the colchicine-binding region of tubulin.

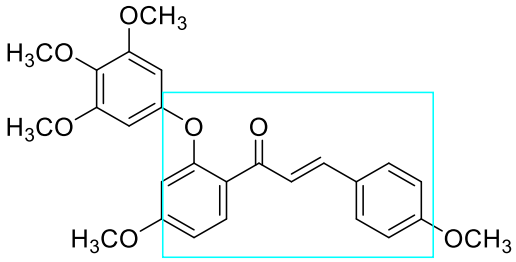


42



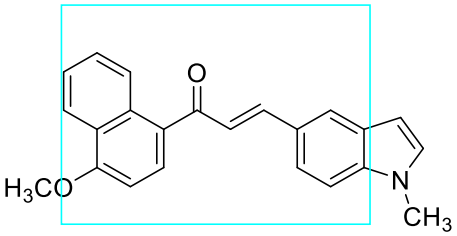
43

A novel series of chalcone derivatives containing diaryl ether moiety was produced and screened for their anti-tubulin polymerization activities and anticancer activities. Among them, compound **44** was found to be most active on MCF-7, HepG2 and HCT116 cancer cell lines, with IC_{50} values of 3.44 ± 0.19, 4.64 ± 0.23, and 6.31 ± 0.27 μM, respectively. *In vitro* tubulin polymerization assay demonstrated that **44** significantly inhibited tubulin polymerization. Further research demonstrated that compound **44** could cause G2/M phase arrest and apoptosis, also molecular docking studies demonstrated that this compound interacts and binds at the tubulin's colchicine binding region.(G. Wang et al., 2020)



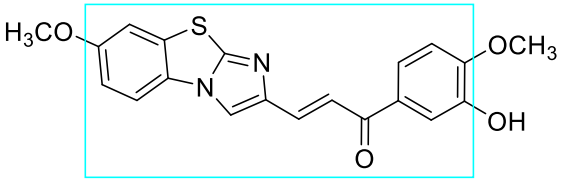
44

A series of novel chalcone derivatives containing indole and naphthalene moieties was synthesized by wang and co-workers.(G. Wang, Peng, & Li, 2019) All compounds were evaluated for their *in vitro* cytotoxic potential against HepG2, HCT116 and MCF-7 cell lines. Among them, compound **45** had the most potent cytotoxic activity against HepG2, HCT116 and MCF-7 with IC_{50} values of 0.65, 1.13 and 0.82 μM, respectively. Further studies showed that compound **45** arrested cancer cells in G2/M phase and also displayed remarkable inhibition of tubulin polymerization (IC_{50} = 3.9 μM).

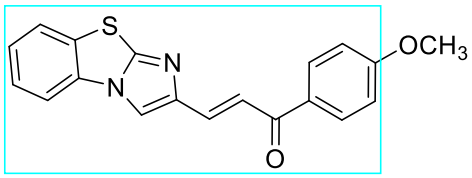


45

A series of benzo[*d*]imidazo[2,1-*b*]thiazole-chalcone conjugates was synthesized and tested for their cytotoxic activity against a panel of human cancer cell lines including A-549, DU-145, HT-29 and MDA MB-231 (human breast cancer cell line). Compounds **46** and **47** showed substantial antiproliferative effect against MDA MB-231 with IC_{50} values of 1.3 and 1.2 μM respectively. These conjugates were able to induce cell-cycle arrest in the G2/M phase and inhibit microtubule assembly. Additionally, Compounds **46** and **47** induce apoptosis. Furthermore, molecular docking studies have been performed to predict the binding modes of these conjugates with the tubulin protein.(Sultana et al., 2018)

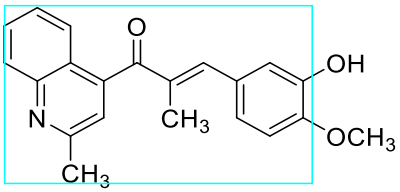


46



47

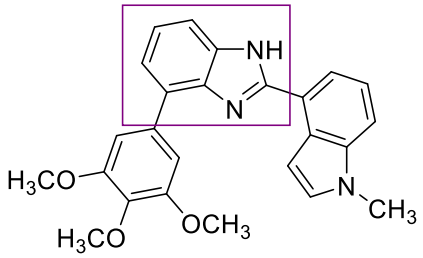
A series of novel quinoline–chalcone derivatives was produced and screened for their antiproliferative activity. Among them, compound **48** exhibited the most powerful activity with IC_{50} values ranging from 0.009 to 0.016 μM in a panel of cancer cell lines. Compound **48** also displayed a good safety profile with an LD_{50} value of 665.62 mg/kg by intravenous injection, and its hydrochloride salt significantly inhibited tumor growth in H22 xenograft models without observable toxic effects, which was more potent than that of CA-4. Mechanism studies revealed that **48** bound to the colchicine site of tubulin, arrested the cell cycle at the G2/M phase, induced apoptosis, depolarized mitochondria, and induced reactive oxidative stress generation in K562 cells (myelogenous leukemia cells). Furthermore, **48** has potent *in vitro* antimetastasis and in vitro and *in vivo* antivascular activities.(W. Li et al., 2018)



48

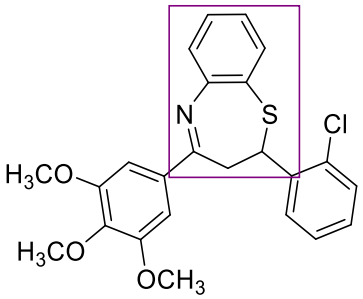
2.5. *Benzofused derivatives:*

Novel analogues of benzimidazole were created as tubulin inhibitors with strong antiproliferative properties. With an average IC_{50} value of 50 nM, compound **49** stood out among them as having the most potent inhibitory effects on cancer cells. This makes it somewhat more effective than colchicine. *In vitro* paclitaxel resistance was successfully overcome by compound **49**, which shown approximately identical effectiveness against both a paclitaxel-resistant cancer cell line (A2780/T, IC_{50} = 9.7 nM) and the matching parental cell line (A2780S, IC_{50} = 6.2 nM). Furthermore, **49** demonstrated significant *in vivo* antitumor efficacy in a melanoma tumor model with tumor growth inhibition rates of 78.70% (15 mg/kg) and 84.32% (30 mg/kg). X-ray crystallography, confirmed its direct binding to the colchicine site.(Ren, Wang, et al., 2021)



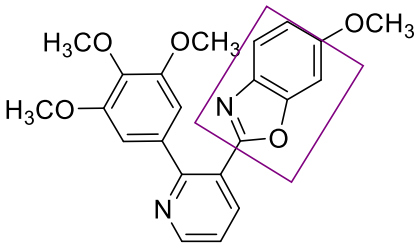
49

A series of diaryl benzo[*b*][1,4]thiazepine derivatives was synthesized and screened as tubulin polymerization inhibitors with anti-tumor potency. Among them, the hit compound **50** showed potential on inhibiting the growth of several cancer cell lines (IC_{50} values: 1.48 μM for HeLa, 1.47 μM for MCF-7, 1.52 μM for HT29 and 1.94 μM for A549), being comparable with the positive controls Colchicine and CA-4(P). IC_{50} of compound **50** as an inhibitor of tubulin polymerization was 1.20 μM. The results of the flow cytometry assay showed that **50** could cause cancer cells apoptosis. The potential for creating interactions with the neighboring tubulin chain was hinted by the docking simulation, which also suggested the binding pattern that might be used.(B. Wang et al., 2021)



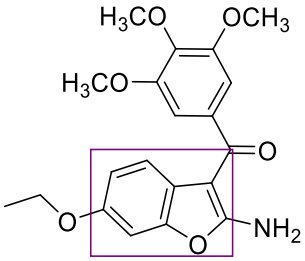
50

A novel series of arylpyridine derivatives was produced and biologically tested for their anticancer activity. Some compounds displayed selective antiproliferative activities against HT-29 cells. Compound **51** with a 6-methoxybenzo[d]oxazole group displayed similar activities to CA-4 and lower cytotoxicities than CA-4 and 5-Fu. Through disruption of the microtubule network, compound **51** successfully binds to the colchicine binding site and arrests the cell cycle of A549 in the G2/M phase, according to further research. **51** exhibits high tubulin polymerization inhibitory activity (IC_{50} = 2.1 μM).(He et al., 2020)



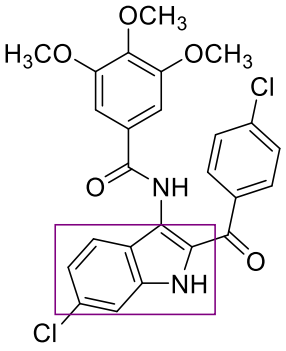
51

Oliva *et al.*(Oliva et al., 2020) Have synthesized a new class of tubulin polymerization inhibitors based on the 2-amino-3-(3′,4′,5′-trimethoxybenzoyl)benzo[*b*]furan molecular scaffold. The synthesized compounds have been evaluated for *in vivo* and *in vitro* biological activity. Derivative **52** was the most promising compound of the series, it showed significant antiproliferative activity (IC_{50}: 5 pM) against the Daoy medulloblastoma cell line, and was nearly devoid of toxicity on healthy human lymphocytes and astrocytes. The powerful antiproliferative properties of **52** come from its ability to bind to the colchicine site and prevent tubulin polymerization. The substance was further tested for *in vivo* action, and results revealed that it was more effective against a syngeneic murine mammary tumor at 15 mg/kg compared to 30 mg/kg of combretastatin A-4 phosphate.



52

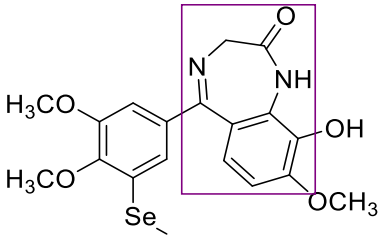
A series of novel 3-amidoindole derivatives possessing 3,4,5-trimethoxylphenyl groups was synthesized and evaluated for their antiproliferative and tubulin polymerization inhibitory activities. The most active compound **53** inhibited the growth of T47D, BT549, and MDA-MB-231 cell lines (human breast cancer cell lines), with IC_{50} values at 0.04, 3.17, and 6.43 μM, respectively. In addition, compound **53** also exhibited the most potent anti-tubulin activity with IC_{50} values of 9.5 μM, which was significant, compared to CA-4. Furthermore, molecular docking studies confirmed the interaction of the compound **53** at the colchicine-binding site of tubulin.(P. Chen et al., 2019)



53

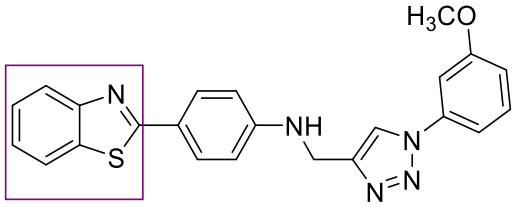
In 2019, Pang *et al.*(Pang et al., 2019) have created a series of novel benzodiazepine derivatives. These derivatives have been evaluated for anticancer activity as tubulin polymerization inhibitors. Most compounds exhibited potent antiproliferative activity against a panel of cancer cell lines. Among these compounds, the optimal compound, **54**, possessed the most potent activity, including cytotoxicity against five cancer cell lines A549, MDA-MB231, HepG2, Hela and HCT116 (IC_{50} = 6–15 nM) and inhibition of tubulin polymerization (IC_{50} = 1.65 ± 0.11 μM). Further studies revealed that **54** could disrupt intracellular microtubule

organization, arrest cell cycle at the G_2/M phase and leads to cell apoptosis. Compound **54** exhibited good metabolic stability with a $t_{1/2}$ of 161.2 min, which was much better than the reference compound CA-4.



54

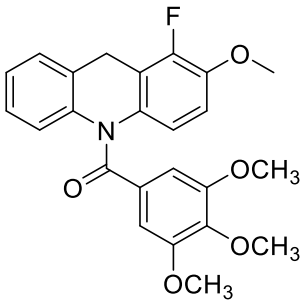
A series of novel 2-(4-aminophenyl)benzothiazole derivatives has been synthesized and evaluated for their antiproliferative activity over a group of three human cancer cell lines, A549, HeLa and MDA-MB-231, using sulforhodamine B assay method. Some of the synthesized molecules showed effective growth inhibition (GI_{50}) activity against the tested human cancer cell lines at lower micromolar concentration (GI_{50}) values in the range 0.2–1.7 μM. Remarkably, compound **55** exhibited good activity in all three cancer cell lines in the GI_{50} range 0.55–1.2 μM. Further, when **55** was assayed for tubulin polymerization inhibition, it displayed more than 55% inhibition at concentration of 5.0 μM. The molecular docking studies explained the molecular interactions of the derivative with the targeted receptor.(Narva et al., 2019)



55

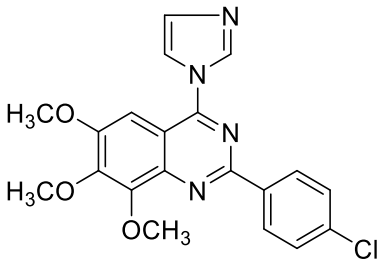
2.6. *Miscellaneous derivatives:*

In 2022, Novel tubulin polymerization inhibitors based on acridane were developed, produced, and bioevaluated as anticancer drugs. The most effective molecule, **56**, demonstrated high tubulin polymerization inhibitory activity (IC_{50} = 1.5 μM) and remarkable antiproliferative potency against four cancer cell lines with an average IC_{50} of 30 nM, better than colchicine IC_{50} of 65 nM. Additionally, in a melanoma tumor model, **56** (10 mg/kg) demonstrated remarkable anticancer activity with tumor growth inhibition (TGI) of 65.1% and no discernible harm.(Peng, Ren, Pan, Liu, & Chen, 2022)



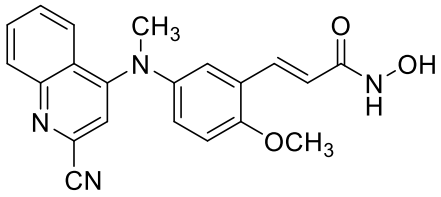
56

Dwivedi and his team(Dwivedi et al., 2022) have synthesized novel 4-*N*-heterocyclic-2-aryl-6,7,8-trimethoxyquinazolines. The synthesized compounds were evaluated for antiproliferative and tubulin polymerization inhibitory activity against MCF-7, HeLa and HT-29 cancer cell lines and normal cell line HEK-293 T. The IC_{50} values for compound **57** against the cancer cell lines MCF-7, HELA, and HT29 were 2.16 μM, 8.53 μM, and 10.42 μM, respectively. Significant tubulin polymerization inhibitory activity was seen in the compound **57** which also demonstrated strong binding affinities in the colchicine domain in the docking studies.



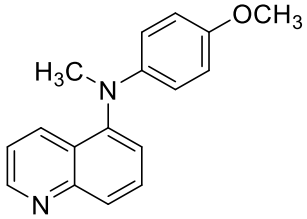
57

As new inhibitors of histone deacetylases (HDAC) and tubulin polymerization (TP), a number of quinoline and quinazoline analogs were created. With an average IC_{50} value of 0.6 and 0.7 nM, compounds **58** demonstrated the best cytotoxicity effects against a panel of human cancer cell lines. Additionally, it showed positive activity against cells that were resistant to many drugs and CA-4 against colon cancer and leukemia. Additionally, through mitochondrial malfunction, compound **58** caused HT29 cell cycle arrest in the G2/M phase and caspase-induced death in HT29 cells. HDAC8 activities were also reduced by compound **58**.(Hauguel et al., 2022)



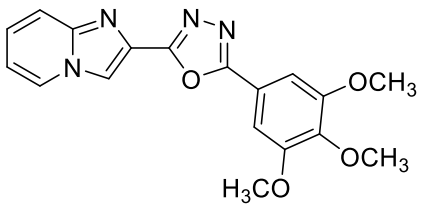
58

In 2021, in order to create novel tubulin inhibitors that target the colchicine binding site, a variety of acridine and quinoline compounds were designed and synthesized. With an IC_{50} of 261 nM against HepG-2 cells, compound **59** had the highest antiproliferative activity of the group. Compound **59** also had the ability to prevent the growth of HepG-2 colonies. According to mechanism studies, compound **59** significantly reduced tubulin polymerization *in vitro* and interfered with microtubule dynamics in HepG-2 cells. Finally, docking tests showed that compound **59** fitted well with CA-4 and fitted perfectly in the tubulin colchicine binding site.(Ren, Ruan, et al., 2021)



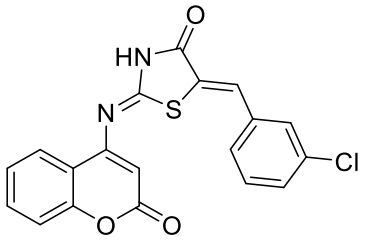
59

Sigalapalli *et al.* (Sigalapalli, Kiranmai, et al., 2021)have synthesized a new series of imidazo[1,2-*a*]pyridine-oxadiazoles . The new compounds were evaluated for their *in vitro* anticancer activity against A549, PC-3 and DU-145 cell lines. Compound **60** showed the highest potency on A549 cells with an IC_{50} value of 2.8 ± 0.02 μM. Target-based research has shown that **60** effectively binds to CT-DNA and inhibits tubulin polymerization with an IC_{50} value of 3.45 ±0.51 μM. Furthermore, the molecular modeling investigations showed that **60** has excellent physico-chemical properties and a strong binding affinity for the tubulin receptor.



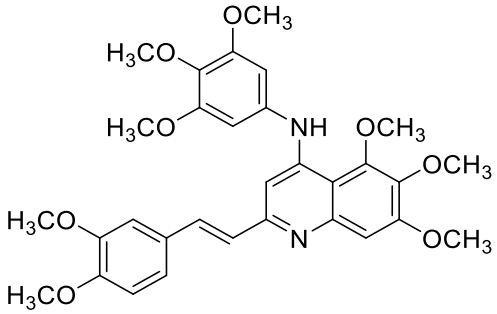
60

As tubulin polymerization inhibitors, a novel 2-iminothiazolidin-4-one derivatives based on chromenyl were designed and produced. Five cancer cell lines A549, MDA-MB-231 and, HepG2, HCT-11 and BT-4716 (breast cancer cell lines) were used as test subjects for the newly synthesized compounds *in vitro* cytotoxicities. Compound **61** was confirmed to be safe in normal human bronchial epithelial cells (Beas-2B) and demonstrated good anticancer activity against the MDA-MB-231 cell line with an IC_{50} value of 0.95 ±1.88 µM. The MDA-MB-231 cells were also shown to be arrested at sub-G2/M phase and to have IC_{50} value of 3.54 ±0.2 µM for tubulin polymerization inhibition, according to cell cycle analyses. To support the tubulin's powerful anticancer potential, molecular docking simulations were used to pinpoint the key binding modes responsible for the inhibitory activity of the protein.(Sigalapalli, Pooladanda, et al., 2021)

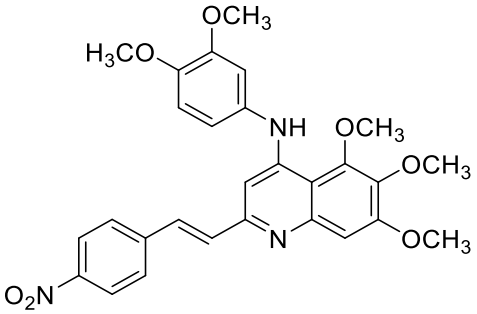


61

In 2020, a new series of styrylquinolines was designed and produced as anticancer agents and tubulin polymerization inhibitors. The *in vitro* anticancer activity of the synthesized quinolines was evaluated against four human cancer cell lines including A-2780 (human ovarian carcinoma), A-2780/RCIS (cisplatin resistant human ovarian carcinoma), MCF-7, MCF-7/MX and normal Huvec cells. In comparison to reference medication CA-4, compound **62** had higher cytotoxic action, with IC_{50} values against all four cancer cell lines ranging from 0.38 to 5.01 µM. Similar to CA4, compounds **62** and **63** suppressed tubulin polymerization. Also, molecular docking analyses of **62** and **63** into the tubulin colchicine-binding site revealed potential interactions between these molecules and tubulin.(Mirzaei et al., 2020)



62



63

Conclusion

Microtubules are well established target for anticancer drugs and an extensive research has been reported in this field. There are numerous instances of active molecules from various chemical classes that have demonstrated outstanding tubulin polymerization inhibitory activities. It is now quite straightforward to identify the necessary properties that must be present in the drug-like molecule to inhibit tubulin polymerization using the crystal structure of tubulin. Furthermore, numerous changes, such as cyclic or heterocyclic moieties, have been incorporated to improve the biological activity profile of the molecules. Microtubules have been designated as a "Privileged" target for the development of a powerful anticancer drug. Various analogues of important classes like thiazole derivatives, triazole and tetrazole derivatives, pyrazole derivatives, chalcone derivatives, may serve as a potential lead for the synthesis of clinically important candidates in near future.

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