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# A review on synthetic chalcone derivatives as tubulin polymerisation inhibitors

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**ABSTRACT**

Microtubules play an important role in the process of cell mitosis and can form a spindle in the mitotic prophase of the cell, which can pull chromosomes to the ends of the cell and then divide into two daughter cells to complete the process of mitosis. Tubulin inhibitors suppress cell proliferation by inhibiting microtubule dynamics and disrupting microtubule homeostasis. Thereby inducing a cell cycle arrest at the G2/M phase and interfering with the mitotic process. It has been found that a variety of chalcone derivatives can bind to microtubule proteins and disrupt the dynamic balance of microtubules, inhibit the proliferation of tumour cells, and exert anti-tumour effects. Consequently, a great number of studies have been conducted on chalcone derivatives targeting microtubule proteins. In this review, synthetic or natural chalcone microtubule inhibitors in recent years are described, along with their structure-activity relationship (SAR) for anticancer activity.

## 1. Introduction

Tubulin is a globular protein mainly in kinetoplastid protozoa, which consisting of the *alpha* ($\alpha$), *beta* ($\beta$), *gamma* ($\gamma$), *delta* ($\delta$), *epsilon* ($\varepsilon$), and *zeta* ($\zeta$) tubulin families[1]. Meanwhile, $\alpha$-tubulin and $\beta$-tubulin form a heterodimer, which in turn forms long-chain fibres[2]. Then 13 parallel long-chain fibres are interlinked to form a hollow tube, which is called microtubule[1,3]. Microtubules are key components of the cytoskeleton and are involved in a variety of cellular functions such as cell signalling, intracellular transport, secretion, formation and maintenance of cell shape, motor regulation, and cell division[4,5]. The heterodimers are bound to each other by non-covalent bonds, so the heterodimers can be continuously bound and separated. And the two heterodimers at the two ends of the microtubule can be continuously added and released so that the microtubule can be continuously polymerised and discrete, so there is a dynamic balance state in this process[6]. When the microtubule is in dynamic balance, one end of the microtubule will release a set of heterodimers, and the other end will bind a set of heterodimers, which can not only keep the length of the microtubule constant but also enable the microtubule to move from one side to the other, thus completing the necessary physiological function.

In mammalian cells, microtubules are closely related to cell division. Tubulin inhibitors can disturb the dynamic balance of microtubules by inhibiting the kinetic properties of microtubules. Thus, leading to the induction of cell cycle arrest at the G2/M phase and interfering with the mitotic process to inhibit cell proliferation[7]. Four targets of action have been identified in the study of microtubule proteins, which can be divided into two classes: microtubule-stabilizing binder including taxanes binding site and laurimalide binding sites; and microtubule destabilising binder including vinca alkaloids binding site and colchicine binding site. The former can promote the polymerisation of microtubule proteins, while the latter inhibits the polymerisation of tubulin, both of which can arrest the process of tumour cell growth in the cell division phase[8]. Many anti-mitotic drugs, such as vinblastine alkaloids, taxanes, and nocodazole, have been used clinically for many years[9], while problems such as drug resistance and toxic side effects remain to be solved. Thus, it is of great significance to discover and develop new tubulin polymerisation inhibitors[10,11].

Chalcone is a natural product with the structure of 1,3-diphenyl-2-propene-1-one, which can act on a variety of targets and has antioxidant, antifungal, antitumor, antibacterial, anti-inflammatory, analgesia, immunoregulation, and other biological activities[12–15]. As early as two decades ago, Edwards et al. discovered that chalcone can bind with tubulin and disrupts the formation of blood vessels around the tumour, lead to the tumour tissue with inadequate oxygen and nutrient supply, eventually lead to tumour cell death[16]. With the development of research on chalcone in recent years, it has been found that chalcone has a simple structure, easy to synthesise, and potent antitumor activity[17]. However, there are no clinical antitumor drugs with chalcone structure, so chalcone derivatives have received wide attention and are expected to advance the progress of antitumor drug research[8]. The core structure of chalcone is composed of two aromatic rings where are connected by three carbon $\alpha$, $\beta$-unsaturated carbonyl bridges (Figure 1). Due to its simple chemical synthesis

and ease of modification, most of the current studies on the structural modification of chalcone are focussed on the replacement of the two aromatic rings or the $\alpha$, $\beta$-unsaturated carbonyl bridges. In this review, we focus on the biological activities of chalcone derivatives as tubulin polymerisation inhibitors reported in recent years, classifies them according to the structure type of modified substituents, and summarise the effect of substituent groups on anticancer activity.

## 2. Simple chalcones

Chalcones are widely distributed in natural medicinal plants and generally have hydroxyl or methoxy substituents on their aromatic rings. By the Claisen-Schmidt aldol condensation of an aldehyde with a ketone, a range of chalcones and (E)-4-(4′-hydroxyphenyl)-but-3-en-2-one have been designed and synthesised by Lawrence et al.[18]. In this study, all tested compounds exhibited superior cytotoxicity ($IC_{50} < 3\,\mu M$) in human leukaemia cancer cells (K562), human breast cancer cells (MCF-7), human breast cancer cells (BT20), and various multidrug-resistant cell lines than the lead butenone **I** (Figure 2). Especially, compounds **2b**–**c** (Figure 2) displayed high toxicity against tested cancer cell lines. The structure-activity relationship studies (SARs) showed that the methyl group of **1** is replaced with a substituted aryl group and an additional substitution alternative pattern of the B ring could improve the antitumor activity. Mechanism studies showed these compounds can block cells in the $G_2/M$ phase of the cell cycle. In addition, the microtubule network in untreated cells was destroyed by

incubation with some target compounds, and **2a** (Figure 2) was the most effective inhibitor of tubulin assembly ($IC_{50} = 10\,\mu M$) in this series of compounds.

Tu et al.[19] synthesised a range of novel 2′,5′-dimethoxychalcone derivatives **3** (Figure 3) and tested their cytotoxicities against human bladder cancer cell lines (NTUB1) and human prostate cancer cell line (PC3). Most of them had strong cytotoxic effects on NTUB1 and PC3 cell lines, with the best effects of **3a** and **3b**. Interestingly, Western blot results showed that compound **3b** increased $\alpha$-tubulin levels in a dose-dependent manner, while 500 nM paclitaxel showed similar effects in NTUB1 cells. These results demonstrated that the substituted carbamoyl group on C-4 position of B-ring may be an important structural feature of the binding to tubulin. In addition, **3b** has the specificity to inhibit the growth of urinary cancer cells and minor toxicity for normal cells.

Kong et al.[20] compared the bioactivity of novel boronic acid analogs **4** (Figure 4) of chalcones with tubulin polymerisation inhibitor combretastatin A-4 (CA-4). The results showed that compound **4b** was the most potent compound in this series, and its inhibitory activity was similar to that of CA-4 in the tubulin assay. However, **4b** was 3–4 times less active against the growth of MCF-7 cells than CA-4 and was even less active in destroying the microtubules in A-10 cells. In contrast, the inhibitory activity of **4a** in colchicine binding and tubulin polymerisation was weak, whereas showed effective cytotoxic activity against MCF-7 cancer cells ($IC_{50}$, 0.9 $\mu M$). Besides, compound **4a** has a 10–200 nM inhibitory effect on 16 kinds of human cancer cell lines (e.g. colon cancer HCT-15, CNS cancer SNB-19, and ovarian cancer SK-OV-3) with $GI_{50}$ values below 10 nM. It also has a significant anti-angiogenesis effect on the human umbilical vein endothelial cells HUVEC. The SAR study showed that the carbonyl adjacent to the A-ring is more active than B-ring.

Based on the classical models of colchicine ligand and colchicine binding sites to $\beta$-tubulin, two series of chalcones were
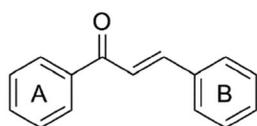


**Figure 1.** Chemical structure of chalcone.



**Butenone 1**      **2a**

2b $R_1 = R_4 = H$, $R_2 = R_3 = OCH_2CH_2O$
2c $R_1 = R_3 = R_4 = OMe$, $R_2 = H$

**Figure 2.** Simple chalcone compounds of **1** and **2**.



R = OH, $OCH_3$, $OCH_2CH_3$, $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH_2C_6H_5$, $OCH_2CH=CH(CH_3)_2$, $NHCH_3$, $NHCH_2CH_3$, $NHCH_2CH_2CH_3$, $N(CH_3)_2$, $N(CH_2CH_3)_2$,
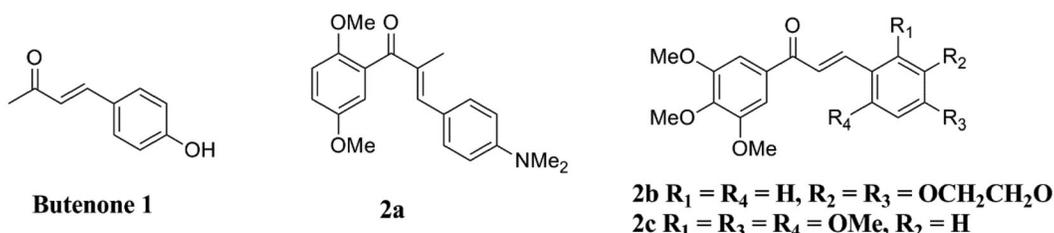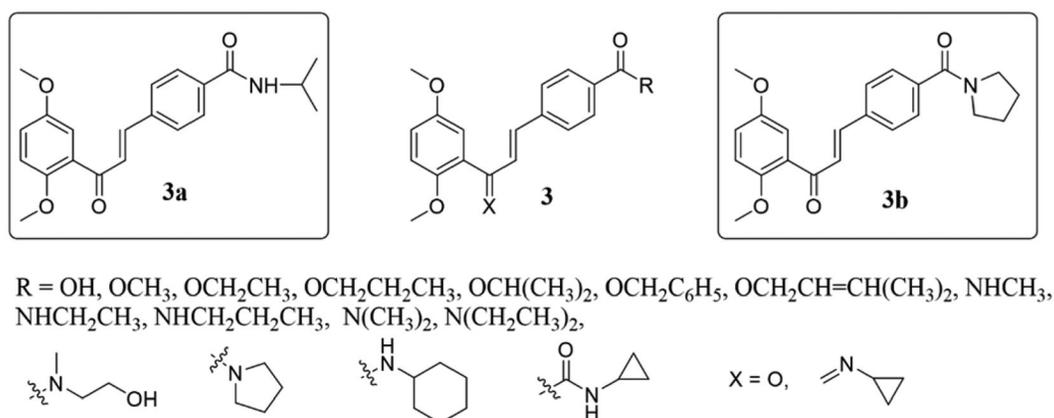
X = O,

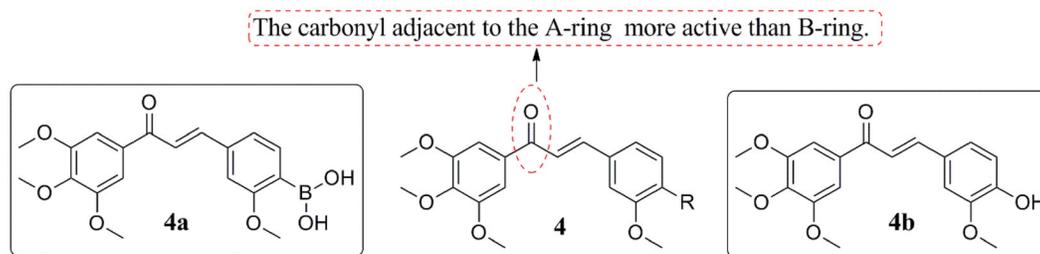**Figure 3.** Simple chalcone compounds of **3**.
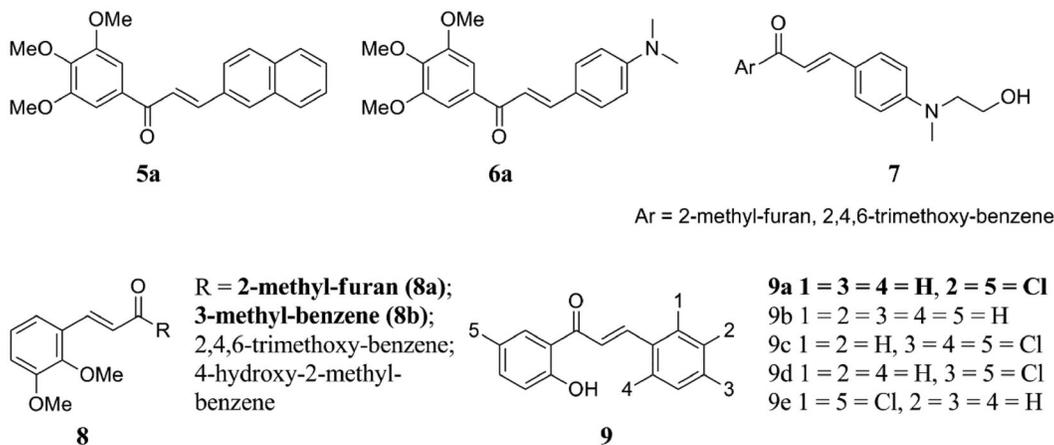
Figure 4. Simple chalcone compounds of 4.



Figure 5. Simple chalcone compounds of 5–9.

designed and synthesised, and their effects on the inhibition of tubulin assembly and toxicity in human cancer cell lines were investigated[21]. Among them, compounds **5a** and **6a** (Figure 5) inhibited tubulin assembly comparable to colchicine *in vitro* and were only one order of magnitude weaker than colchicine in human cancer cell toxicity tests. Docking studies showed that the direction of binding of the chalcone scaffold to the colchicine site of tubulin was similar to that of natural products. Particularly, the 3,4,5-trimethoxyphenyl moiety closed to the carbonyl group seems to favour the ligand's interaction with tubulin, occupying the same position as the colchicine corresponding part. Furthermore, **5a** and **6a** inhibited the growth of human leukaemia cell lines at nanomolar concentration, caused microtubule instability and mitosis arrest in human cervical cancer cells, and inhibited the migration of human breast cancer cells in scratch wounds and Boyden chamber assays.

Two series of chalcones (**7** and **8**) were evaluated for their antiproliferative inhibition and promotion of tubulin polymerisation activities[22]. Thereinto, compounds in series **8** (Figure 5) showed strong inhibitory effect on human colon cancer stage II (HT-29) cells, with $IC_{50}$ value of 19.26–36.95 µM, which was much lower than that of the control compound (153.96 µM). Especially, compounds **8a** and **8b** showed activities as inhibitors and promoters of microtubule dynamics, respectively. Meanwhile, Tajuddin and co-workers have found that compounds containing dimethoxybenzene on ring B and furan on ring A inhibited tubulin polymerisation and HT-29 cell growth.

Aryapour and co-workers prepared a series of chloro-substituted-2′-hydroxychalcones **9** (Figure 5) and tested their cytotoxic effects on K562 and human neuroepithelioma cell lines (SK-N-MC), as well as their role as inhibitors of tubulin polymerisation[23]. Compared with curcumin, they had better tubulin inhibition and cytotoxic activity against K562 and SK-N-MC. Meanwhile,

compound **9a** inhibited the assembly of the protofilaments by 89% and could bind to tubulin with a dissociation constant of 3.7 µM and change the far-ultraviolet circular dichroism spectra of tubulin. By comparing the unsubstituted analogs and chlorine-substituted analogs, it was found that the addition of chlorine-substituted analogs to the A/B ring increased the hydrophobic interaction and bioactivity of the compounds. While, the additional chlorine substitution (2-chlorine) reduces the bioactivity, which may be due to the steric hindrance of the substitution.

Several novel chalcones **10** (Figure 6) containing fluoro atom at B ring were prepared and evaluated for their antiproliferative activity against a panel of human tumour cell lines[24]. Some of them showed potent antiproliferative effects to certain human tumour cells, and compound **10a** displayed the most promising anticancer activity against most cell lines. However, none of them showed any cytotoxicity to normal human embryonic kidney cell lines (HEK 293). Acridine orange staining data indicated that these compounds could induce cytotoxicity and anti-proliferation of tumour cells by inducing apoptosis, and further studies showed that compounds **10a** and **10b** exhibited a concentration-dependent antiproliferative activity on human hepatocellular carcinoma cell lines. Meanwhile, the silico molecular docking study of the compounds on tubulin suggested that they could interfere with cell division. SAR analysis showed that the antiproliferative activity of chalcone structure can be improved by removing the ketone or double bond on the two benzene ring linking groups, and the presence of fluoro substituents in the B ring increased the antitumor potency, but at least two fluoro substituents were in the 2,5 position of the B ring.

Abosalim et al.[25] synthesised a class of new chalcone analogs **11** (Figure 7) and evaluated their anticancer activity against breast cancer and liver cancer. Several of them were revealed a broad superlative inhibitory activity against both HepG2 and MCF-7 cell
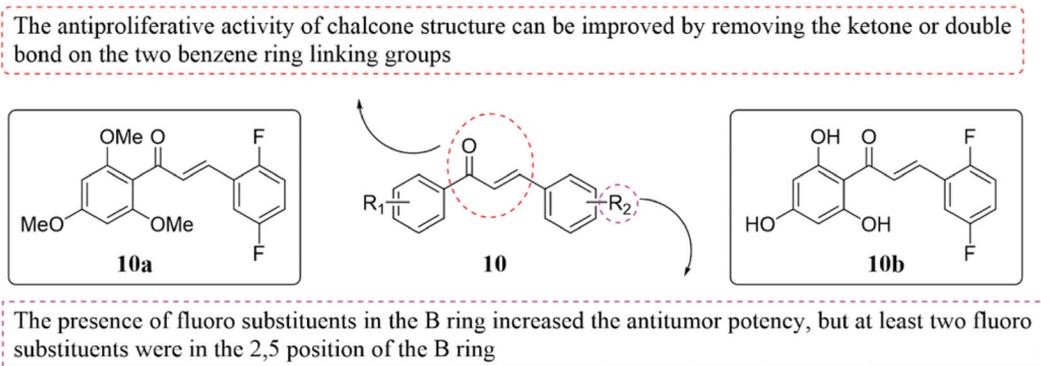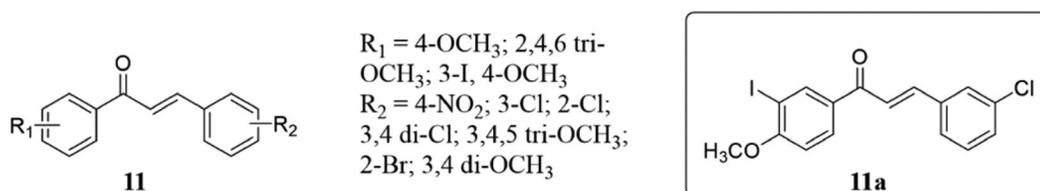
Figure 6. Simple chalcone compounds of **10**.



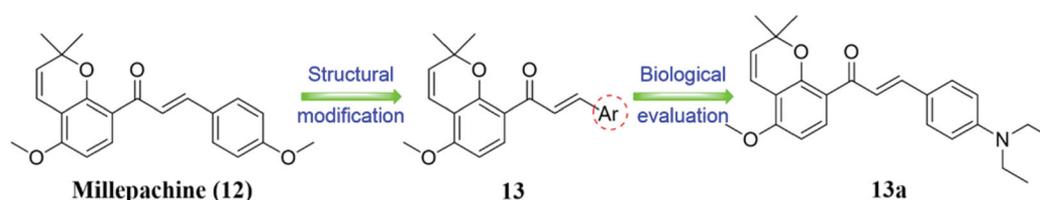Figure 7. Simple chalcone compounds of **11**.



Figure 8. Benzopyran-chalcone compounds of millepachine and **13**.

lines. Particularly, **11a** was the best compound in both MCF-7 and HepG2 cell lines (GI$_{50}$: $5.43 \pm 0.170 \,\mu M$, $1.80 \pm 0.50 \,\mu M$, respectively), besides the tubulin polymerisation inhibition with IC$_{50}$ of $4.51 \pm 0.13 \,\mu M$. Staining analysis and DNA flow cytometry indicated that **11a** arrested the cell cycle arrest in G2/M phase and stimulated apoptosis by effectively inhibiting microtubule polymerisation. In addition, molecular docking results showed that **11a** interacts with various amino acid residues at tubulin binding sites, indicating that **11a** has a good binding pattern.

## 3. Aromatic ring modifications

### 3.1. Benzopyran-chalcones

Millepachine (**12**) is a bioactive natural product with the structure of benzopyran (Figure 8), which exhibits significant potential for cancer treatment[26,27], and its derivatives have the potential to become new anticancer agents. In 2012, Wang et al.[28] designed and synthesised a novel series of millepachine derivatives **13**, and evaluated for their *in vitro* and *in vivo* antiproliferative activity (Figure 8). Among them, compound **13a** with 4-diethylamino substitute on the B ring was found to be superior antiproliferative activity than millepachine against HepG2, K562, human ovarian cancer cells (SKOV3), HCT116, HT29, and SW620 tumour cells (mean IC$_{50}$ = 0.64 *vs.* 2.86 $\mu M$, respectively). In addition, **13a** could significantly suppress tubulin aggregation in HepG2 cells and arrest the HepG2 cell cycle in the G2/M phase in a concentration-dependent manner. *In vivo* studies demonstrated that **13a** could significantly inhibit the growth of tumour, and its anticancer effect

was stronger than millepachine and cisplatin. SAR analysis showed that the introduction of EWG into the right phenyl ring was detrimental to anti-tumour activity, while the introduction of dialkyl-amine groups into the para-position of the right ring remarkably improved the anti-tumour activity. Besides, the introduction of electron-donating groups (EDG) at para-position was more beneficial to inhibit the activity than that of EWGs.

Duo to the poor solubility and low bioavailability (7.08%) of compound **13a** possibly limited the druggability. To obtain new tubulin inhibitors with better antiproliferative activity and oral bioavailability, a range of novel millepachine derivatives **14** (Figure 9) were designed and synthesised based on the SAR of compound **13a**[29]. All newly synthesised compounds showed strong tubulin polymerisation inhibition and anti-proliferation activities. Especially, compound **14a** exhibited the most potent inhibition activity which inhibited the growth of HepG2, A549, human malignant melanoma (A375), human hepatocellular carcinoma (SMMC-7221), and K562 cancer cell lines with IC$_{50}$ values in the range of 0.15–0.52 $\mu M$. In addition, compound **14a** inhibited the G2/M phase of the cell cycle and tubulin aggregation. A molecular docking study suggested that compound **14a** binds into the colchicine binding site of tubulin. Together, these findings suggested that **14a** was a promising new anti-mitotic compound that may be used in the treatment of cancer. Importantly, oral efficacy results show that **14a** displays modest to excellent oral bioavailability (oral bioavailability of 26.25% at a dose of 12 mg/kg) as compared to **13a** (oral bioavailability of 7.08% at a dose of 12 mg/kg).

Wang and colleagues also synthesised a series of novel pyrano chalcone derivatives containing indole moiety **15** (Figure 10) and
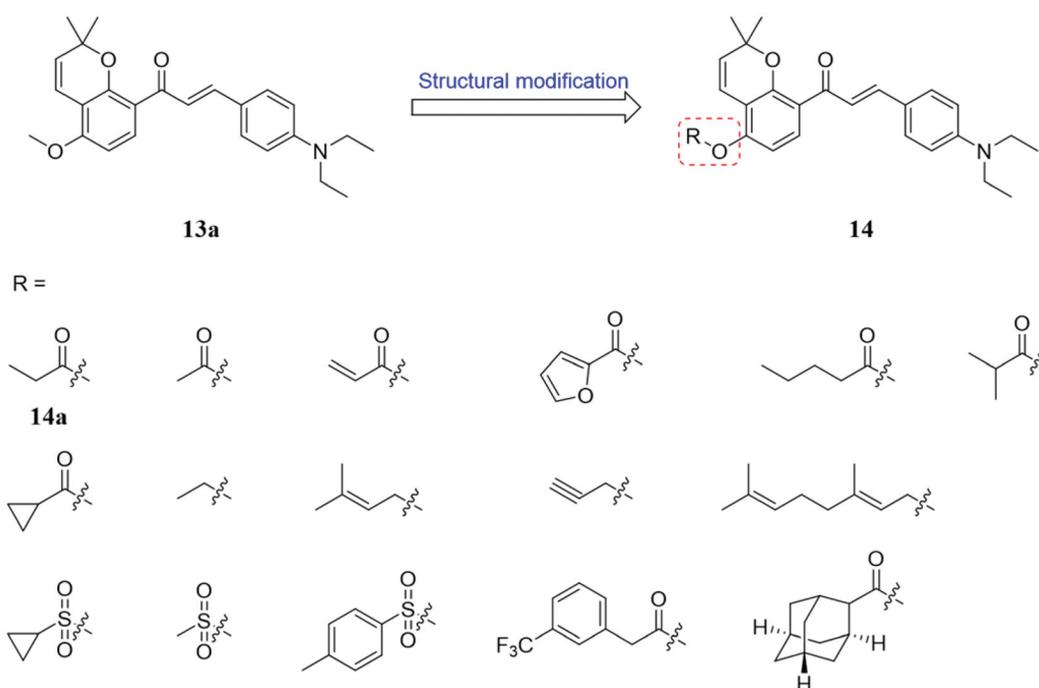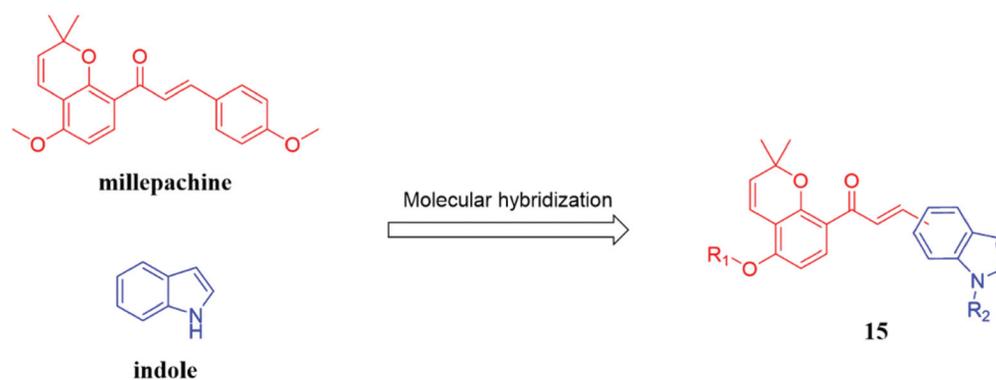
**Figure 9.** Benzopyran-chalcone compounds of **14**.



**Indole position** = 3, 4, 5, 6, 7
**R₁** = Propionyl (**15a**), acetyl, isobutyryl, pentanoyl, ethyl, isopropyl, 3,5-difluorobenzenesulfonyl, 4-bromo-2-(trifluoromethyl)benzenesulfonyl, pentafluorobenzenesulfonyl, n-propyl, n-butyl, 4-methylbenzenesulfonyl, 3-methoxybenzenesulfonyl, 4-isopropylbenzenesulfonyl, n-amyl, 3-(trifluoromethyl)benzenesulfonyl, 2-methoxy-4-nitrobenzenesulfonyl, isobutyl
**R₂** = H, methoxymethyl, methyl (**15a**), ethyl, isobutyl, prenyl, benzyl, 2-morpholinoethyl, 2-bromobenzyl 3-fluorobenzyl, 4-fluorobenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 4-trifluoromethylbenzyl 3-bromobenzyl, 4-bromobenzyl, 2-trifluoromethylbenzyl, 3-trifluoromethylbenzyl, 2-fluorobenzyl

**Figure 10.** Benzopyran-chalcone compounds of **15**.

evaluated their antiproliferative activities[30]. Most of them displayed moderate to strong cytotoxic activity against HepG2 cancer cell lines. Thereinto, compound **15a** with a propionyloxy group at the 4-position of the left phenyl ring and *N*-methyl-5-indoly on the right ring displayed the most potent cytotoxic activity against SMMC-7221, HepG2, PC-3, A549, K562, HCT116, SKOV3, MCF-7, vincristine resistant HCT-8 (HCT-8/V) and taxol resistant HCT-8 (HCT-8/T) with IC$_{50}$ values ranging from 0.22 to 1.80 μM and low cytotoxicity on the normal human cell line Lo2. In addition, **15a** was found to significantly induce cell cycle arrest in the G2/M phase and suppressed the polymerisation of tubulin. Docking studies showed that the interaction of **15a** at the colchicine binding site

of tubulin. SAR analysis indicated that *N*-methyl-5-indoly on the right ring and propionyloxy group at the 4-position of the left phenyl ring could increase the cytotoxicity.

In the same year, Yang et al.[31] synthesised a series of novel millepachine derivatives and their anti-proliferation activities *in vitro* were evaluated. Among them, the IC$_{50}$ value of compound **16** (Figure 11) on three cancer cells (HepG2, A375, and K562) was 163–253 times higher than that of millepachine, and the IC$_{50}$ value was between 8 nM and 26 nM. Preliminary studies on the mode of action showed that **16** induced cell accumulation in the G2/M phase of the cell cycle and induced apoptosis of HepG2 cells, and it was not a substrate for the glycoprotein drug pump,
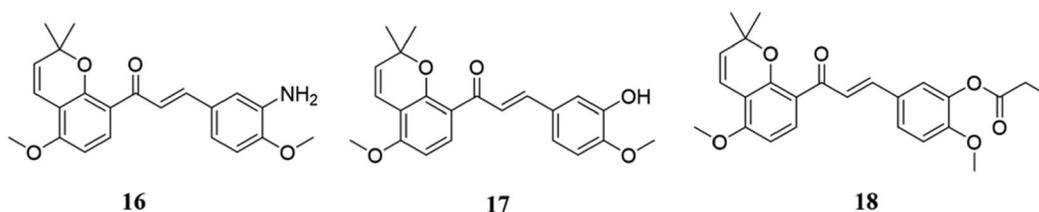
Figure 11. Benzopyran-chalcone compounds of 16–18.



Figure 12. Naphthalene-chalcone compounds of 19 and 20.

nor was it affected by the $\beta$-tubulin III gene. Compound **16** also exhibited anti-vascular activity. Microtubule dynamics confirmed **16** could bind to the colchicine site. Moreover, the hydrochloride salt of **16** (**16**·HCl) effectively increased the bioavailability up to 47% and maintained the antiproliferative activity. Importantly, **16**·HCl was a promising oral anticancer drug because **16**·HCl significantly inhibited tumour growth in four xenograft tumour models (including drug-resistant tumour-bearing mice) without causing significant weight loss in mice.

Through modification of millepachine, a new class of chalcone tubulin inhibitors **17** (Figure 11) were designed and synthesised[32]. Compounds **16** and **17** showed potent and similar growth inhibition against both sensitive and multidrug drug-resistance (MDR)1 or multidrug resistance-associate protein (MRP)1 (A2780/T, HCT-8/T and HCT-8V) cell lines, with a resistance factor ranging from 1.5 to 2.5. By biotinylation of millepachine and pull-down experiments, Yang et al. finally identified $\beta$-tubulins as cellular targets for millepachine and its derivatives and showed that these compounds bind to colchicine sites in an irreversible manner. Besides, since chalcone occupies colchicine in the trans-conformation (s-trans), naturally occurring chalcone with the s-trans conformation was more active.

Many novel millepachine derivatives induce apoptosis in the double-digit nanomolar range of multiple tumour cell lines by attacking microtubules[33]. Among them, compound **18** (Figure 11) showed strong antitumor activity against 21 tumour cell lines including multi-drug resistant phenotypes, with $IC_{50}$ values in the range of 0.09 to 1.30 μM. Moreover, **18** could induce G2/M phase cell cycle arrest, promote tubulin aggregation into microtubules, and stabilise the microtubule. Besides during in vivo study, compound **18** exhibited significantly inhibitory activity in human HepG2 xenograft tumour models. Thus, as a new microtubule stabiliser, **18** has good antitumor activity.

## 3.2. Naphthalene-chalcones

The naphthalene ring system is found widely in natural products and has a variety of biological activities, including antitumor[34], anti-inflammatory[35], antibacterial[36], antifungal[37], and antioxidant[38]. Notably, a large number of naphthalene-substituted chalcones derivatives have also been reported in recent years due to their good biological activity, which prompted researchers to study naphthalene-substituted chalcones in the hope of obtaining compounds with strong anti-tumour activities.

In 2014, Lee et al.[39] synthesised a group of methoxylchalcones and discussed the mechanisms by which these compounds inhibit tumour growth. Among them, the newly synthesised methoxychalcone HMNC-74 **19** (Figure 12) showed the best clonogenicity inhibitory effect on SW620 colon cancer cells. Further study showed that **19** can inhibit tubulin polymerisation and trigger mitotic arrest, in turn causing both p53-dependent and caspase-2-mediated apoptosis.
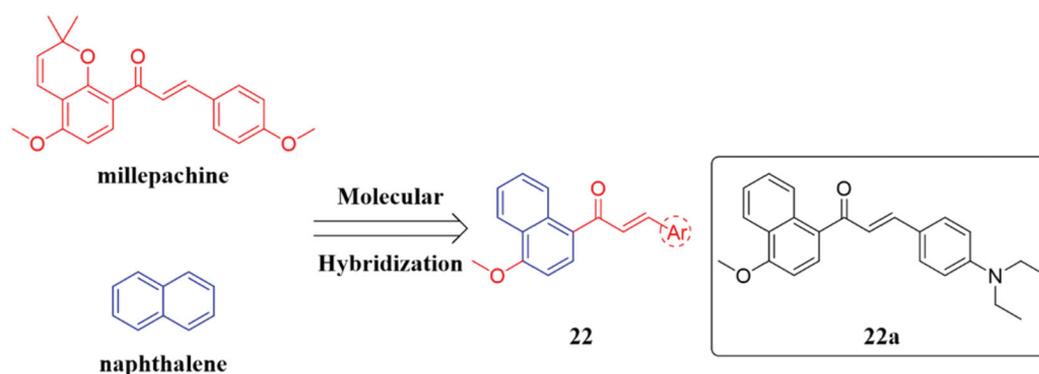
To study the antitumor mechanism of benzochalcone derivatives, Shin et al.[40] synthesised a range of benzochalone derivatives. In this study, 2-hydroxy-4-methoxy-2′,3′-benzochalcone (HymnPro) **20** (Figure 12) showed the best activities against the clonogenicity of Capan-1 human pancreatic cancer cells, and also suppressed cell proliferation in some human solid tumour cell lines as well as inhibited tumour growth in nude mice. The molecular mechanisms showed that compound **20** exerts antitumor activity by disrupting microtubule assembly, which leads to mitotic arrest and sequential activation of the caspase pathway, resulting in apoptosis. In 2016, Lee and colleagues observed that treatment CaPan-1 pancreatic cancer cells with **20** resulted in a dose-dependent accumulation of ROS due to intracellular glutathione depletion[41]. Meanwhile, N-acetylcysteine, a ROS scavenging agent, can inhibit Hymnpro-induced caspase activation and cell death, but G2/M cell cycle arrest and microtubule assembly were not significantly affected.

Based on the lead compound HMNC-74 (**19**), Wang et al.[42] prepared a new class of naphthalene-chalcone derivatives **21** (Figure 13) and evaluated their inhibitory activities against breast cancer MCF-7 cell line. Most of them show strong antiproliferative activity. Particularly, compound **21a** was the most active with an $IC_{50}$ value of 1.42 ± 0.15 μM, as superior to cisplatin ($IC_{50}$ = 15.24 ± 1.27 μM). In addition, **21a** showed lower cytotoxicity to normal cells (HEK 293) than tumour cells. Besides, **21a** could significantly induce cell cycle arrest at the G2/M phase and cell apoptosis. Compound **21a** showed a strong tubulin polymerisation inhibition activity with an $IC_{50}$ value of 8.4 μM, which was slightly higher than that of colchicine ($IC_{50}$ = 10.6 μM). Docking studies indicated that **21a** interact and bind at the colchicine binding site of the tubulin.

**Figure 13.** Naphthalene-chalcone compounds of **21**.
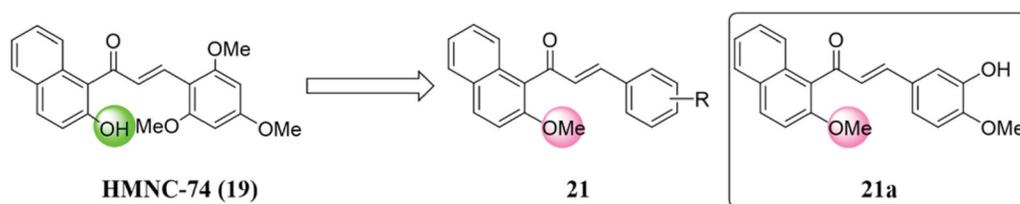
Ar = **3-hydroxy-4-methoxyphenyl (21a)**, 4-bromophenyl, 2,3,4-trimethoxyphenyl, 20bromophenyl, naphthalen-1-yl, naphthalen-2-yl, 3-bromo-4-methoxyphenyl, 3-methoxyphenyl, 4-methoxy-3-nitrophenyl, thiophen-2-yl, 4-chlorophenyl, 2,4,5-trimethoxyphenyl, 2-fluorophenyl, phenyl, 3-fluorophenyl, 3,4,5-trimethoxyphenyl, 2-methoxyphenyl, 4-(dimethylamino)phenyl, 4-(diethylamino)phenyl, 3-amino-4-methoxyphenyl



**Figure 14.** Naphthalene-chalcone compounds of **22**.

Ar = **4-(diethylamino)phenyl (22a)**, 4-chlorophenyl, 3-chlorophenyl, 4-bromophenyl, 3-bromophenyl, phenyl, 4-(dimethylamino)phenyl, 4-methoxyphenyl, 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 2-methoxyphenyl, naphthalen-2-yl, naphthalen-1-yl, 4-methoxy-3-nitrophenyl, 2-chlorophenyl, 2-bromophenyl

To discover novel tubulin polymerisation inhibitors, Wang et al.[43] designed and synthesised a hybrid scaffold by incorporating millepachine with naphthalene moiety in a single molecule **22** (Figure 14). These compounds were evaluated for their *in vitro* anticancer activity on selected human cancer cell lines. Compared with the lead compound millepachine, compound **22a** showed the most effective anti-tumour activity against human colon cancer cells (HCT116) and human hepatocellular liver carcinoma cells (HepG2) with $IC_{50}$ values of 1.20 and 1.02 $\mu$M, respectively. Furthermore, tubulin polymerisation experiments showed that **22a** could effectively inhibit tubulin polymerisation, and flow cytometry indicated that **22a** could inhibit the activity of HepG2 cells in the G2/M phase in a dose-dependent manner. SAR analysis summarised that the change of substituent affects anticancer activity. First, introducing the electron-withdrawing group (EWG) into the meta position of the benzene ring significantly increased its anticancer activity, while moving the EWG to the ortho or para-position destroyed its anticancer activity. Additionally, the introduction of methoxy in the phenyl ring can improve the biological activity, but too much is not good for the antitumor activity. Furthermore, molecular docking studies suggested that **22a** binds to the colchicine binding site of tubulin.

Many previous studies have indicated that the 3-amino-4-methoxy phenyl moiety is an essential pharmacophoric group for binding to the colchicine site of tubulin and enhance antiproliferative activity[44–47]. Based on the structure of compound **22a**, Wang et al. design and synthesis a novel series of aminochalcones by

the introduction of 3-amino-4-methoxyphenyl moiety into the molecules (Figure 15)[48]. The antiproliferative activity of this series of amino-chalcone derivatives **23** against HepG2 and HCT116 human cancer cell lines was evaluated. Most of the newly synthesised compounds showed moderate to strong antiproliferative activity against tested cancer cell lines. Among the derivatives, compound **23a** had the strongest inhibitory activity against HepG2 and HCT116 with $IC_{50}$ in the range of 0.15–0.34 $\mu$M. And this compound showed low cytotoxicity on normal human cell lines (Lo2). Compared with colchicine ($IC_{50} = 9.0 \mu$M), compound **23a** exhibited concentration-dependent tubulin assembly with $IC_{50}$ value of 7.1 $\mu$M *in vitro*. The further biological evaluation showed that compound **23a** could arrest the cell cycle in the G2/M phase. SAR analysis indicated that the antiproliferative activity of the amino group was significantly reduced when the alkyl substituents were introduced into the amino group and the normal or branched-chain alkyl substituents were introduced into the phenyl ring amino group. Docking study demonstrated the interaction of compound **23a** at the colchicine binding site of tubulin.

## 3.3. Benzofuran-chalcones

Furan structures are widely found in bioactive compounds and possess a wide range of biological activities such as antitumor[49], anti-inflammatory[50], antifungal[51], anti-tuberculosis[52], histamine antagonism[53], anti-bacterial[54–56]. Because of such a wide range of pharmacological activities of furans, researchers introduced them
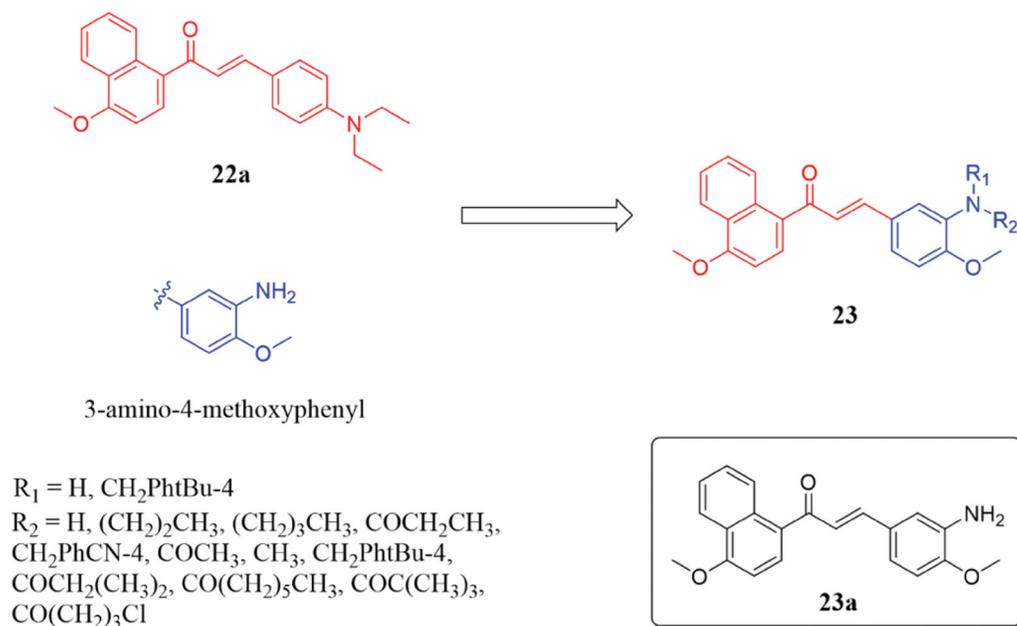
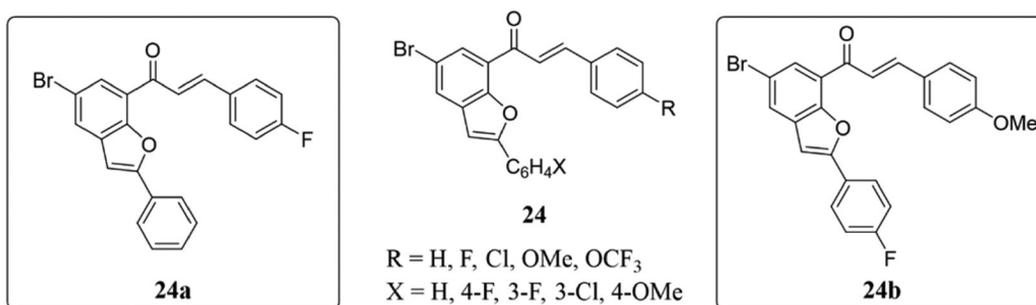Figure 15. Naphthalene-chalcone compounds of 23.



Figure 16. Benzofuran-chalcone compounds of 24.

into the chalcone molecule to modify the structure to obtain derivatives with better antitumor activity.

To investigated the antiproliferative activities against MCF-7 cell line and the potential to induce apoptosis and also to inhibit tubulin polymerisation and/or epidermal growth factor receptor-tyrosine kinase (EGFR-TK) phosphorylation, Mphahlele et al. synthesised a range of 2-arylbenzo[c]furan-chalcones 24 (Figure 16)[57]. Compared with control actinomycin D, several of them showed moderate to significant antigrowth effects on MCF-7 cell lines in vitro. Thereinto compounds 24a and 24b displayed the strongest activities (IC$_{50}$ = 3.55 × 10$^{-4}$– 0.79 µM). Docking studies showed that the compounds have the potential to inhibit tubulin polymerisation and EGFR-TK phosphorylation. Besides, the model structures of 24a and 24b suggested hydrophobic interactions and the bonding of hydrogen and/or halogens to protein residues, which may contribute to the increased binding affinity of both receptors and their significant antigrowth effects on MCF-7 cell lines.

## 3.4. Indole-chalcones

Indole ring is a very important heterocyclic, widely exists in active natural products and synthetic compounds, such as strychnine and Vinblastine[58,59]. Moreover, indole is often used as a component of many natural products and drugs and attracted wide attention for its anti-inflammatory[60], antifungal[61], antibacterial[62], antitumor, and other biological activities[63].

Mirzaei et al. have demonstrated that a series of indole-derived methoxylated chalcones 25 (Figure 17) as anti-dermatophyte agents[64]. Most of the compounds showed strong antifungal activity in the antifungal susceptibility tests of different dermatophytes in vitro. Particularly, the 4-ethoxy analog 25a with minimum inhibitory concentration (MIC) values of 0.25–2 µg/mL was the most active against Trichophyton interdigitale, Trichophyton veruccosum and Microsporum fulvum. Furthermore, the 4-butoxy compound 25b showed the most potent activities against Trichophyton mentagrophytes, Microsporum canis, and Arthroderma benhamiae (MIC values of 1 − 16 µg/mL). Model docking assay showed that compound 25a could bind with tubulin, and by molecular dynamics (MD) simulation, the interaction between compound 25b and the active site of the target protein was stable.

Boumendjel et al. reported that compound 26 (Figure 18) had the potential to treat the tumours of the central nervous system (CNS)[65]. In the four human cell lines (U118, U138, U373, and LN229) and one mouse glioblastoma cell line (GL26), compound 26 suppressed proliferation and arrested cells in the M phase of the cell cycle at the concentration of 10 µM. The mechanism of action shows that this compound is a microtubule depolymerising agent, which binds to the colchicine binding site of tubulin with an association constant of 2 × 10$^5$ M$^{-1}$. Compound 26 also suppressed the activity of the P-glycoprotein (P-gp/ABCB1) and the breast cancer resistance protein (BCRP/ABCG2), without being a
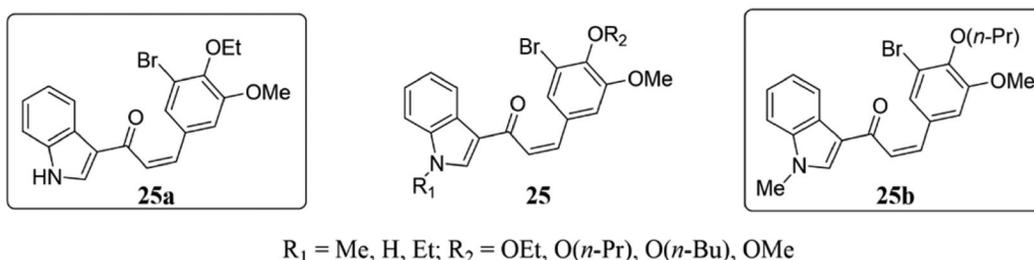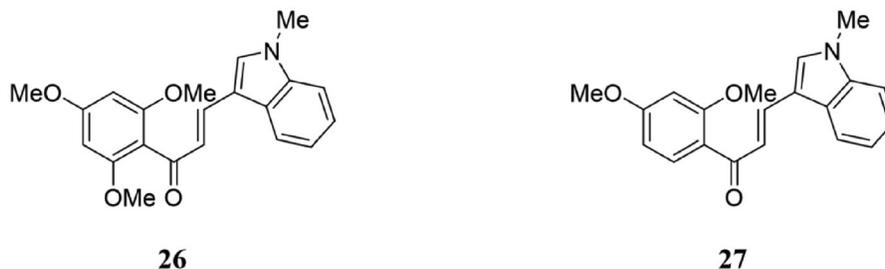
Figure 17. Indole-chalcone compounds of 25.



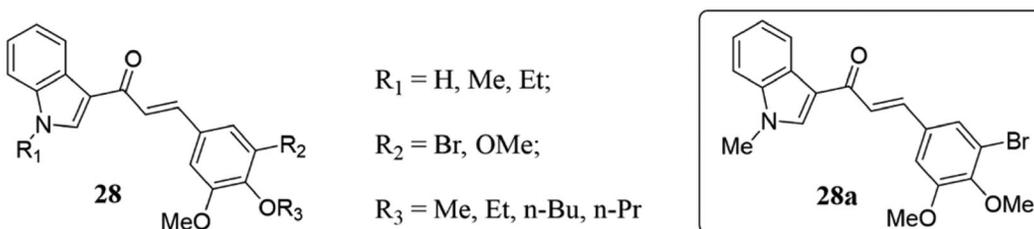Figure 18. Indole-chalcone compounds of 26 and 27.



Figure 19. Indole-chalcone compounds of 28.

substrate of these efflux pumps. Moreover, 26 was found to induce the delay of tumour onset and inhibition of tumour growth *in vivo* studies and was detected in tumours of treated animals by the high-performance liquid chromatography method (HPLC). Besides, 26 is also able to cross the blood-brain barrier (BBB).

Chalcone derivative 27 (Figure 18) is considered a potential anticancer drug because of its ability to kill bladder cancer cells through caspase-dependent apoptosis following cell cycle arrest at the prometaphase stage. Studies have shown that 27 can inhibit the proliferation of bladder and cervical cancer cells without toxicity to normal urothelial and pulmonary cells, which prompted the authors to further investigate the characteristics of 27 and to identify its cell targets[66]. They demonstrated that 27 selectively inhibits tumour-derived cell proliferation and interferes with spindle formation and mitotic chromosomal alignment. In cells, 27 induced an increase in soluble tubulin and showed anti-angiogenic activity. The results of docking studies displayed that the indole group of 27 could be adapted to the colchicine binding site of tubulin, and it could compete with colchicine for soluble tubulin. Furthermore, in a human bladder xenograft mouse model, 27 inhibited tumour growth and was non-toxic to normal cells. In summary, 27 was a novel microtubule-targeting drug with potential chemotherapeutic value.

Because of the importance of 3-bromo-3,5-dimethoxyphenyl as a fragment of tubulin inhibitor, Hassan and co-workers prepared new indole-derived chalcones 28 (Figure 19) as tubulin-targeting antiproliferative agents[67]. Cytotoxicity tests on tumour cell lines showed that several analogs were superior to or as effective as

the control drug etoposide. Particularly, the $IC_{50}$ value of compound 28a was 4.3 μg/mL, which was stronger than etoposide in inhibiting A549 cell line with adenocarcinoma. The further biological evaluation showed that compound 28a induced apoptosis of cancer cells by inhibiting tubulin aggregation and reducing mitochondrial thiol content. The docking model with tubulin showed that 28a could bind to the colchicine binding site. SAR analysis showed that the unsubstituted indole substances were superior to *N*-methyl indole congeners in MCF-7 cells, but the pattern of functionalities on the phenyl ring would affect the cytotoxicity for A549 *N*-Substitution and SKOV3.

A class of novel chalcone derivatives containing 3′,4′,5′-trimethylyl and indole fragments 29 (Figure 20) have been obtained, and their antiproliferative activity against a panel of HeLa, human colon carcinoma (HT29), MCF-7, and human lymphoma (HL-60) cells ($3-5 \times 10^4$ cells) lines also tested[68]. The majority of the synthesised compounds showed superior activities to the reference drug. Especially, compound 29a showed the most potent antiproliferative activity against HeLa, HT29, and MCF-7 cancer cell lines, with $IC_{50}$ values of 0.37, 0.16, and 0.17 μM, respectively, and with considerably lower activity against HL-60 cells ($IC_{50}$:18 μM). In the human myeloid leukaemia U-937 cell line overexpressing human Bcl-2 (U-937/Bcl-2), the cytotoxicity ($IC_{50}$ ~1 μM) of 29a was observed by inhibiting G2-M cell cycle progression and inducing cell apoptosis. Furthermore, the antiproliferative activity of this molecule was found to be associated with the inhibition of tubulin polymerisation. SAR analysis indicated that the introduction of methoxy at the C5- or C6-position of the indole nucleus and the
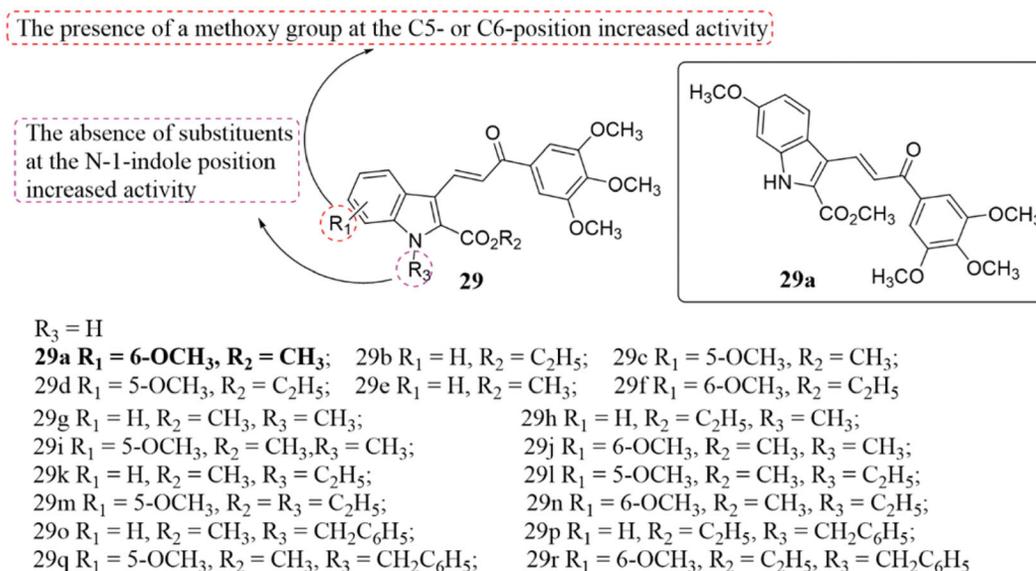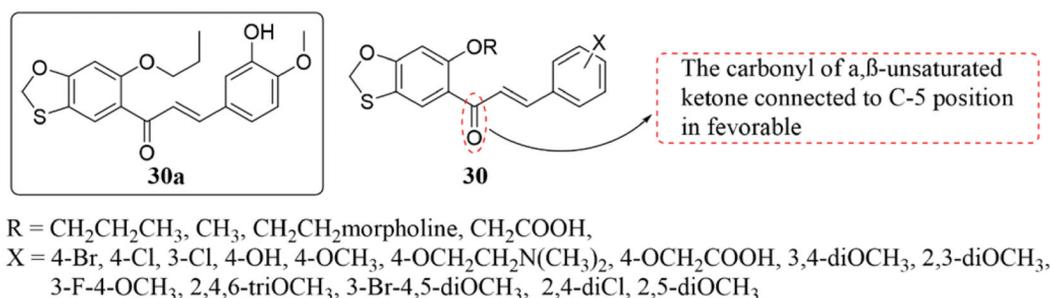
Figure 20. Indole-chalcone compounds of 29.



Figure 21. Benzoxazole-chalcone compounds of 30.

absence of substituents at the *N*-1-indole site favoured the best activity of indole-propenone-3′,4′,5′-trimethoxyphenyl scaffold.

## 3.5. Benzoxazole-chalcones

Benzoxazoles exhibit a variety of effects, and these compounds are widely used in medicine and optical materials[69]. Previous studies have indicated that benzoxazoles have a variety of biological activities such as antitumor[70,71], antiviral[72], antimicrobial[73], antifungal[74], and anti-inflammatory properties[75]. Current clinical drugs that contain this structure fragment include chlorzoxazone, benoxaprofen, and flurbiprofen[70,75].

Konieczny et al.[76] prepared a range of benzoxazole-chalcone derivatives 30 (Figure 21) and tested the antitumor activity of these compounds. Some of them demonstrated cytotoxic activity at sub-micromolar concentrations, and compound 30a showed potent activity against three tumour cell lines (A549, HCT116, and HeLa) with $IC_{50}$ values in the range of $0.0035 \pm 0.015\,\mu M$. The study on the mechanism of activity showed that these compounds interfered with tubulin polymerisation by binding to the colchicine binding site. Moreover, molecular modelling showed that compounds 30 could use two distinct poses in the colchicine binding domain of tubulins according to the substitution pattern of the ring B and alkoxy chain in position 6 of the ring A. SAR studies revealed that, in $\alpha$, $\beta$-unsaturated ketones structures, the activity of the compounds with the carbonyl group attached to the C-5 position of the benzoxazole ring is generally higher than

that of the compounds attached to the C-4 position of the compounds.

Based on previous studies on benzoxazole-chalcone, Konieczny et al.[77] oxidised the sulphur atoms in the oxazole ring to investigate *in vitro* cytotoxic activity and interaction with tubulin of these compounds (31 and 32) (Figure 22). The majority of them displayed cytotoxic activity at submicromolar concentrations, particularly, 31a ($IC_{50} = 0.006\,\mu M$) and 32a ($IC_{50} = 0.003\,\mu M$) showed the strongest cytotoxic activity in A549 cells. In this study, they found that the oxidation and location of sulphur atoms in the oxathiole-fused chalcones greatly influenced the activity of these compounds. Moreover, oxidation of isomers with a sulphur atom at the para-position of chalcone carbonyl increased the activity, while oxidation of isomers with a sulphur atom at the meta-position of carbonyl decreased the activity. Meanwhile, they described that the cytotoxic activity produced by these compounds derives in part from their interaction with the colchicine binding sites of tubulin, but that the profound effect of sulphur atomic oxidation on cytotoxic activity is related only to the interaction of the compounds with tubulin.

## 3.6. Quinoline-chalcones

Quinoline is a structural fragment with strong pharmacological activity, and some compounds exhibit antibacterial, anti-inflammatory and analgesic, anticonvulsant, hypoglycaemic, antihypertensive, antitumor, and antiviral activities when introduced into quinoline[78–81]. And many structurally simple agents can also be
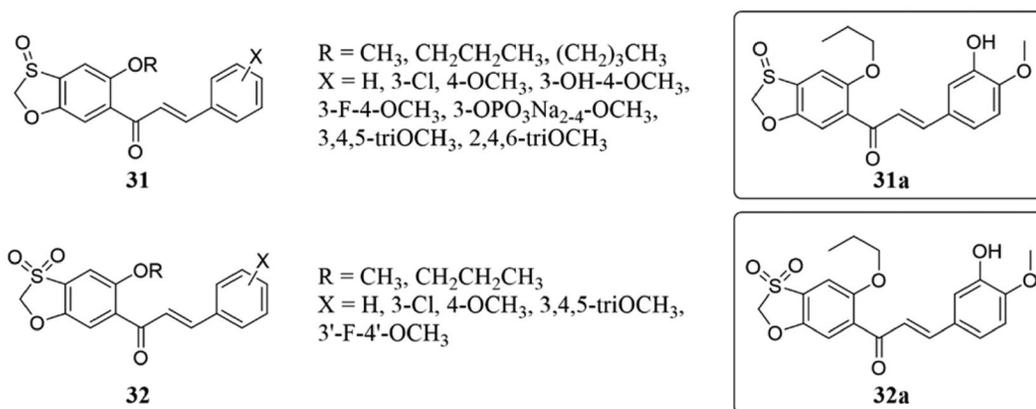
**Figure 22.** Benzoxazole-chalcone compounds of **31** and **32**.
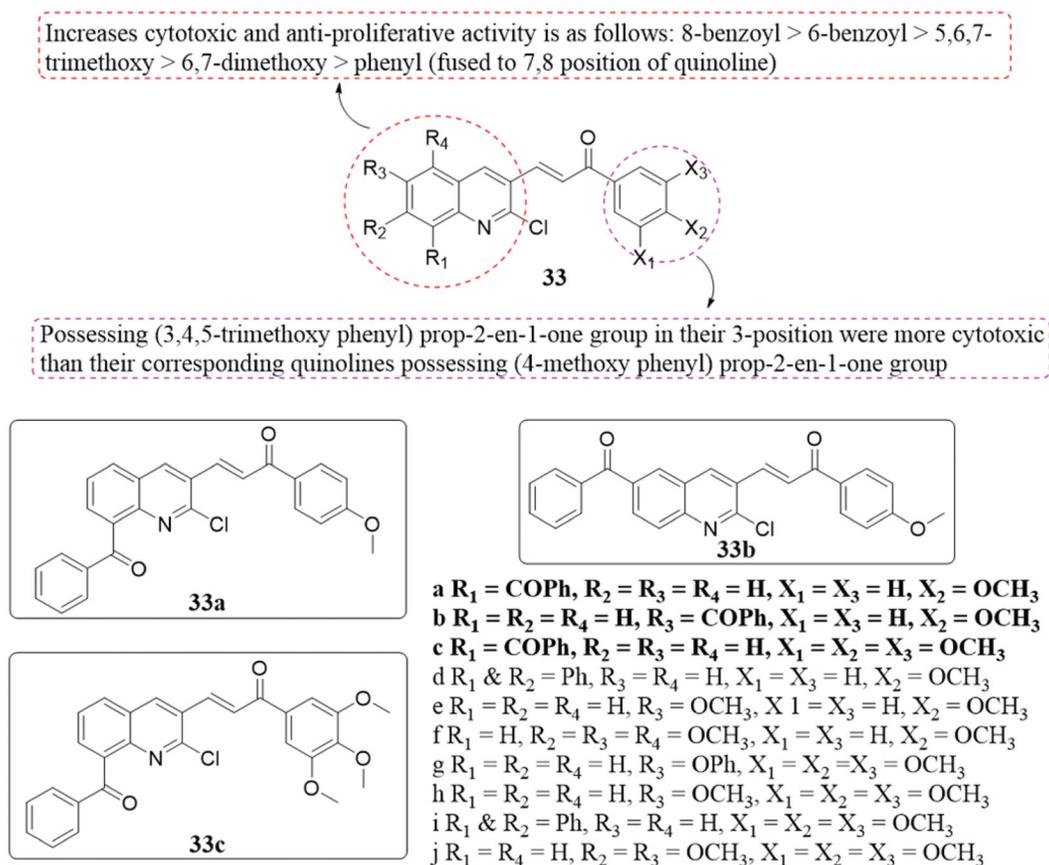


**Figure 23.** Quinoline-chalcone compounds of **33**.

modified by quinoline to increase their physiological activity and efficacy. Thus, the researchers obtained a suggestion that the introduction of quinoline into the chalcone molecule may increase the antitumor activity of chalcone. Mirzaei and co-workers synthesised a series of quinoline-chalcone hybrids **33** (Figure 23) and tested the *in vitro* anticancer activity of those compounds against four human cancer cell lines including human ovarian cancer cell (A-2780), cisplatin-resistant human ovarian cancer cell (A-2780/RCIS), MCF-7, mitoxantrone-resistant human breast cancer cells (MCF-7/MX), and normal cells (HUVEC) using MTT assay[82]. Benzoyl-containing quinolines **33a**–**33c** displayed potent activity against both resistant cancer cells and their parents. In particular, analogs **33b**–**33c** showed the strongest antiproliferative activity with $IC_{50}$ values range from 2.32 to 22.4 μM. They have also been evaluated

as tubulin inhibitors that arrested the cell cycle in the G2/M phase and induced apoptosis. SAR analysis indicated that the groups on the quinoline ring increase cytotoxicity and antiproliferative activity in the following order: 8-benzoyl > 6-benzoyl > 5,6,7-trimethoxy > 6,7-dimethoxy > phenyl (fused to 7,8 position of quinoline), and which with (3,4,5-trimethoxy phenyl) propy-2-en-1-one group is generally more cytotoxic at 3-position than the corresponding quinoline with (4-methoxy phenyl) propy-2-en-1-one group. In addition, molecular dynamics simulation and molecular docking studies on the binding site of **33c** with colchicine indicated that the compound might interact with the active site of tubulin.

Lindamulage and colleagues synthesised and tested a small chemical library of novel quinolone chalcones based on the

**Figure 24.** Quinoline-chalcone compounds of **34a-35a**.



$R_1$ = H, Ph, OCH$_3$; $R_2$ = Br, F, Cl, NO$_2$, OPh, CH$_3$, Ph, OCH$_3$; $R_3$ = H, OCH$_3$; $R_4$ = SMe, SEt
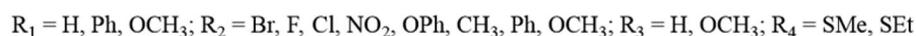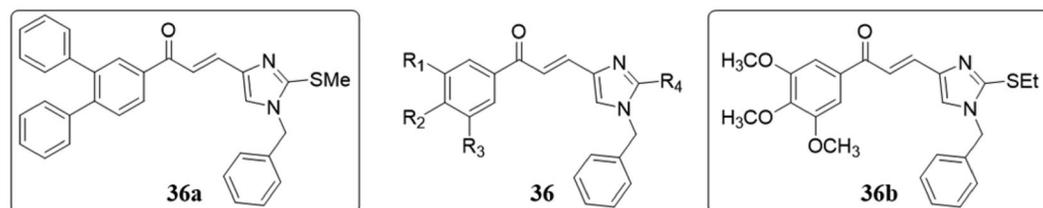
**Figure 25.** imidazole-chalcone compounds of **36**.

postulation that certain chalcones could effectively overcome multidrug resistance problems with minimal side effects[83]. They found that these compounds were generally quite effective in killing cancer cells, and they chose to study **34a** and **34b** (Figure 24) in more detail as they significantly and preferentially killed a wide range of cancer over non-cancer cells. Thereinto, both **34a** and **34b** were resistively bound to colchicine binding pouches on $\beta$-tubulin, and **34b** had strong inhibitory activity against 65 different tumour cell lines from 12 different tissues mainly in a cancer-specific manner. Interestingly, these two compounds significantly inhibited multidrug-resistant cancer cells compared with colchicine, paclitaxel, and vinblastine. Furthermore, **34b** overcame multidrug resistance by inhibiting the function of MRP1 rather than maintaining strong anti-microtubule activity. Data obtained f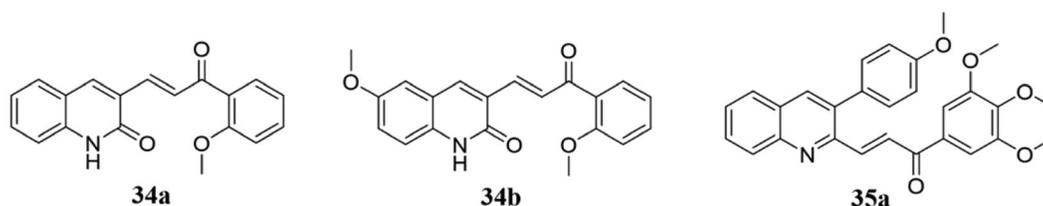rom mice engrafted with the human breast cancer cells (MDA-MB-231) triple-negative breast cancer cells exhibited that **34a** and **34b** exhibited potent antitumor activity and not causing any significant side effects, either alone or in combination with paclitaxel. As a result, these two compounds are potent and safe in the treatment of various kinds of cancers, especially multidrug-resistant tumours.

Tseng et al.[84] evaluated the antiproliferative activity of several 3-phenylquinolinyl chalcone analogs in vitro. Thereinto, **35a** (Figure 24) showed inhibitory activity against the growth of MCF-7, MDA-MB-231, and SKBR-3 with IC$_{50}$ values of 1.05, 0.75, and 0.78 μM, respectively, and no obvious cytotoxicity to normal cells. The mechanism of action of compound **35a** was further studied. It was found that **35a** induced G2/M cell cycle arrest by regulating cyclins B1, CDK1, and CDC25, inhibiting tubulin aggregation. Compound **35a** finally induced apoptosis by increasing the apoptotic protein Bax and decreasing the anti-apoptotic protein Bcl-2. Besides, compound **35a** was found to induce cell cycle arrest at the G2/M phase via cleavage of PARP, induces caspase-3 and -8 activities, and consequently cause cell death.

## 3.7. Other heterocyclic chalcones

Besides the above widely reported hybrid chalcone derivatives, researchers have also introduced pyridine, benzothiazole imidazole, and other structures into the molecular structure of chalcone, designed and synthesised many novel chalcone derivatives.

A series of novel imidazole-chalcone derivatives **36** (Figure 25) were prepared as tubulin polymerisation inhibitors and anticancer agents by Sara et al.[85] In this series, all derivatives exhibited more cytotoxicity on A549 cancer cells in comparison to the other three cell lines (MCF-7, MCF/7-MX, and HEPG2). Particularly, **36a–b** displayed potent cytotoxicity with IC$_{50}$ values ranging from 7.05 to 63.43 μM against all the four human cancer cells. By flow cytometry, they found that low concentrations of **36a–b** induced G2/M arrest in A549 cells, while high concentrations of them increased the number of apoptotic cells (cells in subG1 phase). Further experiments showed that **36b** induced apoptosis of A549 cells in a dose-dependent manner, and **36a–b**, inhibited tubulin polymerisation similar to CA-4. The molecular docking study of **36b** binding sites with tubulin colchicine indicated that this compound might interact with tubulin.

A range of benzo[d]imidazo[2,1-b]thiazole–chalcone derivatives **37** (Figure 26) was obtained and tested the anticancer potential[86]. Most conjugates showed effective activities against the tested cancer cell lines. Thereinto, **37a** and **37b** displayed potent antiproliferative effects against MDA MB-231 with IC$_{50}$ values of 1.3 and 1.2 μM respectively. SAR analysis revealed that the order of enhancement of different substituents on the A ring could be expressed as H > OMe > OEt > F > Me, while the order was expressed as 4-OMe > 3-OH-4-OMe > 3, 4-diOMe > 3, 4, 5-triOMe > 2-Br-3, 4, 5-triOMe > 3, 5-diOMe on ring D. The flow cytometric analysis results indicated that **37** could induce cell-cycle arrest in the G2/M phase, and they could effectively inhibit microtubule assembly in the tubulin polymerisation assay. Furthermore, morphological changes, reactive oxygen species (ROS) detection by 2′,7′–dichlorofluorescein diacetate (DCFDA), and annexin V-FITC/PI assays as well as revealed that **37a** and **37b** induces apoptosis.

A range of Combretastatin A-4-based chalones **38** (Figure 27) were synthesised and their antiproliferation of human lung (A549), breast (MCF-7), melanoma (A375) and colon (HT-29) carcinoma cells were examined[87]. Some of them showed significant antiproliferative activity with IC$_{50}$ < 2 μM and the compound **38a**, the best one, which showed the strongest inhibitory activities against MCF-7 (IC$_{50}$: 10 nM), A375 (IC$_{50}$: 12 nM), and A549 (IC$_{50}$: 65 nM) cell lines, and was 18 times that of CA-4. Moreover, all the tested compounds have shown stronger binding affinity than CA-4 in the colchicine binding site of tubulin dimer.
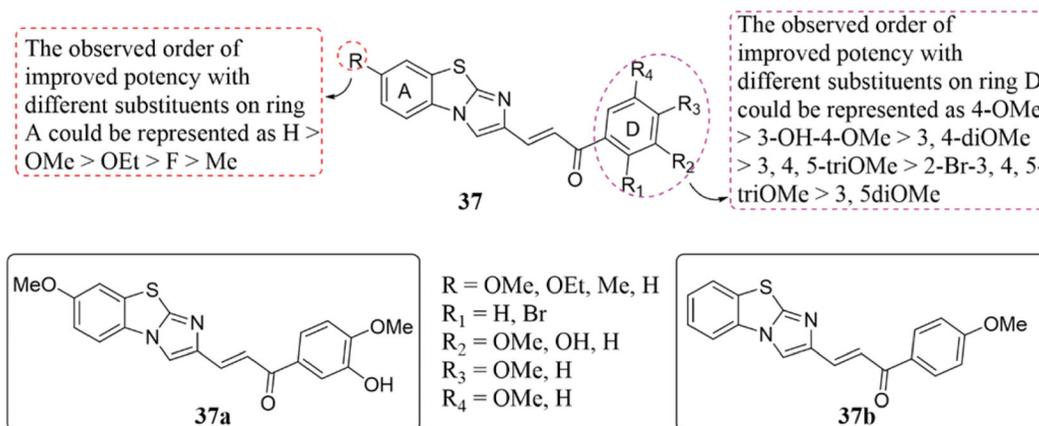
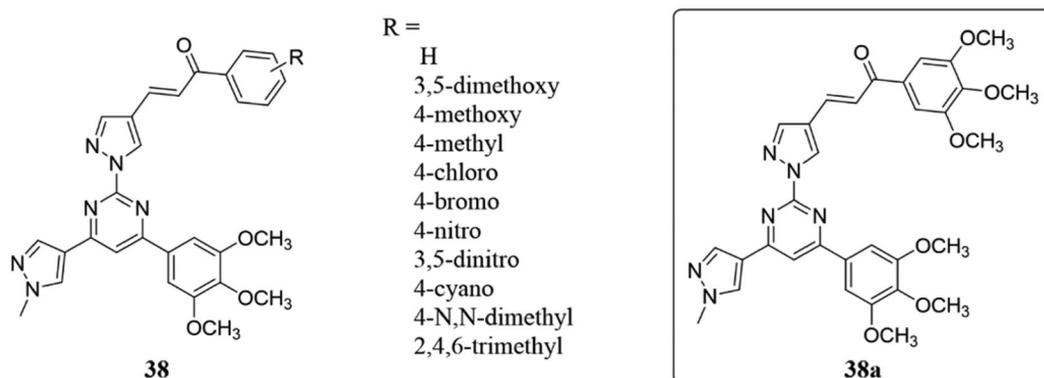**Figure 26.** Benzo[*d*]imidazo[2,1-*b*]thiazole-chalcone compounds of **37**.



**Figure 27.** Combretastatin A-4-based chalone compounds of **38**.

# 4. Modification of the α, β-unsaturated carbonyl bridges of chalcone

## 4.1. Double bond modification

The relative position of the two aryl rings in chalcone plays a crucial role in the activity of the binding tubulin, which allows the ketene bridge to be modified. It has been reported that hydrogenation or bromination significantly reduces chalcone activity through carbon-carbon double bonds or halogenation or conversion to corresponding epoxides. Therefore, the double bond in the ketene structure may be the key site affecting the bioactivity of the compound. However, when the double bond of chalcone remains unchanged, it is unstable *in vivo* due to its ability to undergo Michael addition reactions with biological nucleophiles. Thus, the researchers substituted the ketene-bridge double bond with a heterocyclic ring, which not only maintained the relative tertiary stability of the two aryl rings but also avoided nucleophilic reactions *in vivo*, thus improving the stability of chalcones[88].

Based on the crystal structure of the newly discovered tubulin inhibitor VERU-111 and its analog DJ101, Wang et al.[89] synthesised several new VERU-111 analogs **39** (Figure 28) and investigated their SAR evaluation by focussing on the 3,4,5-trimethoxyphenyl (TMP) moiety modification. In this study, the antiproliferative activity of compound **39a** with 3-methoxybenzo[4,5]dioxyclic ring in melanoma cell lines was better than that of VERU-111 with IC$_{50}$ of 1.1–3.3 μM, and without observable toxicity to normal cells. In addition, the resistance index of **39a** was significantly increased in parental and taxane resistance prostate cancer cell lines compared to paclitaxel,

and the isosteric (conformationally restricted) replacement of TMP could significantly increase the antiproliferative activity of the scaffolds. Compound **39a** inhibits tubulin polymerisation by the same mechanism as VERU-111. Overall, they demonstrated that modifying the TMP component of CBSIs in this scaffold could improve antiproliferative activity without affecting the mechanism or safety.

In 2010, Han and colleagues have been found that chalcone epoxides (A, B-epoxone) **40** (Figure 29) were the growth inhibitors of two pancreatic cancer cell lines, BXPC-3 and MIA PACA-2[90]. Three of them were active, with GI$_{50}$ values of 5.6 to 15.8 μM. The average GI$_{50}$ of compound **40a** on the NCI 60-cell-line panel was 14.1 μM, and it interfered with the enhancement effect of paclitaxel on tubulin polymerisation, suggesting that the microtubules were its active site. Furthermore, **40a** was found to block the cell cycle at the G2/M checkpoint or during mitosis leading to apoptosis. Therefore, chalcone epoxides are worthy of further study as a potential drug for the treatment of cancer.

A range of chalcone-like derivatives **41** (Figure 29) have been obtained and tested for their biological activity and mechanism[88]. All of the synthesised compounds inhibited cancer cell growth at nanomolar to low micromolar concentrations. Among them, compound **41a** had the strongest anti-proliferation activity, which inhibited the assembly and binding of colchicine and tubulin. Generally, all of them with potent antiproliferative activity inhibited tubulin polymerisation with IC$_{50}$ < 2 μM. Some of them could arrest the G2/M phase of the cell cycle of K562 cells. SAR studies revealed that using a thiophene nucleus between the aryl and 3′,4′,5′-trimethoxybenzoyl moieties of chalcones replace the
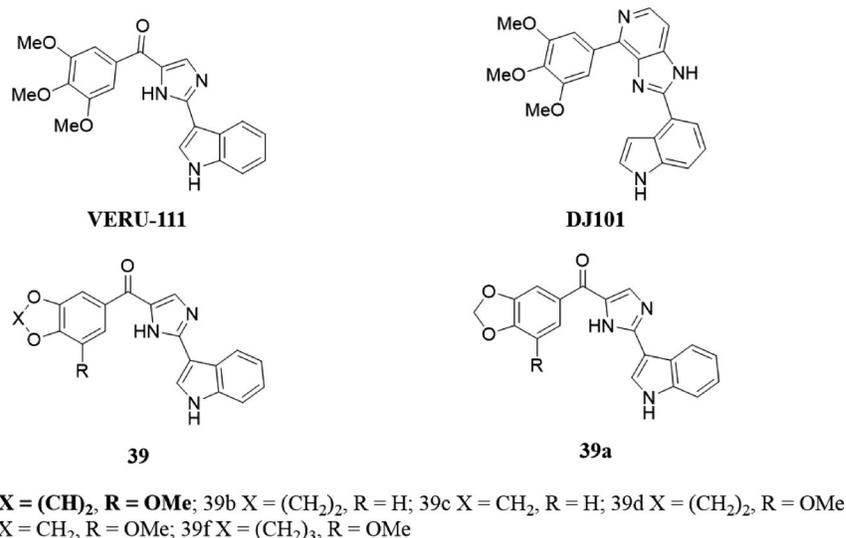
Figure 28. VERU-111 analogs of 39.
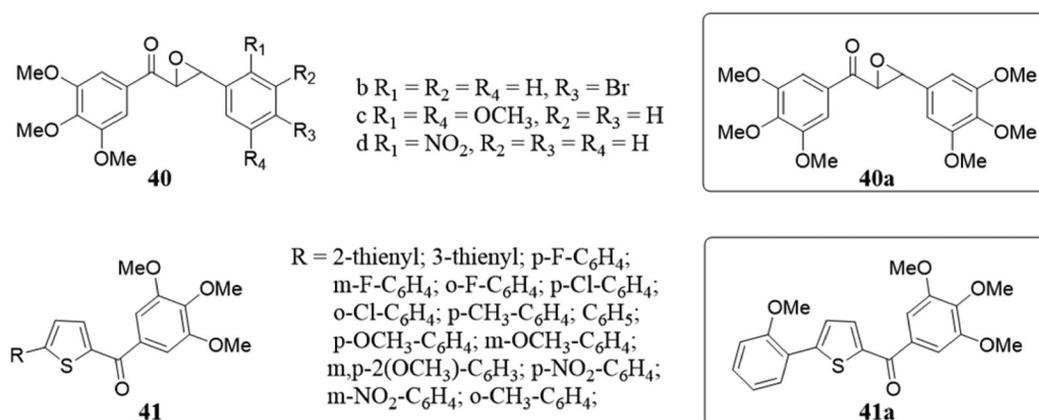


Figure 29. Chemical structure of compounds 40 and 41.

double bond of the enone system will enhance the anti-proliferation activity of these compounds.

## 4.2. Carbonyl modification

Guanidine analogs were widely reported as their significant antiviral and anticancer activities[91,92]. Methylglyoxal-bisguanylhydrazone (MAGA) has been used as a single drug in clinical therapy for malignant lymphoma, carcinoma of the head, oesophageal cancer, and non-small cell lung cancer[93]. A series of novel chalcone guanidine analogs 42 (Figure 30) were studied as part of the study of novel anti-tubulin polymerisation inhibitors[94]. Some of them exhibited remarked effects on antiproliferative activities. Compared with the positive control, compound 42a showed the strongest inhibitory activity against the growth of MCF-7 cells with $IC_{50}$ of $0.09 \pm 0.01\,\mu M$ and the polymerisation of tubulin with $IC_{50}$ of $8.4 \pm 0.6\,\mu M$. On the A ring, the SAR analysis indicated that the para-substituted compounds are more active than the ortho-substituted and meta-substituted compounds. Meanwhile, the halogen-substituted compounds on B-ring showed good antiproliferative activity, and p-Br substituted 42a showed the strongest antiproliferative activity. Besides, docking simulation revealed that 42a could bind to the colchicine site of tubulin.

Wang et al. reported a range of novel chalcone oxime derivatives 43 (Figure 31) have the activity of inhibiting tubulin polymerisation[95]. Most of them showed effective antiproliferative activity and tubulin polymerisation inhibition activity. Thereinto, substance 43a exhibited the most potent inhibitory activity (tubulin $IC_{50} = 1.6\,\mu M$) and showed significant antiproliferative activity against A549, Hela, and MCF-7 with $GI_{50}$ values of 2.1, 3.5, and $3.6\,\mu M$, respectively in the antiproliferative assay. SARs showed that compounds with the p-substituted group showed slightly more potent activities than those of o-substituted, and the more substituted groups in the A ring, the better antiproliferative activity it was. However, the antiproliferative activity of the compounds with meta-substituted in the B ring is slightly better than that of the compounds with para-substituted, and the ERG of the B ring may have better antiproliferative activity. Studies on the mechanism of action have confirmed that chalcone oxime derivatives could still inhibit tubulin aggregation at colchicine binding sites, block cells in the G2/M phase, and induce cell apoptosis.

## 4.3. Rigidified chalcone derivatives

Chalcone has an $\alpha, \beta$-unsaturated ketones with a single bond that can be rotated when a group is introduced into the $\alpha$ position of

Compounds with substitution at the para position showed more potent activities than those with substitution at the ortho and meta position in the A-ring

The compounds with para halogen substituted in the B-ring exhibited excellent antiproliferative activity, **42a** with substituent of p-Br showed the most potent activity in these compounds

**a** $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Br$; b $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = F$; c $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = Cl$; d $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = Br$; e $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = Ph$; f $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = OCH_2Ph$; g $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = Me$; h $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = OMe$; i $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = NO_2$; j $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = F$; k $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OMe$; l $R_1 = F$, $R_2 = R_3 = R_4 = R_5 = H$; m $R_1 = Cl$, $R_2 = R_3 = R_4 = R_5 = H$; n $R_1 = Br$, $R_2 = R_3 = R_4 = R_5 = H$; o $R_1 = OMe$, $R_2 = R_3 = R_4 = R_5 = H$; p $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Cl$; q $R_1 = R_2 = R_3 = R_4 = R_5 = H$
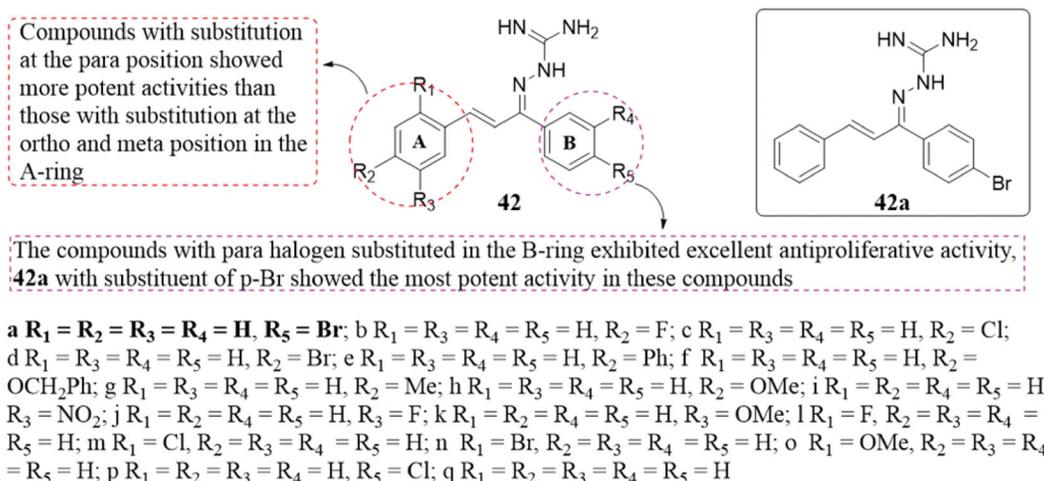
**Figure 30.** Chalcone guanidine compounds of **42**.



compounds with p-substituted group showed slightly more potent activities than those of o-substituted

ERG in B ring may have slightly improved antiproliferative activity

more substituted groups in the A ring, the better antiproliferative activity it was

the compounds with meta-substituted exhibited slightly better antiproliferative activity than para substituted compounds separately

**a** $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_7 = H$, $R_6 = NH_2$; b $R_1 = R_4 = R_5 = R_6 = H$, $R_2 = R_3 = R_7 = OCH_3$; c $R_3 = R_4 = R_7 = OCH_3$, $R_2 = R_5 = R_6 = H$; d $R_1 = R_3 = R_4 = OCH_3$, $R_2 = R_5 = R_6 = H$, $R_7 = OC_2H_5$; e $R_1 = R_3 = R_5 = R_6 = OCH_3$, $R_2 = R_4 = R_7 = H$; f $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_7 = H$, $R_6 = Br$; g $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = H$, $R_6 = R_7 = Cl$; h $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_7 = H$, $R_6 = NO_2$; i $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_6 = H$, $R_7 = Br$; j $R_1 = R_5 = R_6 = H$, $R_2 = R_3 = R_4 = OCH_3$, $R_7 = OC_2H_5$; k $R_1 = R_2 = R_4 = R_5 = R_6 = H$, $R_3 = OCH_3$, $R_7 = OC_2H_5$; l $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OCH_3$, $R_6 = R_7 = Cl$; m $R_1 = OCH_3$, $R_2 = R_3 = R_4 = R_5 = R_6 = H$, $R_7 = OC_2H_5$; n $R_1 = R_3 = R_4 = R_5 = R_6 = H$, $R_2 = R_7 = OCH_3$; o $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_6 = R_7 = H$; p $R_1 = R_3 = R_5 = OCH_3$, $R_2 = R_4 = R_6 = H$, $R_7 = F$; q $R_1 = R_3 = R_5 = R_7 = OCH_3$, $R_2 = R_4 = R_6 = H$; r $R_1 = R_3 = R_4 = OCH_3$, $R_2 = R_5 = H$, $R_6 = R_7 = Cl$
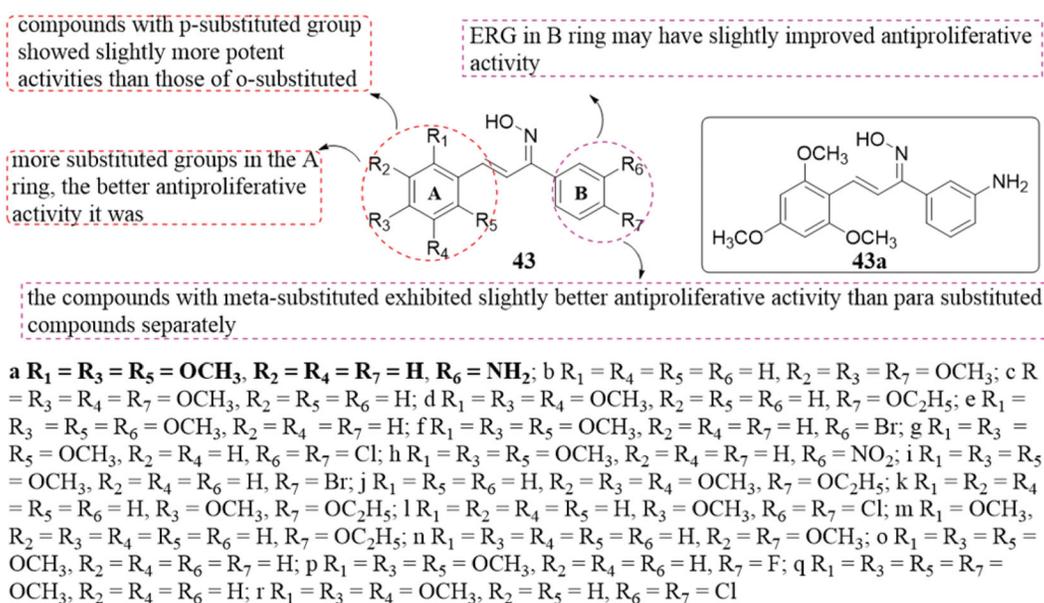
**Figure 31.** Chalcone oxime compounds of **43**.

the carbonyl group making it easier for the chalcone to maintain the trans configuration. The $\alpha$ position of the compound MDL-27048 is methyl, which is a typical alpha-substituted chalcone compound. Earlier years, Peyrot et al.[96,97] demonstrated that MDL-27048 effectively and reversibly binds to the colchicine binding site of tubulin and has antitumor activity. In addition, Lawrence et al.[98] revealed the effects of different $\alpha$-substitutions, in particular $\alpha$-fluorination, on the structure and biological activity of several chalcones **44–46** (Figure 32). In this study, x-ray analysis showed that $\alpha$-position fluorine chalcone has *s-trans* conformation, which displayed strong cytotoxicity and tubulin inhibition properties. Moreover, the 3-hydroxy-4-methoxy B-ring was important for improving biological activity, and no alternative model (including 3-fluoro-4-methoxy) could match its effect.

Kerr et al.[99] prepared a series of aryl, hydroxyl substitutions in the $\alpha$ position of chalcone, all of which showed potent inhibitory activities of tubulin polymerisation. But only compound **47** (Figure 33) had activity similar to that of CA-4 at low concentrations but did not exhibit anti-mitotic or cytotoxic effects.

Ducki et al.[100] synthesised a series of chalcone analogs and tested the inhibitory activity against the K562 human chronic myelogenous leukaemia cell line. Among the tested compounds, the most cytotoxic analogs were those most similar to the CA-4 itself, while **48** (Figure 33) with both the A- and B-ring substitutions of CA-4 was the strongest. Interestingly, compound **49** (Figure 33), in the presence of an alkyl group at the $\alpha$-position, increased its activity to 10 times that of **49**. Cell cycle analysis by flow cytometry showed that these drugs had anti-mitotic effects, and they also were found to inhibit tubulin assembly at low concentrations, and competitively binding tubulin with [³H] colchicine.

In the same year, another series of novel $\alpha$-aryl chalcones **50** and **51** (Figure 34) was be reported as mimics of podophyllotoxin by Ducki et al.[101]. These substances showed effective bioactivity, and compound **51a** exhibited the strongest activity against K562 cancer cells with $IC_{50}$ value of 12 nM, and the inhibition of tubulin was higher than that of the control. However, molecular studies have revealed that the B rings of chalcone and CA-4 do not occupy the same pockets of tubulin, which belonged to different
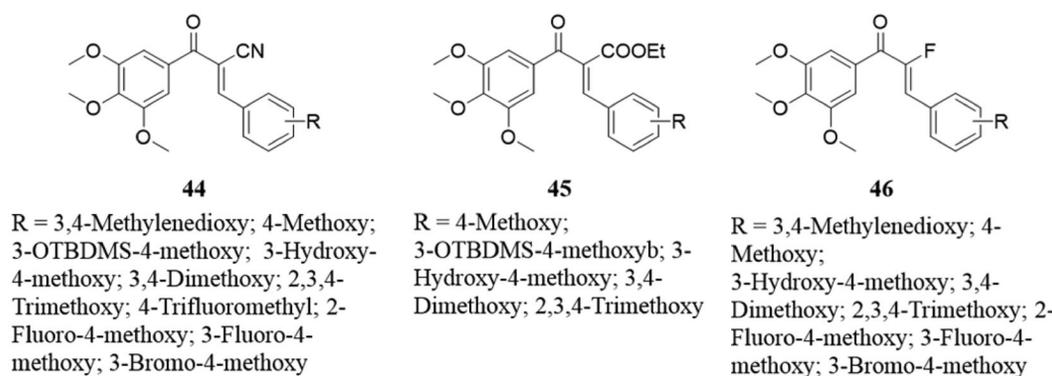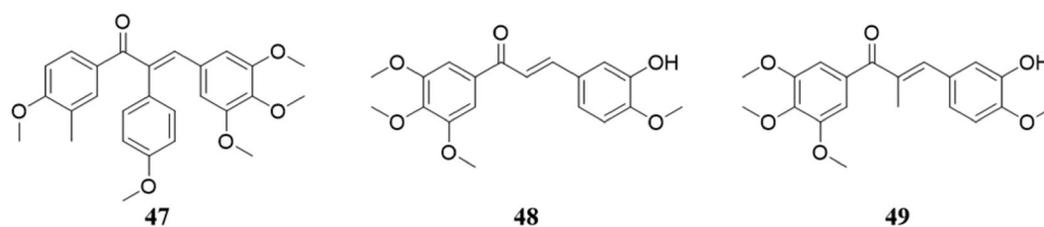
**Figure 32.** Chemical structure of compounds **44–46**.

Compound **44**: R = 3,4-Methylenedioxy; 4-Methoxy; 3-OTBDMS-4-methoxy; 3-Hydroxy-4-methoxy; 3,4-Dimethoxy; 2,3,4-Trimethoxy; 4-Trifluoromethyl; 2-Fluoro-4-methoxy; 3-Fluoro-4-methoxy; 3-Bromo-4-methoxy

Compound **45**: R = 4-Methoxy; 3-OTBDMS-4-methoxyb; 3-Hydroxy-4-methoxy; 3,4-Dimethoxy; 2,3,4-Trimethoxy

Compound **46**: R = 3,4-Methylenedioxy; 4-Methoxy; 3-Hydroxy-4-methoxy; 3,4-Dimethoxy; 2,3,4-Trimethoxy; 2-Fluoro-4-methoxy; 3-Fluoro-4-methoxy; 3-Bromo-4-methoxy



**Figure 33.** Chemical structure of compounds **47–49**.



50a R = $R_2$ = $R_3$ = $R_4$ = H, $R_1$ = OH 50b R = F, $R_2$ = $R_3$ = $R_4$ = H, $R_1$ = OH 50c R = OTBDMS, $R_2$ = $R_3$ = $R_4$ = H, $R_1$ = OH  50d R = $R_1$ = $R_2$ = $R_3$ = $R_4$ = H 50e R = $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = OCH$_3$ 50f R = $R_2$ = $R_4$ = H, $R_1$ = $R_3$ = OCH$_3$  50g R = $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = N(CH$_3$)$_2$  50h, R = $R_1$ = H, $R_2$ = $R_3$ = $R_4$ = OCH$_3$ 50i R = OH, $R_2$ = $R_3$ = $R_4$ = H, $R_1$ = OH

51b $R_1$ = $R_2$ = $R_3$ = $R_4$ = H, $R_5$ = OH, $R_6$ = OCH$_3$ 51c $R_1$ = $R_2$ = $R_3$ = $R_4$ = $R_5$ = $R_6$ = H 51d $R_1$ = $R_2$ = $R_3$ = $R_4$ = H, $R_5$ = NO$_2$, $R_6$ = OCH$_3$ 51e $R_1$ = $R_2$ = $R_3$ = $R_4$ = H, $R_5$ = F, $R_6$ = OCH$_3$ 51f $R_1$ = $R_2$ = $R_4$ = $R_5$ = $R_6$ = H, $R_3$ = CH$_3$ 51g $R_1$ = $R_2$ = $R_4$ = $R_5$ = H, $R_3$ = CH$_3$ , $R_6$ = OCH$_3$ 51h $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = CH$_3$, $R_5$ = OH, $R_6$ = OCH$_3$ 51i $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = CH$_3$, $R_5$ = NO$_2$, $R_6$ = OCH$_3$ 51j $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = CH$_3$, $R_5$ = F, $R_6$ = OCH$_3$ 51k $R_1$ = $R_2$ = $R_4$ = $R_5$ = $R_6$ = H, $R_3$ = OCH$_3$ 51l $R_1$ = $R_2$ = $R_4$ = $R_5$ = H, $R_3$ = $R_6$ = OCH$_3$ 51m $R_1$ = $R_2$ = $R_3$ = $R_4$ = $R_5$ = H, $R_6$ = OCH$_3$ 51n $R_1$ = $R_2$ = $R_4$ = H, $R_5$ = NO$_2$ $R_3$ = $R_6$ = OCH$_3$ 51o $R_1$ = $R_2$ = $R_4$ = H, $R_5$ = F, $R_3$ = $R_6$ = OCH$_3$
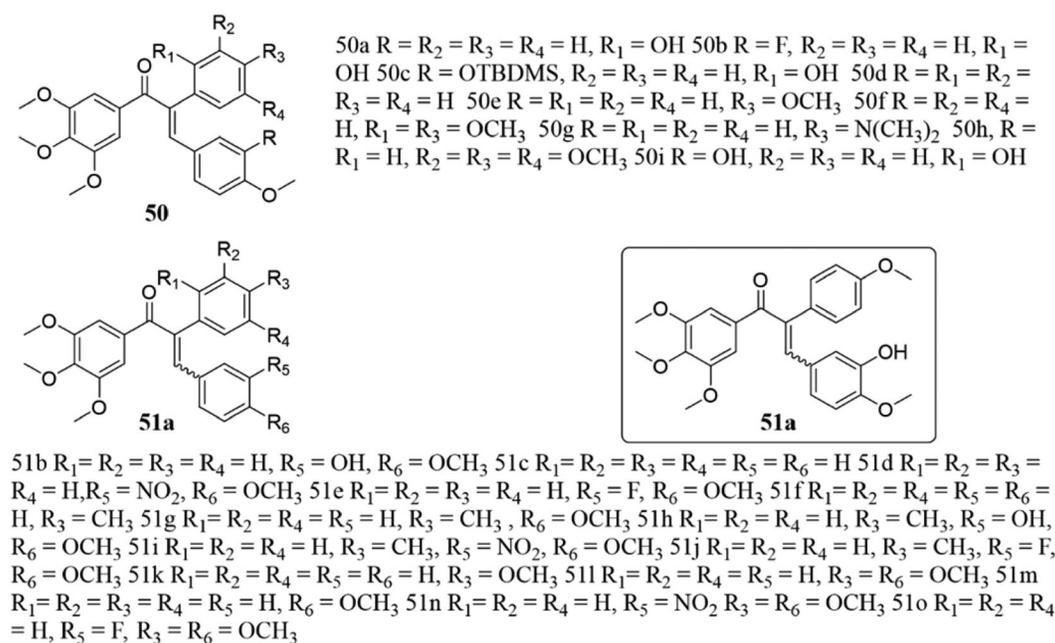
**Figure 34.** Chemical structure of compounds **50** and **51**.

pharmacophore groups. Interestingly, the binding mode of chalcone is very similar to that of podophyllotoxin, and planar hydrophobic groups such as benzene ring are added to the alpha position of chalcone skeleton, which well simulates the B ring of podophyllotoxin.

The antiproliferative activity of some novel indole-chalcone derivatives **52** (Figure 35) against different human cancer cell lines was evaluated using the MTT assay[102]. In this series, analog **52a** showed the strongest bioactivity against six cancer cells (A549, HeLa, Bel-7402, MCF-7, A2780, HCT-8) with IC$_{50}$ values in the range of $3 - 9$ nM. SAR analysis indicated that the position of the methoxy group on indole was very important for its antiproliferative activity, and the order of potency was 6-OCH$_3$ > 5-OCH$_3$ > 4-

OCH$_3$. Interestingly, the methyl group at the $N$-1 position has the opposite effect on the acetophenone and propiophenone analogs. Mechanism studies revealed that **52a** could arrest the cell cycle at the G2/M phase and induce apoptosis along with the decrease of mitochondrial membrane potential. In addition, **52a** was found to be a novel tubulin polymerisation inhibitor that could bind colchicine sites. Importantly, **52a** (including its phosphate) showed better metabolic stability in mouse liver microsomes, as well as exhibited good inhibitory activity against the tumour growth in xenograft models *in vivo* without apparent toxicity, which was superior to the control compound.

Novel pyridine-chalcone conjugates **53** and **54** (Figure 36) were evaluated as microtubule polymerisation inhibitors by Li et
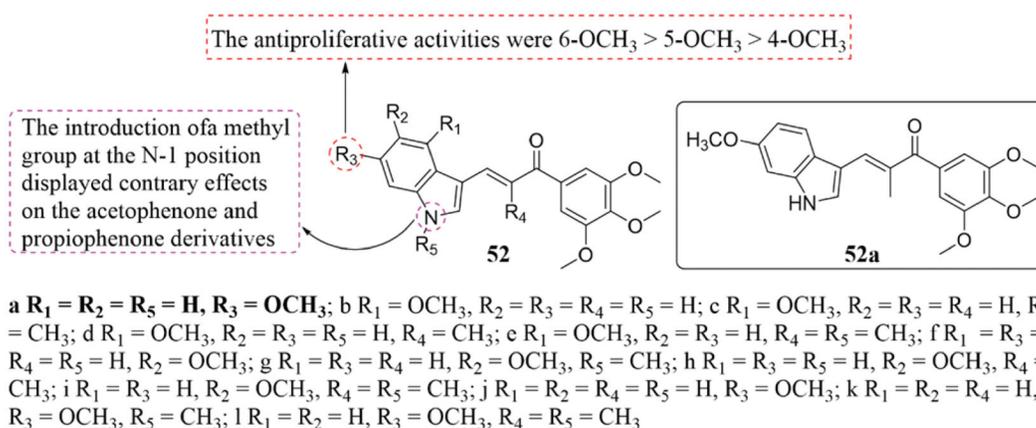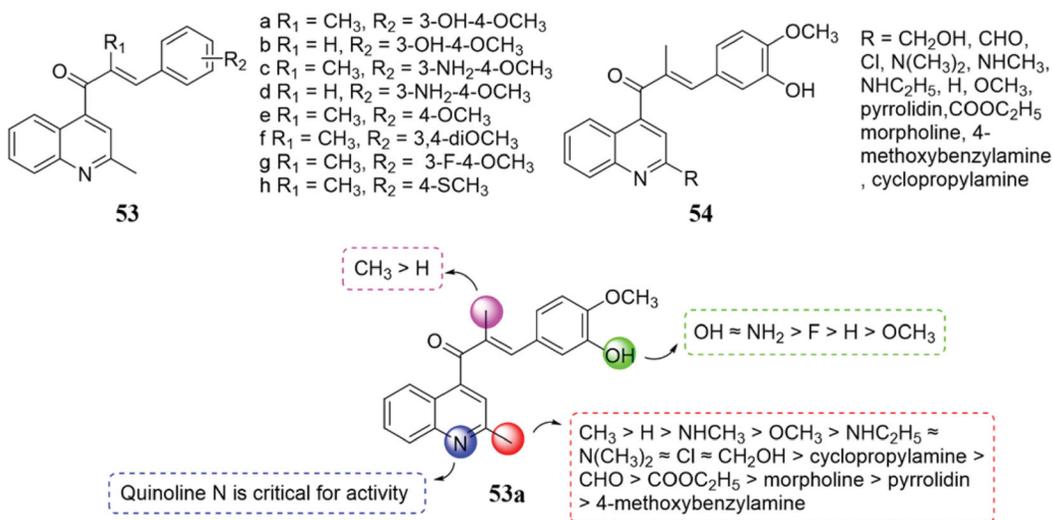
Figure 35. Indole-chalcone compounds of **52**.



Figure 36. Pyridine-chalcone compounds of **53**.

al.[103]. *In vitro* antiproliferative activity screening showed that most of the compounds exhibited potent antiproliferative activities in a nanomolar range. Among them, compound **53a** with 3-amino-4-methoxyphenyl moiety exhibited the most potent activity with $IC_{50}$ values ranging from 0.009 to 0.016 μM in a panel of cancer cell lines. SAR analysis indicated that Quinoline *N* is a critical for activity and the methyl substituent at the α-position of α, β-unsaturated ketone improved the antiproliferative activity. The order of potency of substitute in the C2-position of quinoline moiety was $CH_3 > H > NHCH_3 > OCH_3 > NHC_2H_5 \approx N(CH_3)_2 \approx Cl \approx CH_2OH >$ cyclopropylamine $>$ CHO $>$ COOC$_2$H$_5 >$ morpholine $>$ pyrrolidin $>$ 4-methoxybenzylamine. Mechanism studies demonstrated that **53a** bound to the colchicine site of tubulin, arrested the cell cycle at the G2/M phase, induced apoptosis, depolarised mitochondria, and induced reactive oxidative stress generation in K562 cells. Molecular docking study shown that compound **53a** adopted a location very similar to that of CA-4.

A range of novel pyridine-chalcone derivatives **54** (Figure 37) was found to have potential as anti-tubulin agents, and all of them were evaluated for their antiproliferative activities[104]. In this series, compound **54a** exhibited significantly activities ($IC_{50} =$ 23–45 μM) against five cancer cell lines, HepG2, epidermoid carcinoma of the nasopharynx (KB), HCT-8, MDA-MB-231, and hepatoma 22 cells (H22), which was 3 times superior to the parent compound. SAR analysis showed that the introduction of the methyl at the α-position of unsaturated carbonyl group was beneficial to

the antiproliferative activity, and the exposed hydroxyl on ring B was the key to maintaining the activity. Additionally, the substituents of the EDGs on pyridine are fitted better than that of the EWGs. Studies of mechanism demonstrated that compound **54a** could bind to the colchicine site of tubulin. Besides, cellular mechanism results indicated that **54a** arrested the G2/M phase, induced cell apoptosis, and destroyed the intracellular microtubule network.

Cong and colleagues prepared an αmethyl-substituted indole-cardol **55a** (Figure 38) and found that it exhibited potent inhibitory activity against NCI-60 cell lines (average concentration resulted in 50% growth inhibition = 6 nM)[105]. Interestingly, some multidrug-resistant cancer cell lines were not resistant to **55a**, which also showed significant selective toxicity to cancer cells and normal CD34+ blood progenitor cells. The mechanism studies showed **55a** inhibited the cells associated with tubulin binding and inhibited microtubule dynamics. Moreover, the National Cancer Institute COMPARE analysis and molecular modelling suggested that **55a** acted in a similar way to colchicine.

Canela et al.[106] have studied the tubulin-binding, vascular targeting, anti-tumour, and anti-metastatic activities of such conjugates of chalcone. Compound **56a** (Figure 38) showed the most potent activity against the proliferation of endothelial cells and tumour cells, and the inhibition concentration of 50% was 1–4 nM, which was slightly better than the control substances colchicine and combretastatin A4-phosphate (CA-4P). In addition, they
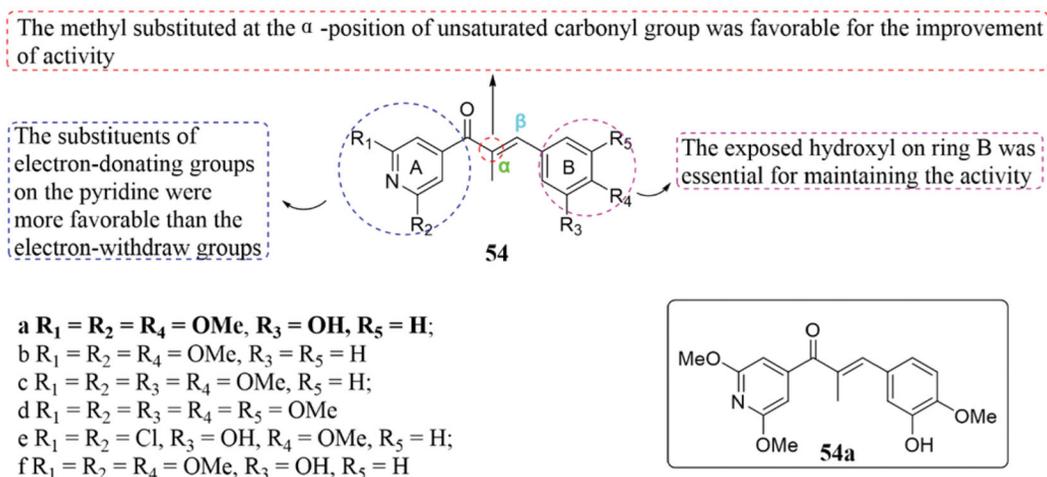
Figure 37. Chemical structure of compounds 54.



Figure 38. Chemical structure of compounds 55 and 56.

showed that these conjugates destabilise microtubules by binding colchicine sites to tubulins, and **56a** arrested tumours and endothelial cells in the G2/M phase and induced apoptosis of these cells at nanomolar concentrations. Meanwhile, In the chicken allantoic membrane (CAM) experiment, **56a** could inhibit endothelial cell migration and angiogenesis. A water-soluble L-lys-L-Pro conjugate of **56a** could result in rapid vascular closure and massive tumour necrosis in melanoma and breast cancer xenograft tumour models. **56a** as well as showed anti-metastatic activity comparable to CA-4P. These data suggested that this novel range of derivatives was an important lead molecule for the design of vascular-destroying agents (VDAs).

## 5. Chalcone hybrids

### 5.1. Platinum-chalcone hybrids

Multi-target drugs have attracted more and more attention. To obtain compounds with high cytotoxicity, the pharmacophore binding to a single compound molecule has become the design method of new anti-tumour drugs[107–109]. Platinum compounds are widely used in clinical practice but have serious toxic and side effects, such as nephrotoxicity, neurotoxicity, and myelosuppression[110]. To overcome these shortcomings, researchers have designed novel platinum-based complexes that combine platinum with other pharmacophore groups to reduce toxic side effects. Prompted by this idea, the researcher's combination of the active groups of microtubule inhibitors with cytotoxic DNA-damaging platinum compounds to obtain complexes can also be considered as an effective strategy to target tubulin and DNA, at least theoretically, to enhance the antitumor activity of platinum drugs and overcome their side effects.

Schobert et al.[111] synthesised a class of chalcone conjugates **57** (Figure 39) by introducing platinum (II) into chalcone. In this

study, the activity level of the compound **57** was the same as that of parent product **48** (Figure 39), showing good activity against all kinds of tumour cells, while **57** could effectively inhibit the reproliferation of tumour cells. In addition, compound **57** could interact with various forms of DNA, but not as tightly as cisplatin. At the same time, cancer cells treated with **48** progressed more slowly from caspase-9 to caspase-3 than those treated with **57**. In 2010, Zoldakova et al.[112] further investigated the mechanism of **57** and found that the compound **57** has strong anti-tumour activity against a variety of cancer cells. Cellular uptake of compound **57** was similar to oxaliplatin and was mainly dependent on the organic cationic transporter (OCT-1/2) and copper transport-associated protein (CTR1), while compound **48** was mainly dependent on endocytosis. Compound **57** has strong anti-proliferative activity against tumour cells with higher P-gp expression but has relatively low activity against corresponding cancer cell lines as the substrate of BCRP, MRP3, and MRP1 ABC-transporter. In conclusion, unlike compound **48**, its platinum complex **57** has a high cell line specificity and induces apoptosis through multiple targets.

The antiproliferative activity of Pt(IV) complexes **58–60** (Figure 40) comprising chalcone compounds was evaluated by MTT assay[113]. All of the synthetic complexes exhibited better and stronger activity against three types of human cancer cells, including cisplatin (CDDP) resistant cells than their parent PT (II) species. Among all of the tested compounds, substances **58a–b** were found to possess superior targeting of cancer cells than CDDP. Compounds **58a–b** also significantly inhibited HUVEC cells migration *in vitro*. Docking study showed that complexes **58a–b** binding to the colchicine site of tubulin. The studies of molecular mechanism have shown that **58a–b** could induce the production of reactive oxygen species (ROS) and cell cycle arrest at the G2/M phase and mediate mitochondrial apoptosis by regulating the expression of Bcl-2 family members.
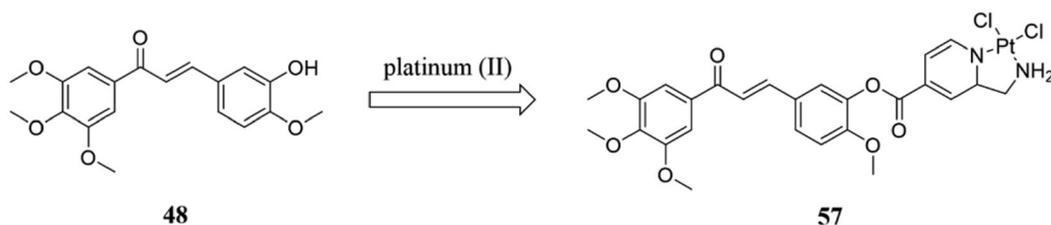
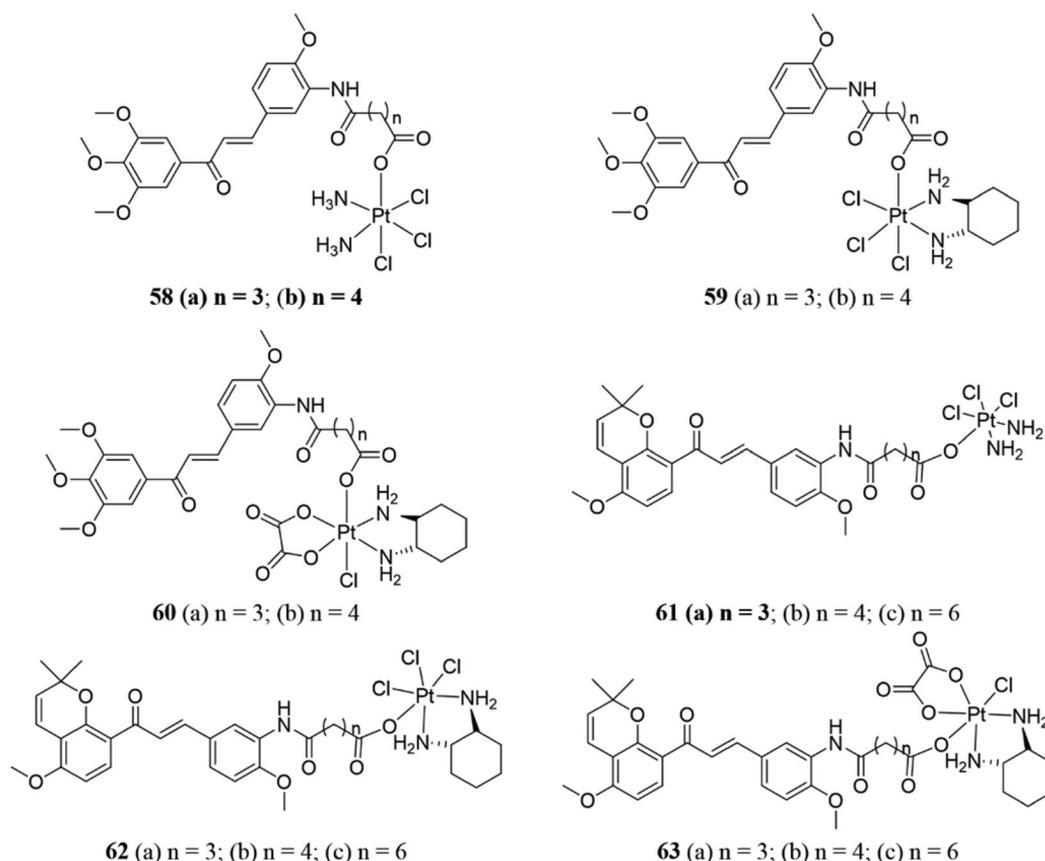**Figure 39.** Chemical structure of compounds **48** and **57**.



**Figure 40.** Platinum-chalcone hybrids of **58–63**.

In 2018, novel Pt(IV) species and millepachine derivatives **61–63** (Figure 40) were tested against human cancer cell lines by Huang et al.[114] The results of *in vitro* biological experiments showed that all the tested substances had better antitumor activity against human tumour cells (including cisplatin sensitive and resistant ones) than Pt(II), and lower cytotoxicity against two kinds of normal cells than Pt(II). Among the evaluated substances, **61a** was the most active, it could arrest the cell cycle at the G2/M phases and finally induce cell apoptosis. Besides, **61a** showed no obvious toxicity to the two normal cells but was sensitive to the two cisplatin-resistant cells. Importantly, **61a** showed good antitumor activity in SK-OV-3 xenograft tumour model, which was superior to cisplatin and its corresponding millepachine analogs.

To overcome the shortcomings of cisplatin, Huang et al.[115] successfully synthesised four tubulin-targeting platinum (IV) prodrugs and tested them for anti-proliferative activity using MTT assay. Compared to the positive drugs cisplatin and **48**, compounds **64** (Figure 41) exhibited stronger antiproliferative activity in human cancer cells tested including multidrug-resistant cancer cell lines, while the cytotoxicity of human normal liver HL-7702 was also lower than that of positive drugs (cisplatin and **48**). Particularly,

compound **64a**, with IC$_{50}$ values of 0.19–0.37 μM, was the best compound against tested human cancer cell lines. Additionally, **64a** could induce G2/M phase arrest and apoptosis of A549 cells, the mechanism of which is related to mitochondrial membrane potential (MMP) collapse, the expression of some apoptosis-related proteins, and the increase of intracellular reactive oxygen species (ROS). More importantly, compound **64a** significantly inhibited tumour growth in the A549 xenograft tumour model, but with no significant toxicity hints (Figure 41).

## 5.2. Nitrogen mustard-chalcone hybrids

Nitrogen mustard is one of the earliest antitumor drugs used clinically. As a representative of bioalkylation agents, nitrogen mustard can stably bind to affinity groups after entering the body, affect cell metabolism and promote cell apoptosis[116,117]. Based on molecular hybridisation strategy[118], Sabina and co-workers synthesised a novel range of p-[N, N-bis(2-chloroethyl)amino]benzaldehyde substituted chalcone conjugates **65** (Figure 42) and evaluated for their inhibitory activity against A549 and HepG2 cancer cells[119]. Compound **65a** displayed more activity in both
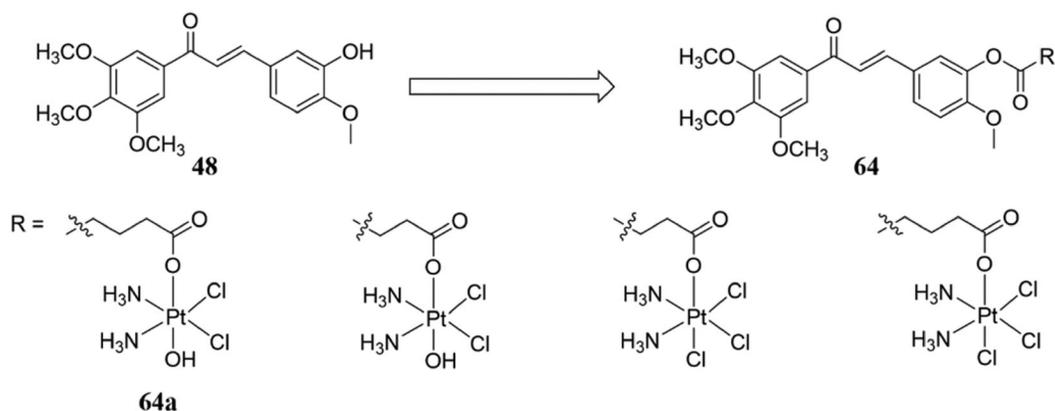
**Figure 41.** Simple chalcone compounds of **48** and **64**.
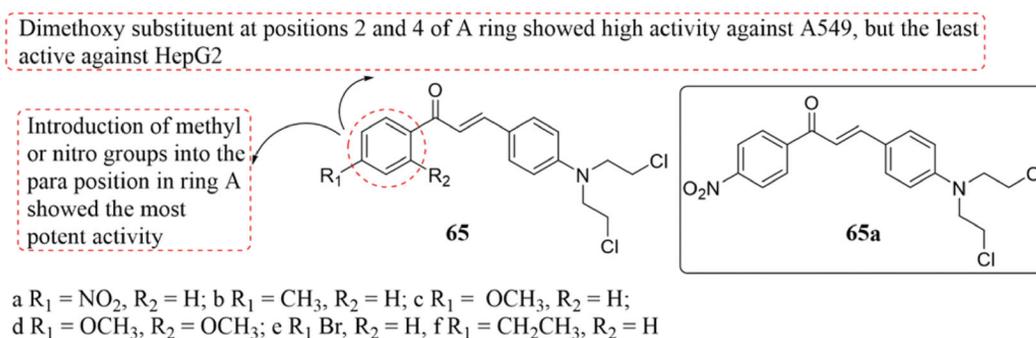


**Figure 42.** Nitrogen mustard-chalcone hybrids of **65**.
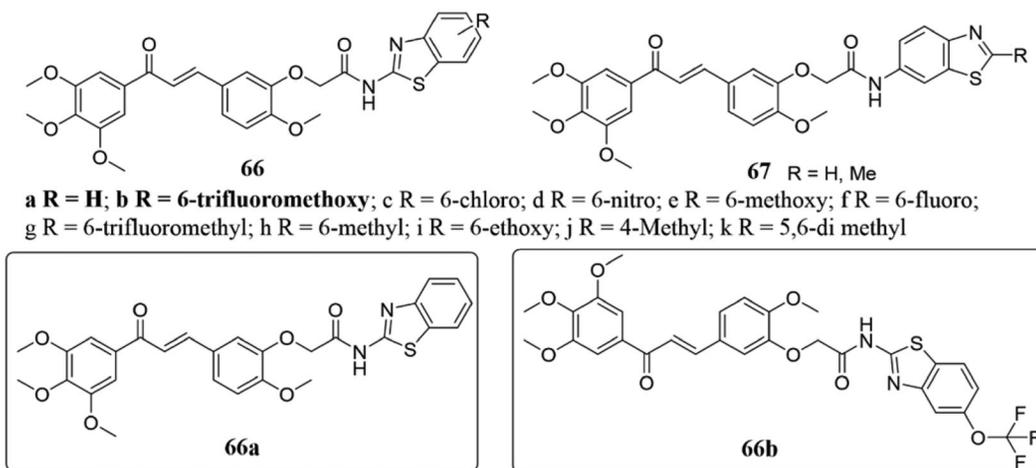


**Figure 43.** Benzothiazole-chalcone hybrids of **66** and **67**.

the cancer cell lines and tubulin than the other substances (IC$_{50}$ in the range of 0.089 to 0.200 μM). Docking results showed that the synergistic effect of nitrogen mustard made compound **65a** bind tightly to tubulin. Besides, SAR analysis showed that the compounds with methyl or nitro groups in the A ring were more active. Interestingly, dimethoxy substituents at the 2 and 4 sites of the A ring showed the highest activity against A549, while the lowest activity against HepG2.

## 5.3. Benzothiazole-chalcone hybrids

Benzothiazole is an important structure for drug development due to its favourable biological activity[120–122]. Benzothiazole

compounds have biological activities such as antitumor[123], anti-inflammatory[124], antioxidation[125], antibacterial[126], and anti-diabetic[127] et al. Benzothiazole-chalcones retain the basic structure of chalcones and replace the A or B rings with benzothiazoles to obtain chalcones with high antitumor activity and low toxicity.

A novel series of chalcone-amidobenzothiazole derivatives **66** and **67** (Figure 43) were synthesised and evaluated as potential antitumor drugs by Kamal et al.[128]. All of them showed potent inhibitory activity against a panel of five human cancer cell lines (A549, A375, MCF-7, HT-29, and ACHN), and the active compounds **66a–b** inhibited the growth of various cancer cell lines with IC$_{50}$ ranging from 0.85 to 3.3 μM. Mechanism studies have shown that the synthesised substances arrested the A549 cell cycle in the G2/M phase, and resulting in the death of caspase-3-dependent
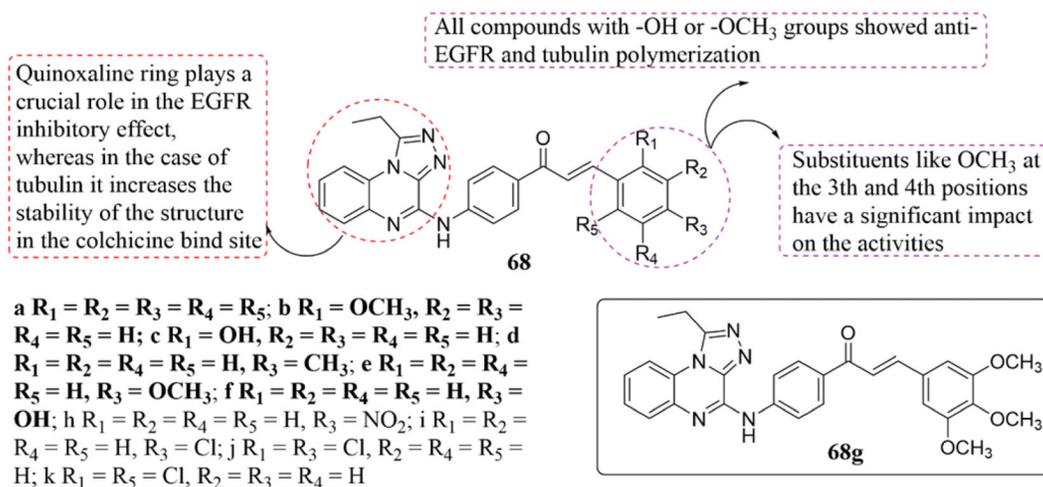
**Figure 44.** Triazole-chalcone hybrids of **68**.

apoptotic cells. The tubulin polymerisation assay (IC$_{50}$: **66a** = 3.5 μM; **66b** = 5.2 μM) and immunofluorescence analysis indicated the synthesised conjugates could significantly inhibit the microtubule assembly at both molecular and cellular levels in A549 cells. Moreover, docking studies demonstrated that they interacted with tubulin and bonded effectively.

## 5.4. Triazole-chalcone hybrids

Triazole compounds have five-membered heterocycles, which are easy to bind to targets *in vivo* and have a wide range of biological activities[129,130]. Triazole compounds were originally mainly used in pesticides, but there are also triazole antibiotics in clinical use, such as triazole, terconazole, itraconazole, and so on. Later studies have found that triazole drugs can be used not only for antibacterial and insecticidal purposes but also for anticonvulsant, antianxiety, anti-inflammatory, and anti-tumour purposes[131–134]. Therefore, they were introduced into chalcones to obtain novel tubulin inhibitors.

A new library of chalcone derivatives **68** (Figure 44) with triazoloquinoxaline-linked moiety was prepared and their anticancer activity also has been tested[135]. Most of them displayed potent activities against EGFR TK and tubulin at micromolar or submicromolar concentrations. Thereinto compounds **68b–c**, and **68e–g** showed superior antiproliferative activities against the majority of the cell lines, with selective or non-selective behaviour (IC$_{50}$ = 1.65–34.28 μM), and compounds **68a–c**, **71e**, and **68g** could effectively inhibit the EGFR TK with IC$_{50}$ ranging from 0.093 to 0.661 μM. Additionally, docking study results revealed that derivative **68g** embedded colchicine binding pocket at the interface of tubulin α and β with lowest binding energy. SAR analysis indicated that quinoxaline rings play a key role in the inhibition of EGFR, and improved the stability of the structure in the colchicine bind site. The compounds which containing -OH or -OCH$_3$ groups showed anti-EGFR and tubulin polymerisation, and the 3rd and 4th substituents, such as OCH$_3$, had potent effects on the activity.

## 5.5. Other chalcone hybrids

The nitro group is a unique functional group. Its strong electron attraction ability produces local or local electron defects in the molecule, which enables the part with a strong electrophilic ability to bind closely to amino acids, proteins, and enzymes. The

introduction of the nitro group enables compounds to have a variety of chemical and biological effects. For example, Zhang and co-workers obtained a series of compounds **69** (Figure 45) by introducing *o*-nitroaromatic rings into chalcone and evaluated for their biological activities as anti-tubulin agents[136]. All of them showed significant activities against tubulin polymerisation and the growth of MCF-7 and A549 cell lines. Thereinto, compound **69a** had the strongest inhibitory activity against MCF-7 and A549 cells (IC$_{50}$ = 0.03 and 0.95 μg/mL) and was the best compound in the anti-tubulin polymerisation assay with IC$_{50}$ of 1.42 μg/mL. SAR analysis indicated that the antiproliferative activity of the electron substituents introduced into the A ring was stronger than that of the electron-withdrawing substituents, and the potency order was CH$_3$ > OCH$_3$. However, compounds of salicylaldehyde have two halogen atoms at position-3 and position-5, their anticancer activity was lower than that of introducing only one halogen atom at position-5. In the molecular docking study, one hydrogen bond and one π-cation of **69a** could interact with the colchicine binding site protein residues, which might play a key role in its anti-tubulin polymerisation and anti-proliferative activities.

The diaryl ether scaffold is frequently found in many natural products and biologically important molecules[137,138], and some molecules with a diaryl ether nucleus possess cytotoxic activity by inhibiting tubulin polymerisation[139–141]. Based on these finding, Wang et al. synthesised several novel chalcone analogs with diaryl ether moiety **70** (Figure 46) and evaluated as inhibitors of tubulin polymerisation and human cancer cell lines[142]. Most of the tested compounds displayed moderate to good antiproliferative activity of three human cancer cell lines of MCF-1, HepG2, and HCT116 (IC$_{50}$ of 3.44 ± 0.19–8.89 ± 0.42 μM), and compound **70a** displayed the most potent activities against three human cancer cell lines with IC$_{50}$ values of 3.44 ± 0.19, 4.64 ± 0.23, and 6.31 ± 0.27 μM, respectively. In addition, **70a** could significantly inhibit tubulin polymerisation *in vitro*, and the mechanism results showed that **70a** could induce G2/M phase arrest and apoptosis. Besides, **70a** was found to interact and bind at the colchicine binding site of the tubulin by the molecular docking study.

A series of novel chalcone-containing shikonin conjugates **71** (Figure 47) have been obtained and evaluated as potential inhibitors of tubulin polymerisation by Qiu et al.[143] Majority of them displayed significant anti-proliferative activity, and **71a** was the best compound with IC$_{50}$ values of 2.98 ± 0.53 μM and MCF-7 cells with IC$_{50}$ values of 2.36 ± 0.32 μM. Mechanism studies showed that **71a** induced MCF-7 cell apoptosis, decreased mitochondrial
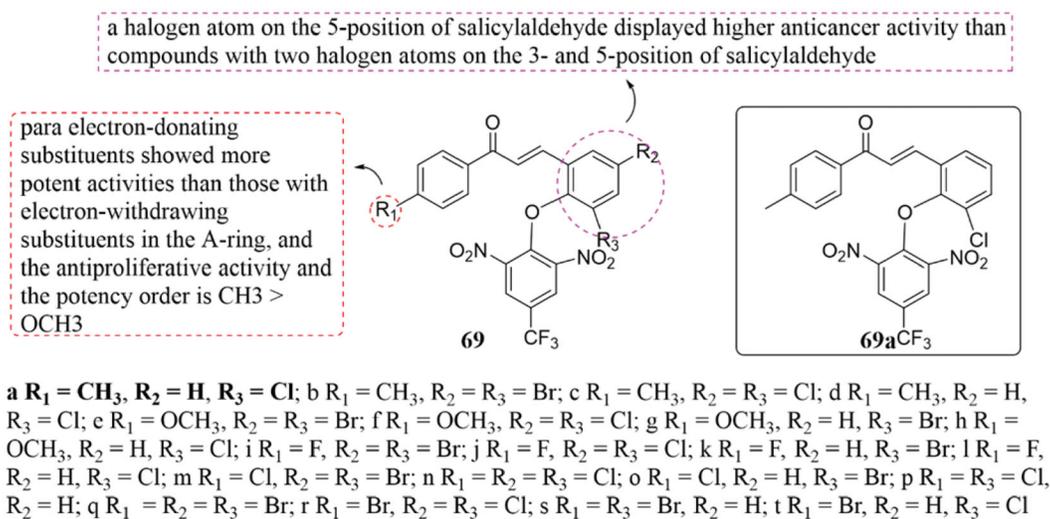
**a R₁ = CH₃, R₂ = H, R₃ = Cl**; b R₁ = CH₃, R₂ = R₃ = Br; c R₁ = CH₃, R₂ = R₃ = Cl; d R₁ = CH₃, R₂ = H, R₃ = Cl; e R₁ = OCH₃, R₂ = R₃ = Br; f R₁ = OCH₃, R₂ = R₃ = Cl; g R₁ = OCH₃, R₂ = H, R₃ = Br; h R₁ = OCH₃, R₂ = H, R₃ = Cl; i R₁ = F, R₂ = R₃ = Br; j R₁ = F, R₂ = R₃ = Cl; k R₁ = F, R₂ = H, R₃ = Br; l R₁ = F, R₂ = H, R₃ = Cl; m R₁ = Cl, R₂ = R₃ = Br; n R₁ = R₂ = R₃ = Cl; o R₁ = Cl, R₂ = H, R₃ = Br; p R₁ = R₃ = Cl, R₂ = H; q R₁ = R₂ = R₃ = Br; r R₁ = Br, R₂ = R₃ = Cl; s R₁ = R₃ = Br, R₂ = H; t R₁ = Br, R₂ = H, R₃ = Cl

**Figure 45.** Chemical structure of compounds **69**.
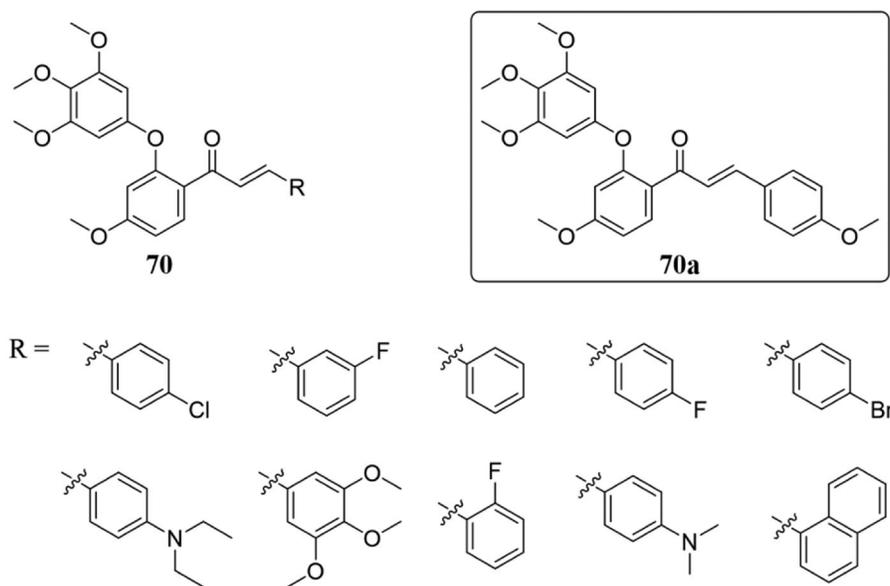


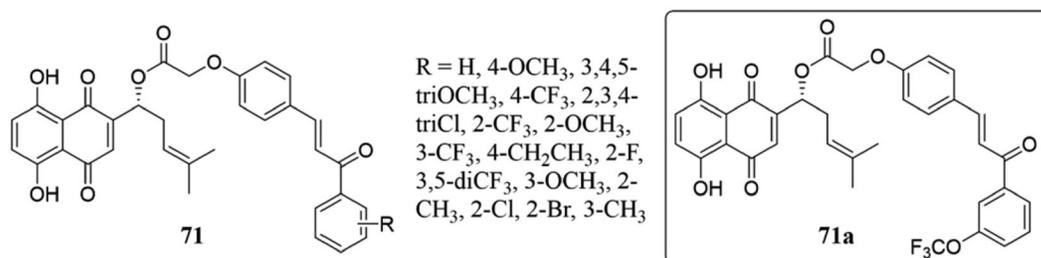**Figure 46.** Chemical structure of compounds **70**.



**Figure 47.** Chemical structure of compounds **71**.

membrane potential, led to cell accumulation in the G2/M phase of the cell cycle, and seriously damaged the microtubule system, which is equivalent to the colchicine. Meanwhile, three hydrogen bonds of **71a** were found in the protein residues of the colchicine binding site, which may be the reason for its resistance to tubulin polymerisation.

Shankaraiah and co-workers have been obtained a range of new heterocycles-linked chalcone conjugates **72** (Figure 48) by varying different alkane spacers, and the *in vitro* cytotoxic

potential against a panel of selected human cancer cell lines of them were be tested[144]. Several of them showed a wide range of inhibitory activities on the tested human cancer cell lines (IC₅₀ = 1.48 ± 0.19 μM-10.98 ± 0.81 μM), and **72a** showed the strongest activity against the NCI-H460 (lung cancer) cells (IC₅₀ of 1.48 ± 0.19 μM). Meanwhile, **72a** was found to effectively inhibit the tubulin polymerisation and destroy microtubule formation with IC₅₀ of 9.66 ± 0.06 μM. SARs of the tested compounds have been exhibited that piperidine and morpholine on the B ring and
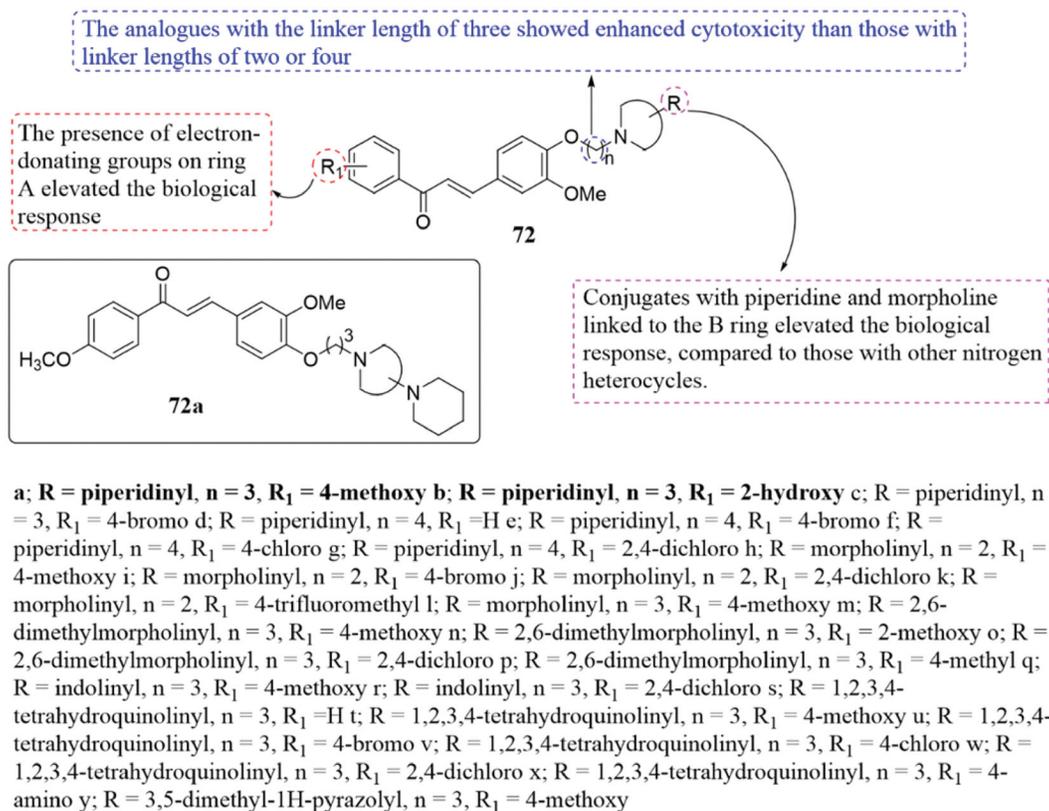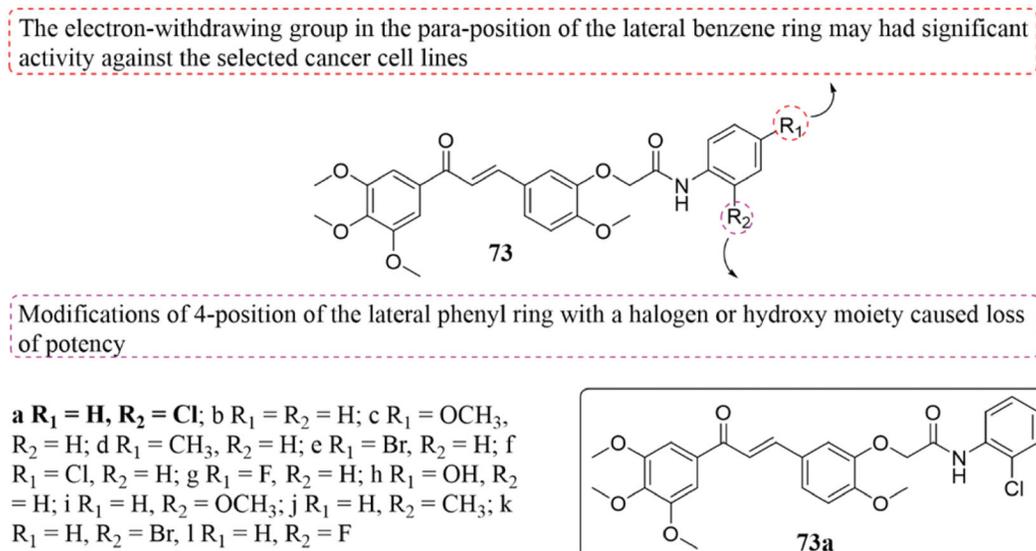
Figure 48. Chemical structure of compounds 72.

a; R = piperidinyl, n = 3, R$_1$ = 4-methoxy b; R = piperidinyl, n = 3, R$_1$ = 2-hydroxy c; R = piperidinyl, n = 3, R$_1$ = 4-bromo d; R = piperidinyl, n = 4, R$_1$ =H e; R = piperidinyl, n = 4, R$_1$ = 4-bromo f; R = piperidinyl, n = 4, R$_1$ = 4-chloro g; R = piperidinyl, n = 4, R$_1$ = 2,4-dichloro h; R = morpholinyl, n = 2, R$_1$ = 4-methoxy i; R = morpholinyl, n = 2, R$_1$ = 4-bromo j; R = morpholinyl, n = 2, R$_1$ = 2,4-dichloro k; R = morpholinyl, n = 2, R$_1$ = 4-trifluoromethyl l; R = morpholinyl, n = 3, R$_1$ = 4-methoxy m; R = 2,6-dimethylmorpholinyl, n = 3, R$_1$ = 4-methoxy n; R = 2,6-dimethylmorpholinyl, n = 3, R$_1$ = 2-methoxy o; R = 2,6-dimethylmorpholinyl, n = 3, R$_1$ = 2,4-dichloro p; R = 2,6-dimethylmorpholinyl, n = 3, R$_1$ = 4-methyl q; R = indolinyl, n = 3, R$_1$ = 4-methoxy r; R = indolinyl, n = 3, R$_1$ = 2,4-dichloro s; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ =H t; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ = 4-methoxy u; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ = 4-bromo v; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ = 4-chloro w; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ = 2,4-dichloro x; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R$_1$ = 4-amino y; R = 3,5-dimethyl-1H-pyrazolyl, n = 3, R$_1$ = 4-methoxy



Figure 49. Chemical structure of compounds 73.

the introduction of EDGs on the A ring enhanced the activity. In addition, the cytotoxicity of the 2 - or 4-link conjugates was lower than that of the 3-link conjugates. Mechanism studies showed that compound 72a could block NCI-H460 cells in the G2/M phase of the cell cycle and induce cell apoptosis. Besides, docking studies have shown that these novel conjugates may dock with colchicine sites of tubulin.

A class of novel chalcone derivatives 73 (Figure 49) were considered as potential antitumor agents by Huang et al.[145]. These newly synthesised chalcones showed good anticancer activity, which the IC$_{50}$ values are mainly at the micromolar level.

Thereinto, derivative 73a showed the most effective activity against tested tumour cell lines, including multidrug-resistant human cancer lines (IC$_{50}$ of 3.75–8.42 µM). SAR analysis indicated that the introduction of EWG in the para-position of the lateral benzene ring may beneficial to the activity of the selected cancer cell lines. While the 4-position of the lateral benzene ring is modified with a halogen or hydroxyl group to render them ineffective. Additionally, the mechanism results showed that 73a induced apoptosis of NCI-H460 cells through the mitochondrial pathway, which also could effectively induce cell cycle arrest in the G2/M phase. Besides, the novel analogs could inhibit tubulin
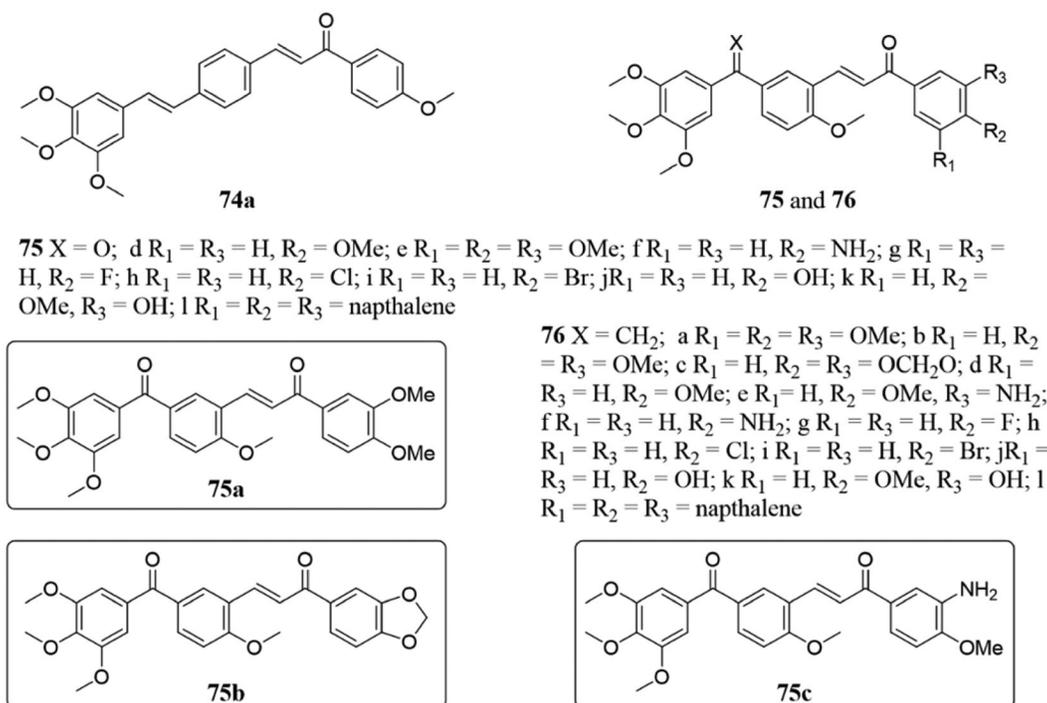
**75** X = O; d R$_1$ = R$_3$ = H, R$_2$ = OMe; e R$_1$ = R$_2$ = R$_3$ = OMe; f R$_1$ = R$_3$ = H, R$_2$ = NH$_2$; g R$_1$ = R$_3$ = H, R$_2$ = F; h R$_1$ = R$_3$ = H, R$_2$ = Cl; i R$_1$ = R$_3$ = H, R$_2$ = Br; j R$_1$ = R$_3$ = H, R$_2$ = OH; k R$_1$ = H, R$_2$ = OMe, R$_3$ = OH; l R$_1$ = R$_2$ = R$_3$ = napthalene

**76** X = CH$_2$; a R$_1$ = R$_2$ = R$_3$ = OMe; b R$_1$ = H, R$_2$ = R$_3$ = OMe; c R$_1$ = H, R$_2$ = R$_3$ = OCH$_2$O; d R$_1$ = R$_3$ = H, R$_2$ = OMe; e R$_1$ = H, R$_2$ = OMe, R$_3$ = NH$_2$; f R$_1$ = R$_3$ = H, R$_2$ = NH$_2$; g R$_1$ = R$_3$ = H, R$_2$ = F; h R$_1$ = R$_3$ = H, R$_2$ = Cl; i R$_1$ = R$_3$ = H, R$_2$ = Br; j R$_1$ = R$_3$ = H, R$_2$ = OH; k R$_1$ = H, R$_2$ = OMe, R$_3$ = OH; l R$_1$ = R$_2$ = R$_3$ = napthalene

**Figure 50.** Chemical structure of compounds 74–76.

polymerisation through the tubulin polymerisation assay, and docking studies revealed that **73a** could bind to the colchicine site of tubulin.

Kumar et al.[146] prepared a range of new resveratrol-chalcone conjugates and tested the anticancer activity against 60 human cancer cell lines. Thereinto, conjugate **74a** (Figure 50) displayed the strongest activity and high selectivity towards certain ovarian cancer, non-small cell lung cancer, and breast cancer cell lines (GI$_{50}$ = 1.28–34.1 μM). Docking studies revealed **74a** could bind to the colchicine site of tubulin, and observe a potent H-bonding interaction (1.852 Å) with Cys-241 amino acid present in the binding pocket. Therefore, compound **74a** might be a precursor for the development of new anticancer drugs.

A series of phenstatin/isocombretastatin-chalcones **75** and **76** (Figure 50) have been prepared and evaluated the anticancer activity against various human cancer cell lines[147]. Several targets chalcones showed potent antiproliferative activity against 60 human cancer cell lines of the NCI (GI$_{50}$ = 0.11–19.0 μM). The three compounds **75a–c** exhibited a broad spectrum of anti-proliferation activities in the submicromolar range for most cell lines, and all of them showed moderate to excellent cytotoxicity against breast cancer cells such as MCF-7 and MDA-MB-231 (IC$_{50}$ of 0.5 to 19.9 μM). By the tubulin polymerisation assay and immunofluorescence analysis, compound **75b–c** effectively inhibited tubulin assembly with IC$_{50}$ values of 0.8 μM and 0.6 μM, respectively. The competitive binding assay indicated that these chalcones bind at the colchicine-binding site of tubulin, and which is blocked in the G2/M phase and leads to apoptosis.
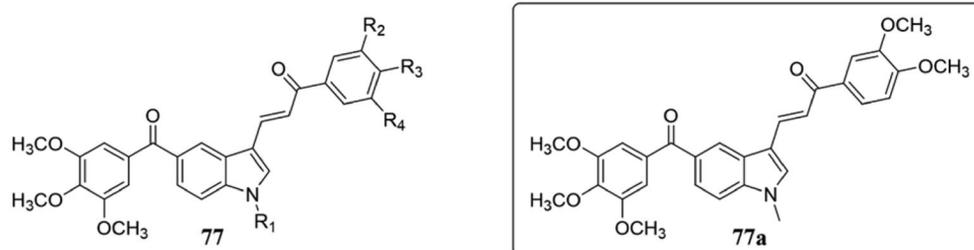
A number of novel phenstatin based indole linked chalcone analogs **77** (Figure 51) were prepared and evaluated for their antiproliferative activity on few cancer cell lines by Kode et al.[148] In this series, analog **77a** showed potent anti-proliferative activity against oral cancer squamous cancer cells as well as oral cancer stem-like spheroids, and the tumour volume of experimental mice was reduced significantly, and there was no toxic effect. Interestingly, **77a** also reduced angiogenesis in mice xenografts,

reduced collagen levels, and led to a significant reduction in cell process, cell integrity, and cytoskeletal organisation. In addition, **77a** successfully eliminated glucose uptake in tumour xenografts. Further studies showed that **77a** could directly interact with tubulin and inhibit tubulin polymerisation, resulting in the formation of tubulin ring intermediates, profilament bending, and destabilisation. Besides, molecular docking results indicated that **77a** showed better binding affinity to target binding sites, thus inhibiting microtubule polymerisation and glucose metabolism.

Ahmed and co-workers have been synthesised a new range of α-phthalimido-substituted chalcones-based hybrids **78** (Figure 52) as dual histone deacetylase (HDAC)/tubulin inhibitors, and the *in vitro* anticancer activity was tested by MTT assay using CA-4 as reference compound[149]. All of them displayed significant antitumor activity with IC$_{50}$ at micromolar concentrations against MCF-7 and HepG2 cell lines. In particular, the antitumor activity of **78a** was 2.57 and 4.51 times that of CA-4, respectively, and could effectively inhibit the activity of β-tubulin polymerase and HDAC 1 and 2. Moreover, the mechanism studies showed that **78a** could successfully arrest the cell cycle in G2/M phase and induce the apoptosis of preG1 cells. Besides, molecular docking studies indicated that these compounds could efficiently bind to the colchicine binding site of both tubulin polymerase enzyme and HDAC2 active sites with energy scores higher than the reference ligands.
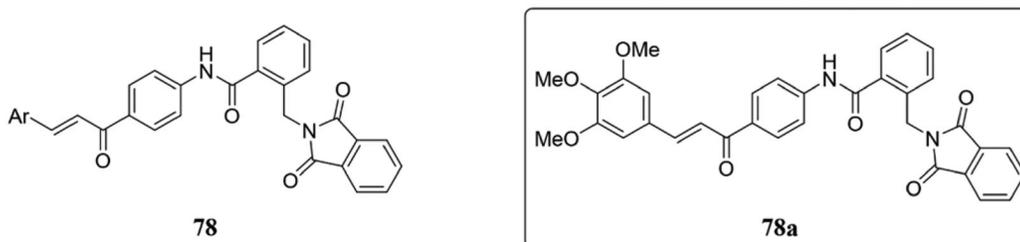
## 6. Conclusion

Chalcone is an important natural product with a variety of biological activities. Some compounds with a chalcone structure exhibit potent antitumor effects, which have attracted a lot of attention. Further studies revealed that the mechanism of antitumor action of chalcone compounds is that they can reversibly or irreversibly bind tubulin, thereby inhibiting or promoting tubulin polymerisation, disturbing the dynamic balance of microtubules, inhibiting cell cycle progression, inducing apoptosis, or inhibiting the formation of capillaries around tumour cells, and thus

**Figure 51.** Chemical structure of compounds **77**.



Ar = 3,4,5-Trimethoxyphenyl; Phenyl; p-Chlorophenyl; p-Methoxyphenyl; 1-Naphthyl; Piperonyl; 4 [2,2]Paracyclophanyl; p-Nitrophenyl; 2,4-Dimethoxyphenyl; 2-Furfuryl

**Figure 52.** Chemical structure of compounds **78**.

inhibiting tumour cell proliferation. The structure of chalcone consists of two aryl groups and an $\alpha$, $\beta$-unsaturated alkenone. Therefore, modification of the two aryl rings and $\alpha$, $\beta$-unsaturated alkenone structure is a major research direction in recent years. In previous studies on CA-4, hydroxyl and methoxy substitutions were critical for the compounds to exhibit a favourable activity, and a variety of methoxy substitution patterns were effective in increasing the antitumor activity of chalcone compounds and their derivatives, but excessive methoxy substitutions may conversely reduce the antitumor activity of the compounds. From some natural products, researchers began to replace the two aromatic rings of chalcone with heterocycles. Many attempts have been made to design heterocyclic substitution of chalcone compounds. It has been found that the substitution of the A or B ring of chalcone with the heterocycle of naphthalene ring, benzopyran, benzofuran, indole, benzothiazole, benzoxazole, quinoline, etc. exhibits excellent cytotoxicity and inhibitory effects against tubulin polymerisation. Moreover, the introduction of methoxy into the heterocyclic ring could improve the antitumor activity of heterocyclic-chalcone derivatives. Chalcone has an $\alpha$, $\beta$-unsaturated ketone structure with a single bond that is rotatable, but the $\alpha$-position priming group in the carbonyl group makes it easier for the compound to maintain the trans configuration and better bind to the colchicine binding site of tubulin. Hence, the introduction of thiophene, thiazole, imidazole, and benzothiophene into the alkenone system likewise forces the double bonds in alkenones to maintain a trans conformation, increasing the affinity of the compounds to tubulin.

Overall, the inhibitory effects of chalcone derivatives against tubulin polymerisation and tumour cells could be enhanced by structurally modifying.

## Disclosure statement

The authors confirm that this article content has no conflict of interest.

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