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Chapter

Chalcones: Potential Anticancer Agents

Adam McCluskey and Cecilia Russell

Abstract

Chalcones in their various guises have been considered either valid and critically important lead compounds in the development of novel anticancer agents or as pan assay interference compounds, PAINS. Medicinal chemistry is replete with exemplars from both "camps" progressing to clinical utility. Chalcones offer a simple starting point for the development of specific compounds with high levels of activity toward key biological targets. Chalcones have been shown to display a wide array of anticancer compounds. This chapter seeks to offer an overview of key examples in an effort to encourage further reading and research in development in this intriguing space.

Keywords: chalcones, biologically active, cancer, structure activity relationship data

1. Introduction

Arguably, cancer represents one of the most serious threats to human health. Its incidence is on the rise, and while there have been an increasing number of new drugs and new targets over the past 50 or so years, it is still responsible for multiple deaths across the globe [1]. The advent of targeted therapies arguably commenced with the discovery and clinical use of the protein kinase inhibitor imatinib [2]. Since this first report, there have been multiple novel protein kinase inhibitor-based drugs entering clinical use [3]. More recently, there has been a significant shift in treatment paradigms to the use of mono-clonal antibodies, with this market predicted to be >$US300 billion by 2030 [4]. Despite this, the survival rates for metastatic breast cancer (Stage IV, 5-year survival is <25%), for pancreatic cancer this is a more dire 7% [5]. Treatment of glioblastoma and other neurological cancers has not advanced in the past 3–4 decades [6, 7].

2. Biological activity of chalcones

Chalcones or analogues or derivatives of (*E*)-1,3-diphenyl-2-propene-1-one represent a very diverse array of molecules. This family of molecules are known to possess a myriad of biological activities spanning (but not limited to) antidiabetic, antimicrobial, antioxidant, anti-inflammatory, anticancer and chemopreventative properties [8]. A number of chalcones are in current clinical use, exemplified by the selected analogues shown in **Figure 1** and in other figures throughout this chapter.

Note that the breadth of the potential applications of chalcones in cancer is expansive and beyond the scope of this chapter, the intent here is to supply a snapshot of chalcones and their targets to encourage further exploration, by the reader, of this area [8–10].

Despite the numerous examples of clinically used chalcones, they are often overlooked for lead development as a function of PAINS filtering [11]. We note the key role here of the medicinal chemist in understanding both the limitations of the lead scaffold, potential promiscuity and the nature of the biological screening conducted. If the scaffold limitations are understood, there is limited rationale in excluding a whole compound class, especially given the current utility of these analogues. However, vigilance is required in SAR examinations. We recommend the removal of PAINS filters from preliminary screening cascades and the introduction of robust orthogonal assay procedures to enable rapid identification of true lead compounds [12, 13]. In so doing, we believe that this will increase the attractiveness of chalcones as leads; potentially matching their use will greatly increase the attractiveness of chalcones as potential starting points for drug discovery [9, 14].

Historically, chalcones, for example **1–5**, have been used in a therapeutic environment for millennia. Typically, through the ingestion of plants and herbs, chalcones have been used in the treatment of a myriad of conditions, spanning but not limited to inflammation, diabetes, and the topic of this chapter, cancer [8, 15–18]. Metochalcone (**1**) and sofalcone (**2**) have been used in the treatment of ulcers and as mucoprotective agents, respectively (**Figure 1**) [15, 16].

Being able to switch between two chalconoid structures (**6a** and **6b**; **Figure 2**) in principle establishes two Michael acceptor possibilities for this class of compounds. It is important to recognize at this point that key researchers view these and molecules such as these as PAINS [11]. As such, caution should be used in determining absolute effects and ascribing them to specific compounds' actions in a

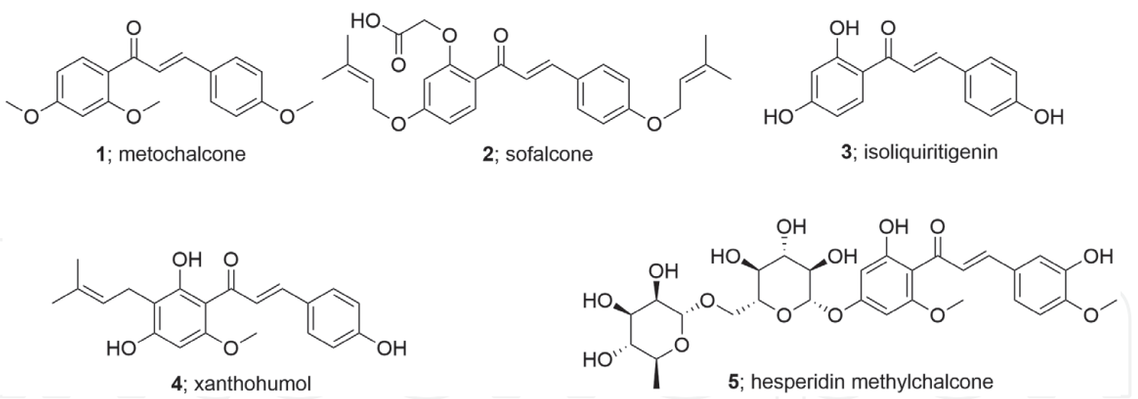


Figure 1.
*Chemical structures of selected clinically used chalcones: metochalcone (**1**), sofalcone (**2**), isoliquiritigenin, xanthohumol (**4**) and hesperidin methylchalcone (**5**).*

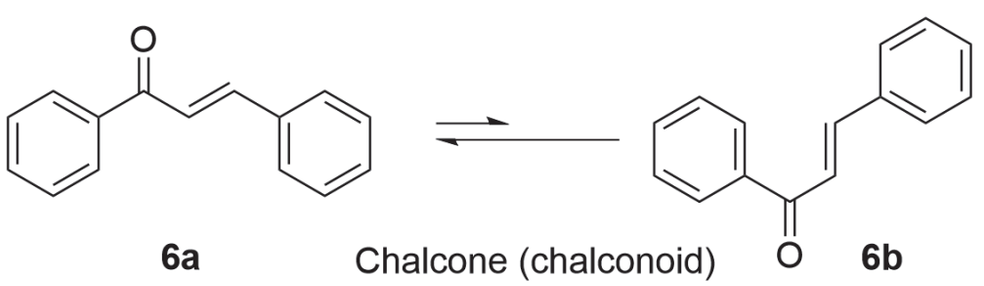


Figure 2.
The interplay between the s-cis and s-trans chalconoid structural motifs available to simple chalcones.

biological system. Clearly, Michael acceptors are generally biologically active. Michael acceptor-type compounds are known to be involved in cell signaling cascades and in many cases these compounds are capable of forming covalent attachments to the sulfhydryl of cysteine or other thiols to obtain the Michael adduct **7** (**Figure 3**), which may play an important role in their biological activities [19–23]. Interestingly, the past reservations about small molecules forming covalent linkages with proteins are subsiding with a wide variety of targeted drugs operating via a covalent interaction mode [24]. This may lead to a resurgence in the examination of chalcones as lead compounds.

Despite the PAINS expectation, there have been a large number of chalcones reported to elicit anticancer activity via specific cell signaling pathways. Of note are those analogues (**8**–**10**) that target the NF-kB pathway. It has been reported that the anticancer activity of 3-hydroxy-4,3′,4′,5′-tetramethoxychalcone (**9**) correlates with its NF-κB inhibitory activity. The reported mechanism of action requires interaction with the IKKb cysteines (46% inhibition; 10 μM) [23] proceeding via a JNK-mediated autophagy pathway triggering c-IAP (**Figure 4**) [25].

A key feature of chalcone **9** is its ability to synergize with existing clinical treatments. As a combination therapy, **9** and the TNF-related apoptosis-inducing ligand (TRAIL) or cisplatin significantly enhanced its cytotoxicity in lung cancer cells. This effect is mediated via the suppression of cellular FLICE (FADD-like IL-1b-converting enzyme)-inhibitory protein large (c-FLIPL) and cellular inhibitor of apoptosis proteins (c-IAPs), which in combination activate autophagy [26, 27].

Within the NF-κB activation pathway the Toll-like receptor 4 (TLR4) and myeloid differentiation 2 (MD2) regulate the downstream signal transduction, such as MAPK phosphorylation. In a LPS-acute lung injury model chalcone **10** inhibited the activity of MD2 reducing the inflammatory effects in this model [28].

The removal of purported PAINS is more frustrating with recent examples where promiscuous inhibitors were not removed or the filters demonstrated an

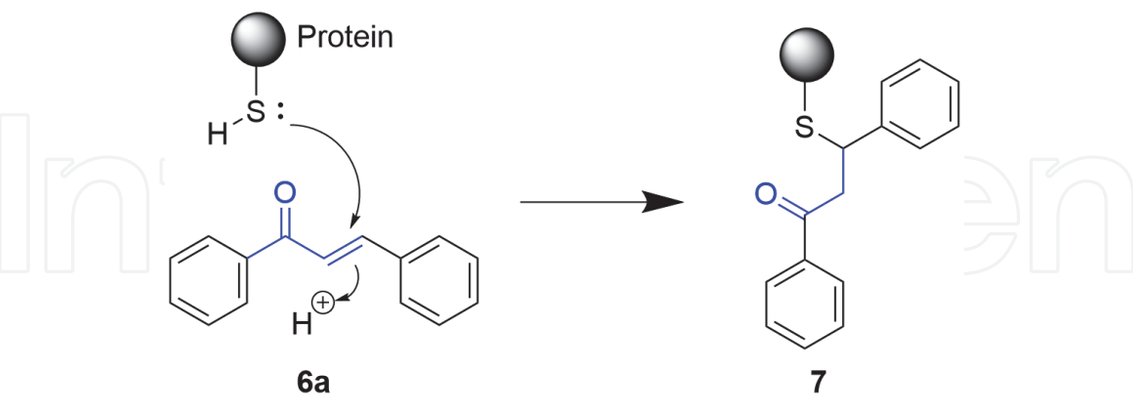


Figure 3.
*Michael addition of a chalcone (**6a**) with cysteine to form the Michael adduct 7.*

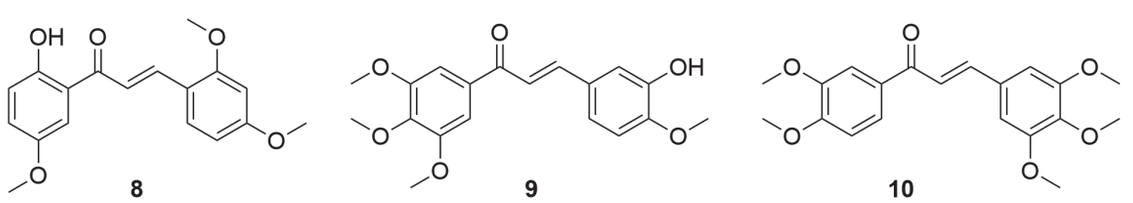
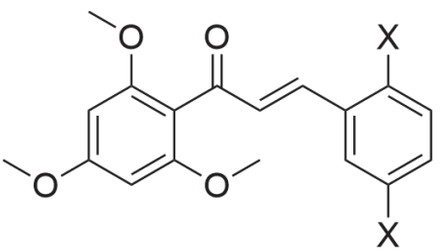


Figure 4.
Exemplar chalcones known to be NF-κB inhibitors [28].

oversensitivity toward key compound types. That is, these filters may reject non-promiscuous compounds [12, 29].

The use of bioisosteric replacements with chalcones has high prevalence. Commencing with 2,4,6-trimethoxychalcone (**11**) a simple H (**11**) to F (**12**) isosteric replacement effected a 2-fold potency increase against HeLa (cervical cancer), A498 (renal cancer), and HepG2 (hepatocellular carcinoma) cells, with retention of activity against the A549 (lung adenocarcinoma epithelial) and A375 (skin malignant melanoma) cell lines with IC_{50} values spanning 0.03–0.120 μM (**Table 1**) [30].

Isosteric replacements have not been limited to simple Grimm's isosteres, but they have been explored in nonclassical isostere space with the replacement of the central olefin with a small heterocyclic compound, such as the thiophene analogues shown in **Table 2**. These modified chalcones, for example, **14** developed from **13**, displayed good levels of cytotoxicity against a range of cancerous cell lines, with activities noted in the sub-μM to mid-nM range (0.160–0.510 μM) against HeLa,



X	HeLa	A549	A498	A375	HepG2
11; H	0.134	0.134	0.067	0.067	0.134
12; F	0.060	0.120	0.030	0.060	0.060

Table 1.
*Effect of H to F bioisosteric modification on the cytotoxicity of a 2,4,6-trimethoxychalcone (**11**). IC_{50} values are expressed in μM.*

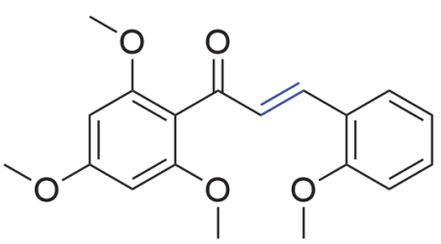
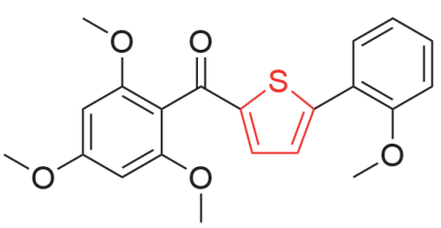
Compound	HeLa	Molt-4	CEM	L1210	FM3A
	1.4	1.9	1.8	2.1	7.9
	0.160	0.180	0.370	0.510	0.440

Table 2.
Bioisosterism represented by the replacing the double bond of the enone (blue) with a thiophene (red) [31]. IC_{50} values are expressed in μM.

Molt-4 (human T-lymphocyte), CEM (human T-lymphocyte), L1210 (murine leukemia), and FM3A cell lines (murine mammary carcinoma) (**Table 2**) [31].

2.1 Chalcone hybrids

The biological activity of chalcones, and study thereof is not limited to the parent structure, but has recently expanded to encapsulate hybrid (chimeric) molecules. These chimeras combine the cytotoxicity of the parent chalcone (**15**) and the biological activity of the second drug. Multiple chimeric partners have been reported including antibiotics (ciprofloxacin, **16**) linking through the N-aryl piperazine moiety. This allows access to the known inhibition of human DNA topoisomerase II, itself a known anticancer drug target (**Figure 5**) [32]. The chalcone-ciprofloxacin hybrid (**17**) inhibits human DNA topoisomerase II with potent in vitro anticancer activity against myriad of cancer cell lines [33–36].

Chalcones themselves are known to inhibit several anticancer targets, including thioredoxin reductase [21], and tubulin polymerization [37, 38]. Based on this there was an expectation (upheld) that chimeric molecules possessing a N-aryl piperazine and chalcone moieties would show higher potencies in the cell lines examined. Indeed, with these molecules considerable synergy arising from the combination of both partners was observed. Of the analogues reported, hybrid **17** displayed the highest activity against cervical cancer (Hela; IC_{50} = 190 nM) and gastric cancer (SGC7901; IC_{50} = 410 nM) cells (**Figure 5**). These data compare favorably with that reported for cisplatin in the same cell lines with IC_{50} values of 20 and 12 μM, respectively [32].

The introduction of an active warhead has been accomplished through the synthesis of a α-bromoacryloylamido chalcones (**Figure 6**). Analogues of this nature are expected to act as covalent modifiers of their target protein [39]. Intriguingly, this combines the once thought of anathema of a covalent inhibitor with a compound classified as a PAINS [11, 24]. Yet, compounds **18** and **19** exhibit the highest activity against tumor cell growth (IC_{50} < 1 μM) and 10- to 100-fold increases in potency relative to the corresponding amide derivatives. Preliminary mechanism of action studies support apoptosis induction via mitochondrial engagement and activation of caspase-3. The related amide-linked dithiocarbamate-chalcone (**20**) also exhibited excellent growth inhibition against SK-N-SH cells, with an IC_{50} value of 2.03 μM, with negligible toxicity toward the normal GES-1 cell line (IC_{50} > 50 mM). However, this effect is via G_0/G_1 arrest and progression through apoptosis. The nature of the linking and pendant moieties affects the compound mode of action [40].

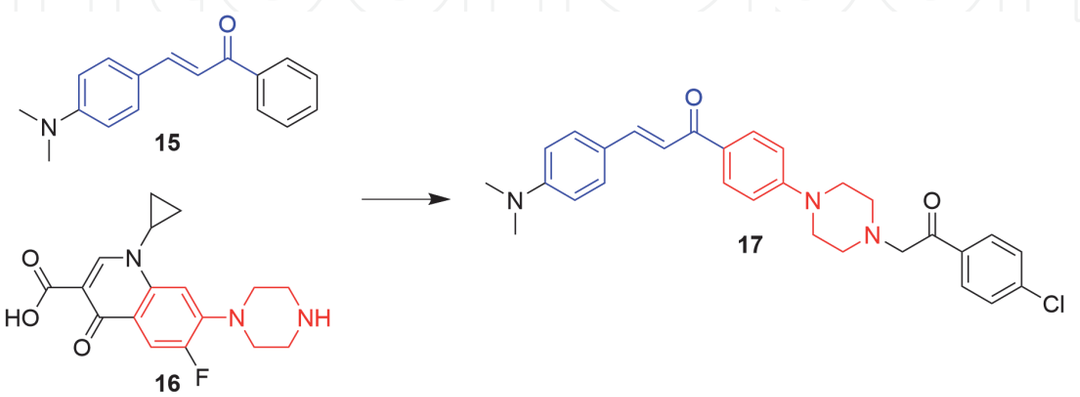


Figure 5.
Molecular hybrid obtained from the combination of chalcones (red) and N-aryl piperazine moiety (blue) [32].

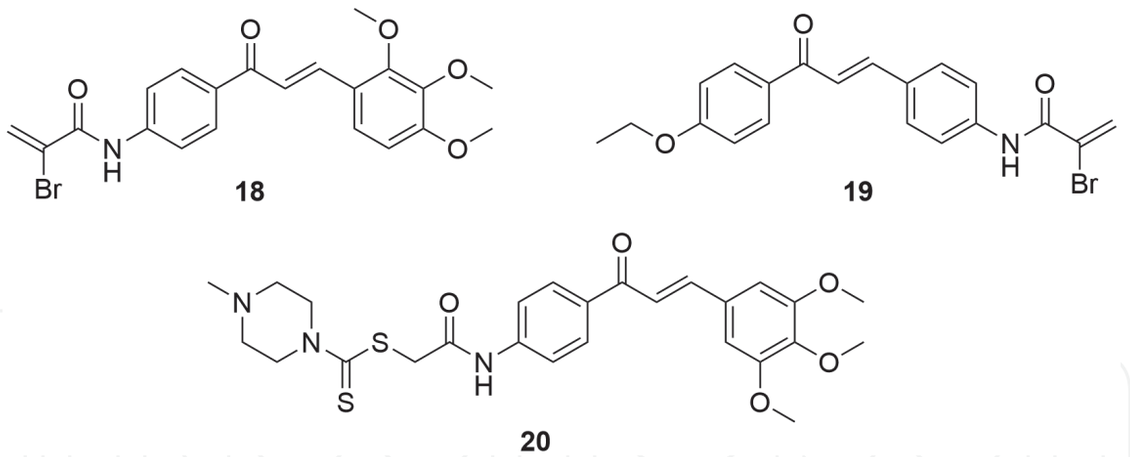


Figure 6.
Chemical structures of selected amide-linked chalcones bearing a covalently active warhead [40].

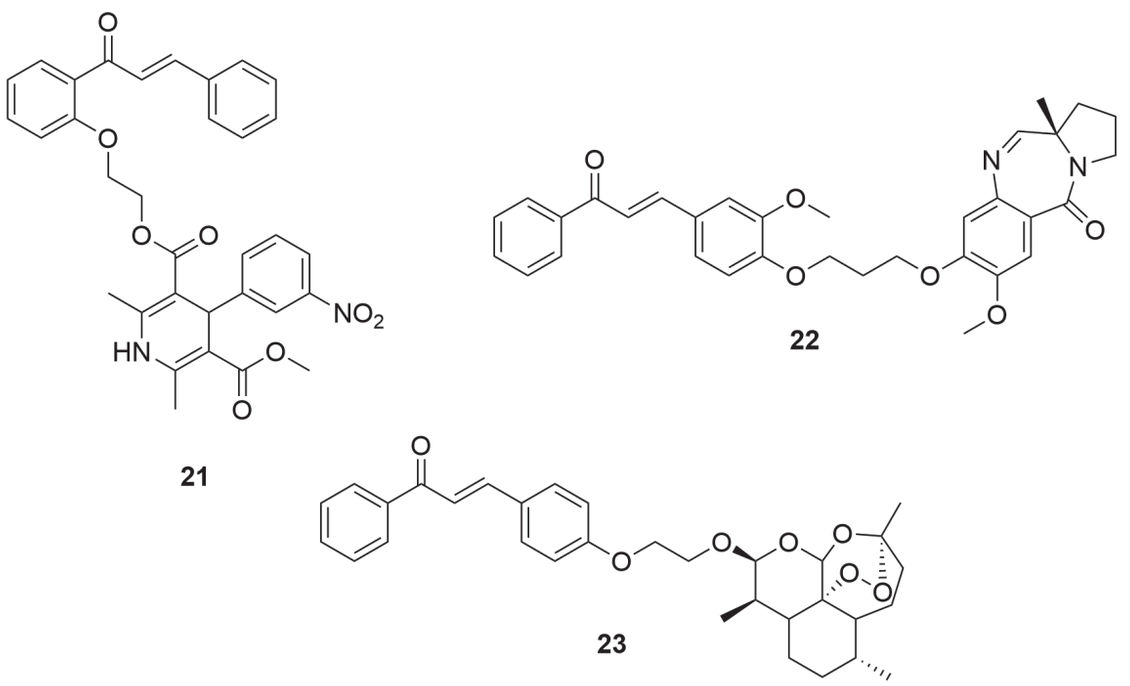


Figure 7.
Chemical structures of example chalcone hybrids linked though a diol linker [43].

Chalcones have been hybridized via an ethylene glycol (or related diol linker) linker (often used in medicinal chemistry to enhance solubility) (**Figure 7**) [41, 42]. Analogues such as the 1,4-dihydropyridyl-chalcones (**21**–**24**) show growth inhibition via an undetermined mechanism of action [43].

Access to chalcones can be via traditional solution phase synthesis approaches, but in some instances there are reports of the use of solid supports. With **22**, a supported intramolecular aza-Wittig reductive cyclization is utilized to afford a compound that showed high activity in the NCI-60 cell line panel. Acidity appears to be related to a high level of DNA binding as determined by thermal denaturing analysis [44, 45]. In keeping with the chimeric theme of this section, the inclusion of the dehydroartemisinin scaffold with and ethylene glycol spacer afforded **23,** which showed a 6-fold potency enhancement relative to the apparent dehydroartemisinin with an IC_{50} of 300 nM [46].

While amide linages have been reported in the development of chalcone hybridism, the use of an ester moiety has the added advantage of allowing a cellular

esterase cleavage of the hybrid to afford the two parent drugs. In principle, this may allow the presentation of three different biologically active compounds simultaneously: the hybrid, the chalcone, and the co-drug. The ester approach to coupling compound pharmacophores has been elegantly demonstrated with chalcone hybrids leveraging the often-present hydroxyl moiety. This approach, obviously, can also afford the corresponding ether (versus ester) linked analogue, which is significantly more cleavage resistant. Within this coupled pharmacophore environment, chalcone-amidobenzothiazole chimeras **24** and **25** are 0.185–3.3 μM potent across a panel of cancer cell lines (**Figure 8**). These analogues induce cell cycle arrest at the G_2/M phase boundary [47]. Platinum incorporation into the amidobenzothiazole moiety with **26** also gave excellent anticancer activity across 21 cell lines, but no real potency boost relative to the parent chalcone, but the chimera did yield a different long-term treatment and mechanism of apoptosis induction pathway that may be beneficial, especially in the onset of resistance should this occur [48].

Like a significant number of other chimeric compounds, "click-approaches" have also been applied in the development of a series of chalcone-coumarin chimeras, for example, **27** and **28** (**Figure 9**). Gratifyingly, these chimeras displayed higher efficacy against HepG2 cells than etoposide combined with negligible toxicity toward normal cells. Molecular docking studies support, but do not confirm, that these hybrids may act through binding with both tubulin and falcipain [49].

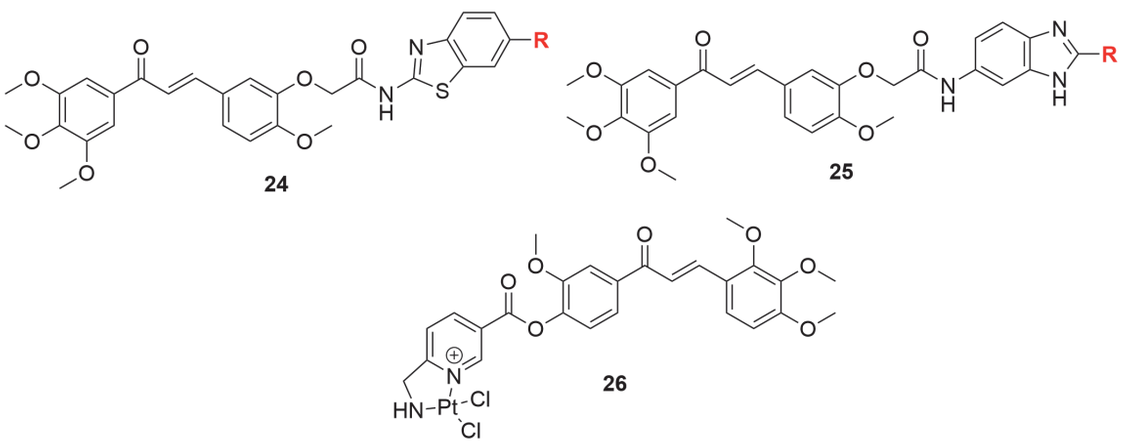


Figure 8.
Selected ester and ether-linked chalcones [47, 48].

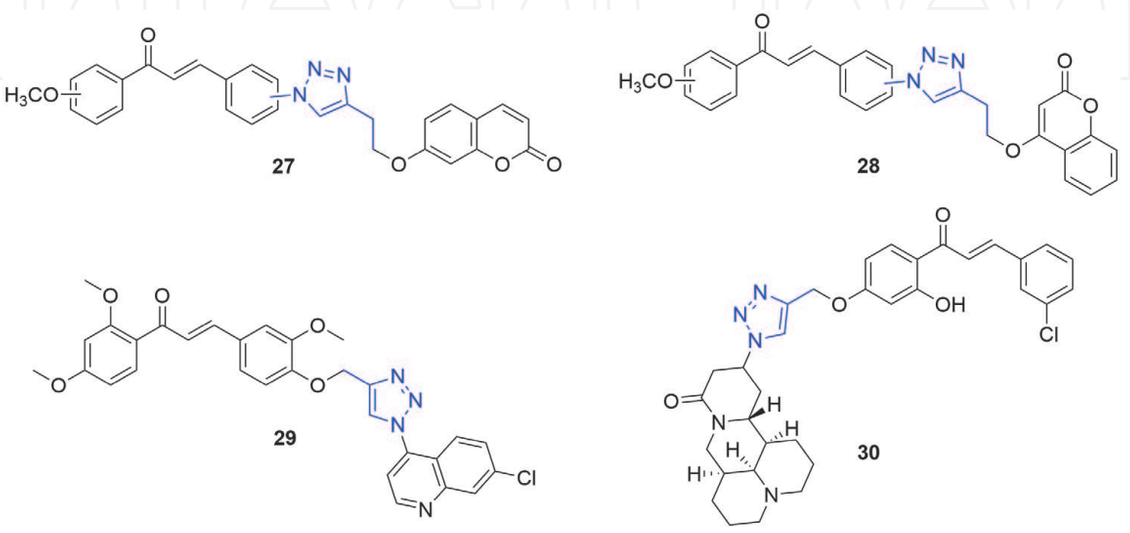


Figure 9.
Selected exemplars of Huisgen "click-linked" chalcone hybrid molecules [50].

Within this subset of click conjugates, **29** demonstrated higher efficacy than the archetypal cytotoxic agent 5-fluorouracil against four human cancer cell lines (A459 (lung), Bel-7402 (hepatocellular), HeLa (cervical), and MCF-7 (breast)) and low cytotoxicity to NIH3T3 normal cells. Chalcone **29** was synergistic with matrine against the A459 cell line demonstrating a favorable in vivo safety profile. Chalcone **29** has been reported as capable of effecting aa ca 90% tumor burden reduction in a A459 mouse xenograft model (10.0 mg/kg/day, 20 days, iv) without any apparent loss of body weight [50].

Continuation of the click-linked chalcone hybrids with β-lactams [51] revealed **30** as the most potent analogue within a discrete library. Of particular note was IC_{50} values < 1 μM against A549 and THP-1 (leukemia) cancer cells [52]. No other noteworthy analogue was reported.

Loch-Neckel et al. reported a further investigation of the mechanism of an analogue (**31**; **Figure 10**) [53]. In vitro and in vivo, it could inhibit glioma cell growth and induce mitochondrial apoptosis in U87-MG glioma cells via the inhibition of MDM2.

Several hybrids besides those discussed above have also been reported to exhibit potent anticancer activities (**Figure 11**). For example, β-carboline–chalcone (**32**) exhibits significant DNA binding interaction and DNA stabilization [54]. Imidazothiazole-chalcone (**33**) exhibits promising cytotoxicity with a microtubule destabilizing mechanism and could compete with colchicine [55, 56]. Anthraquinone-chalcone (**34**) shows high cytotoxicity in HeLa cells [57, 58]. The compound induces the activity of caspase-3 and caspase-8 in HeLa cells and has shown potent inhibition of MMP-2 secretion.

2.2 Inhibition of tubulin

Clinically, targeting microtubules—the multifunctional cytoskeletal proteins comprising α- and β-tubulin heterodimers—has provided considerable success in the treatment of multiple cancers. Archetypal microtubule-targeting compounds include the taxanes used in the treatment of metastatic pancreatic cancer [59], and vinca alkaloids in the treatment of hematological and lymphatic neoplasms [60].

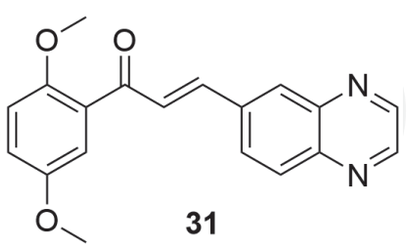


Figure 10.
*Chemical structure of chalcone **31**, (E)-1-(2,5-dimethoxyphenyl)-3-(quinoxalin-6-yl)prop-2-en-1-one [53].*

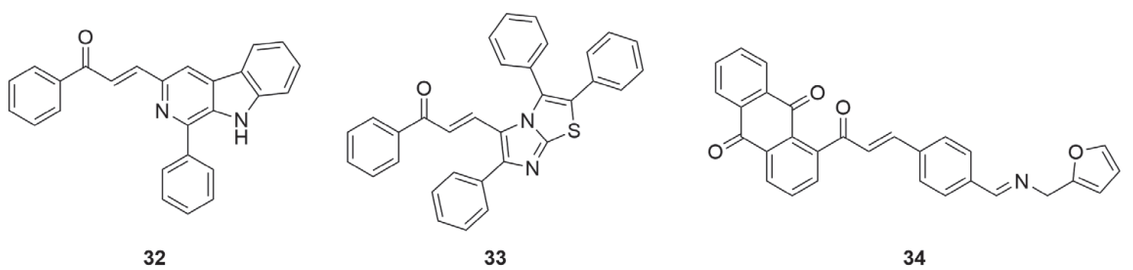


Figure 11.
Representatives of fused chalcone analogues [54].

However, the continued use of these agents, like a significant number of anticancer drugs, is limited by toxicity (here neurotoxicity) and drug resistance [61, 62]. In this target space, multiple natural and synthetic chalcones (**35**-**42**) have been reported with microtubule activity (**Figure 12**).

Given the current toxicity and resistance issues with the taxanes and vinca alkaloids, in combination with the decorated microtubule activity of chalcones, it is not surprising that the hybridization of these compound classes has been explored. Some groups have attempted to develop these combination drugs using rational drug design approaches. Niu approached this via molecular docking and high-correlation quantitative pharmacophore models (40 compounds with experimental data and 800 decoys to discriminate active versus inactive molecules) were generated using the SAR of known tubulin inhibitors [63]. Model validation followed by virtual screening identified ca. 1000 drug-like molecules that were pharmacophore matched. Ultimately, five differentially substituted (**43**–**47**) chalcones with diverse substituents and strong molecular interactions with the key amino acids in the tubulin-binding site were identified (**Figure 13**). Two compounds were of particular note, with **48** and **49** (**Figure 14**) demonstrating potent inhibitory activity against MCF-7 cells with IC_{50} values of 28 and 54 nM respectively [63].

Isolated from *Combretum caffrum*, Combretastatin A-4 is a microtubule-targeting natural product that has been further developed through imidazolone incorporation (**51**) (**Figure 15**) [64]. Evaluation in a broad-spectrum panel containing 53 human tumor cell lines spanning leukemia, non-small cell lung, colon,

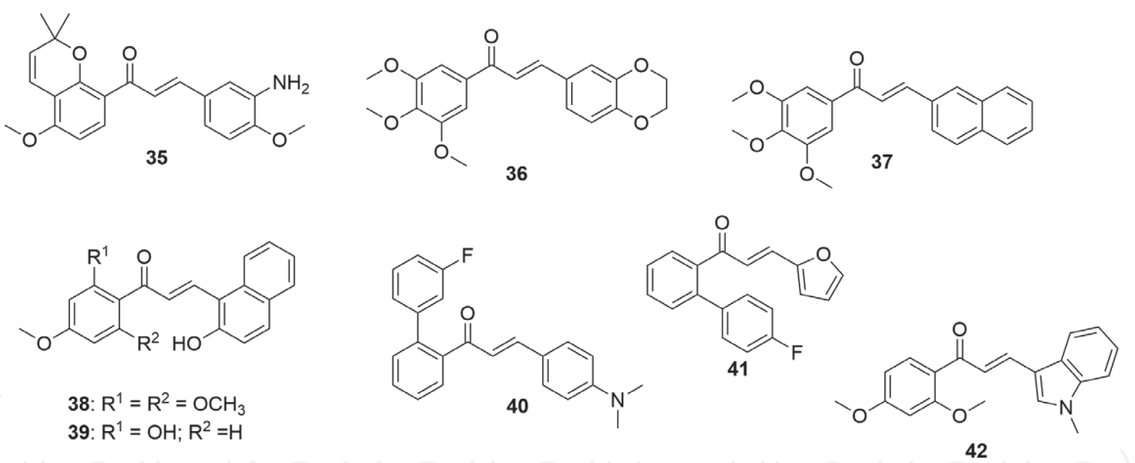


Figure 12.
Examples of natural and synthetic chalcones with reported biological activity against microtubules [60].

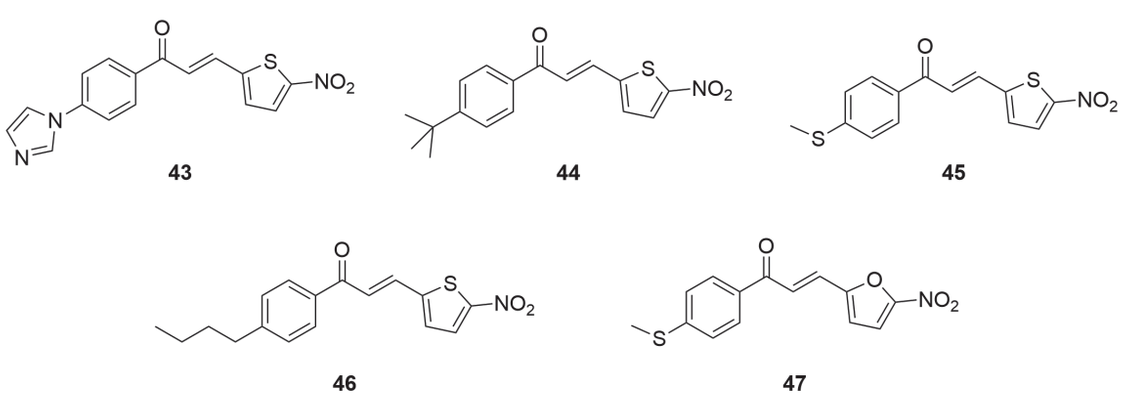


Figure 13.
Selected chalcones arising as hits from QSAR-based drug design strategy [63].

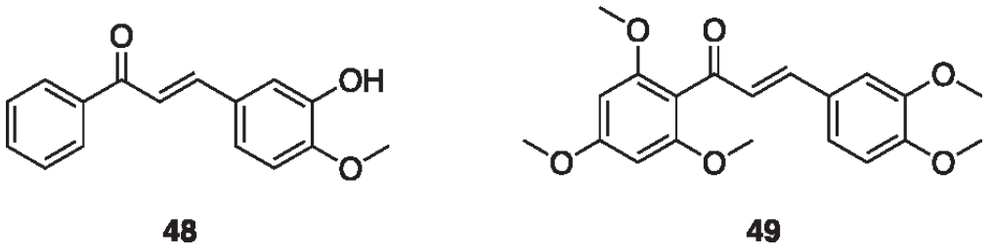


Figure 14.
*Chalcones with anticancer activity identified by integration of ligand-based pharmacophore screening and molecular docking studies [63]. Active against the breast cancer cell line, MCF-7 with IC_{50} = 28 and 54 nM, respectively (**48**, **49**).*

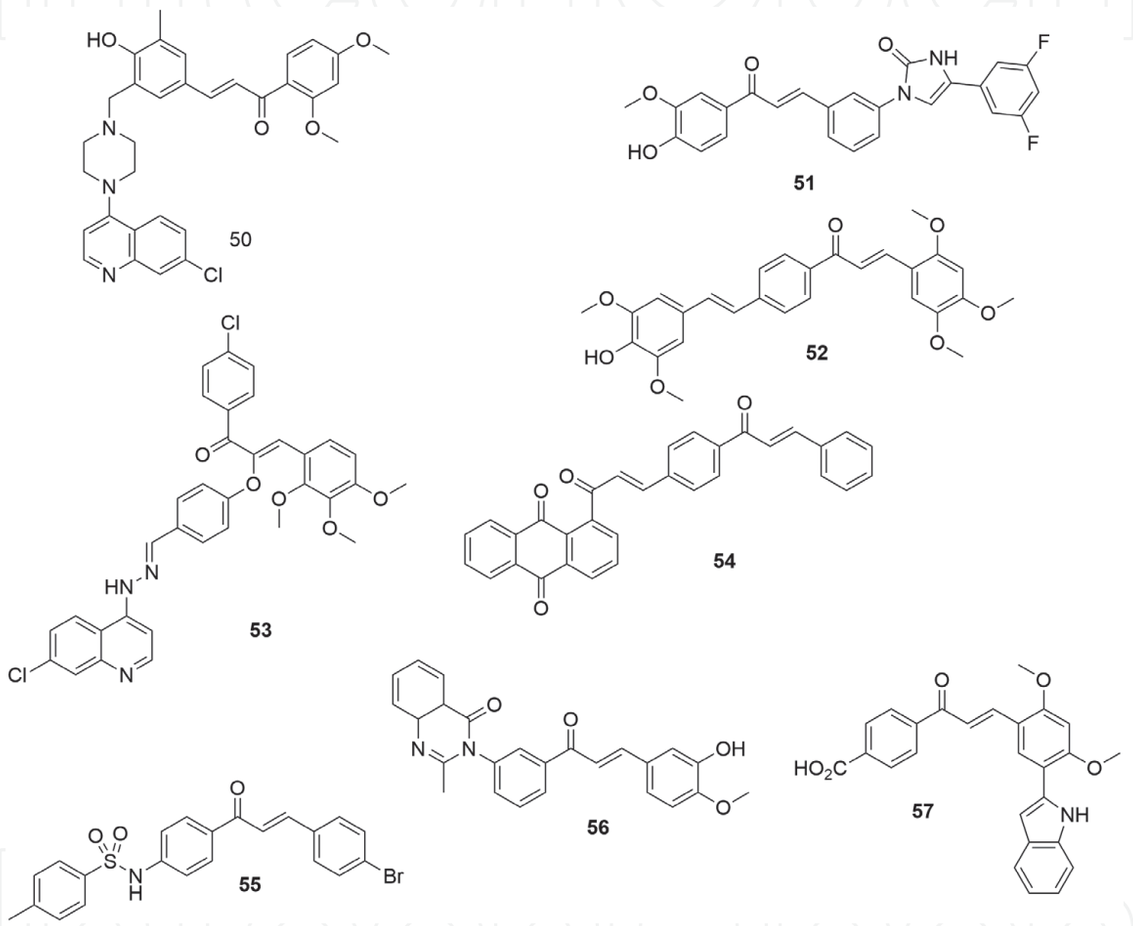


Figure 15.
Variety of linked chalcones including examples that induce apoptosis via the PI3K/Akt/mTOR pathway [64–67].

CNS, renal, prostate, ovarian, breast, and melanoma determined **51** showed good anticancer activity, with GI_{50} values ranging from 1.26 to 10.5 μM and arresting cells at G_2/M phase [65].

Other hybrids, for example, anthraquinone-chalcone (**54**), have high cytotoxicity toward HeLa cells but low toxicity to normal cells [66], as does the quinazolinone chalcone derivative (**56**), which induces mitochondria-dependent apoptosis and inhibits the PI3K/Akt/mTOR signaling pathway [67].

Chalcone modification afforded the amino-substituted **58** with subsequent examination of these analogues for their tubulin-binding, vascular-targeting, antitumor and antimetastatic activities revealing it to be the best compound in the series developed by Canela et al [68]. Chalcone **58** inhibited the proliferation of

endothelial (HMEC-1, microvascular endothelial cell line-1; and BAEC, bovine aortic endothelial cells) and tumor (B16-F10.luc2, melanoma cells expressing firefly luciferase 2; Cem; and HeLa) cell lines with IC_{50} values of 1 and 4 nM [68]. The low solubility of **58** (0.016 mg/mL) was ameliorated through the incorporation of an amino acid-based pro-moiety with the L-Lysine-L-Proline derivative **59** approximately 2000x more soluble than **58** (**Figure 16**). Pro-drug **59** was effective in inhibiting tumor and endothelial cell proliferation, parent **58** was successfully released by the liver, and **59** demonstrated excellent in vivo anticancer activity in melanoma (10 mg/kg) and breast cancer models (15 mg/kg) by causing rapid intertumoral vascular shutdown and massive tumor necrosis [68].

Isolated from *Millettia pachycarpa*, millepachine (**60**) induces cell cycle arrest and apoptosis in human hepatocarcinoma cells in vitro and in vivo [69]. The development of amino-substituted-millepachines was explored in an effort to enhance the antiproliferative activity of the natural product lead (**Figure 17**). Millepachine derivative (**61**) exhibited excellent anticancer activity against a panel of drug-sensitive cancer cell lines and multidrug-resistant cancer cells. Studies support the inhibition of tubulin polymerization as the mechanism of action, by binding at the colchicine site [70–72]. More conformationally restrained analogues **62–65** and **68** in this amino-substituted series retained high levels of activity, again acting as anti-microtubule agents.

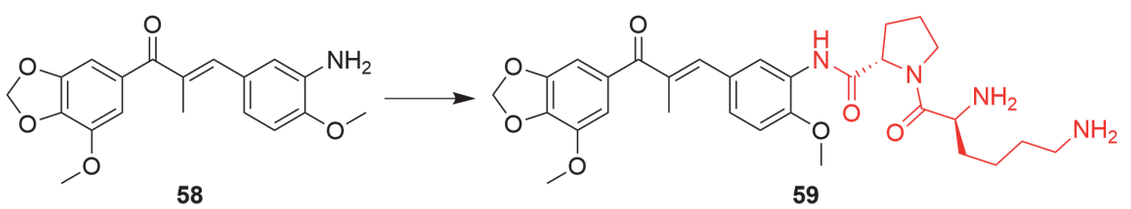


Figure 16.
*Latentiation represented by the replacing the amine of **58** with an L-Lysine-L-Proline (red) [68].*

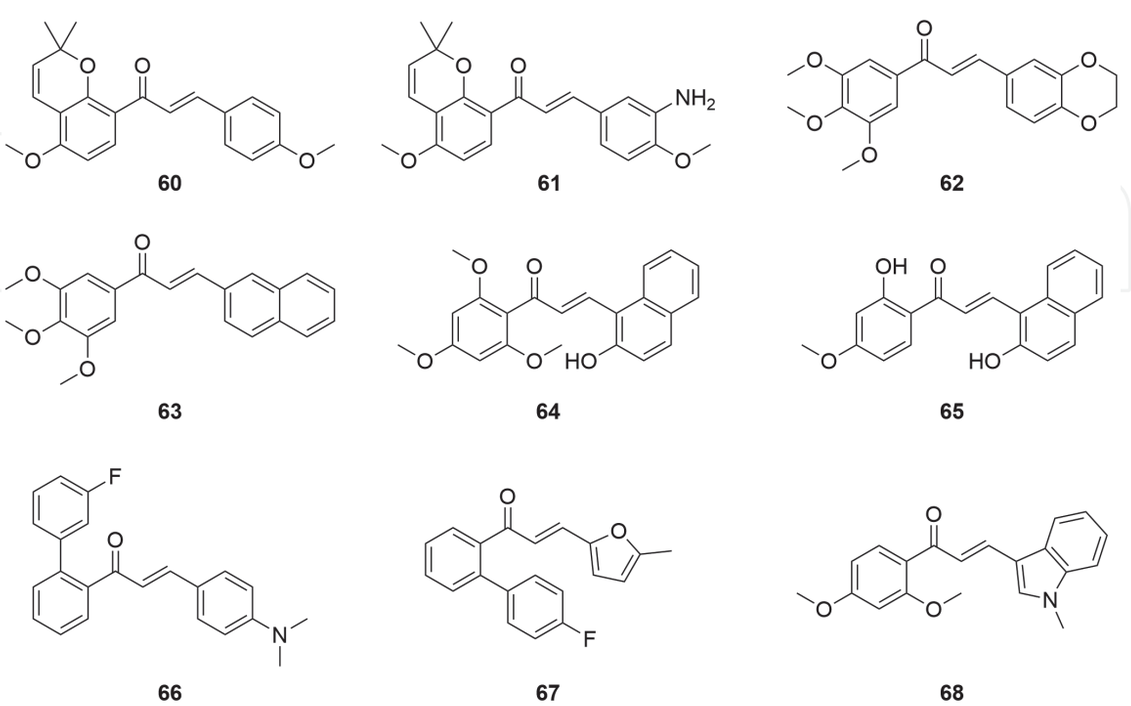


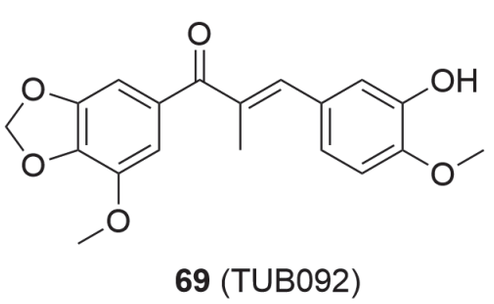
Figure 17.
Family of novel chalcones hypothesised to inhibit tubulin by binding at the colchicine site [70–72].

Novel o-aryl chalcone (**66**) showed potent cytotoxicity against several multidrug-resistant cancer cell lines (paclitaxel-resistant human ovarian carcinoma cells, vincristine-resistant human ileocecum carcinoma cells, and doxorubicin-resistant human breast carcinoma cells) in an extremely low nanomolar range. This has been shown to be G2/M phase cell cycle arrest effect, mediated by **66** binding to the colchicine site of tubulin as was observed with **62**–**65** and **68** [73], which is a key feature of anti-microtubule agents [74]. Acting via the colchicine-binding site [75], **67** was effective in mouse A549 xenograft models with no dose-limiting weight loss observed [37, 73]. An indole-chalcone (**68**), namely, IPP51, induced prometaphase arrest and the subsequent apoptosis of bladder cancer cells and showed a significant inhibition of tumor growth without a great loss in body weight [75].

Confirmation of the tubulin-binding target has been obtained with TUB091 (**58**), TUB092 (**69**), and TUB099 (**59**) series of compounds [68]. TUB092 (**70**) was soaked in the crystals of a protein complex comprising αβ-tubulin (T2) dimers, a stathmin-like protein RB3 (R), and tubulin tyrosine ligase (TTL) (**Figure 18**). The subsequent high-resolution cocrystal structure (2.4 Å) showed the chalcone bound with tubulin at the colchicine-binding site. The chalcone is organized such that the 1,3-benzodioxole ring of **69** is located in the β-tubulin residue-derived hydrophobic pocket, with a water-mediated hydrogen bond to the backbone carbonyl and amide of Gly237 and Cys241. The α,β-unsaturated ketone hydrogen bonds with Asp251 and two additional water-mediated hydrogen bonds are evident from the backbone carbonyls of Thr179 and Asn349 with the hydroxyl and methoxy groups. Optimization of lead solubility via L-Lys-L-Pro dipeptide incorporation yielded pro-drug **59** with a ca. 2000x solubility enhancement (31 mg/mL in PBS versus 0.016 mg/mL in PBS). **59** also inhibited primary tumor growth and spontaneous metastasis in mice (iv injection, 10 mg/kg, 5 days) with 90% or higher inhibition.

2.3 Miscellaneous chalcones

Chalcone-benzoxaborole (**70**), prepared from the intermediates 6-formylbenzoxaborole and the corresponding ketone, has recently been found to inhibit *Trypanosoma brucei* growth and possess antitrypanosomal activity (**Figure 19**) [76]. Boronic-chalcone (**71**) was described early in 2002 as a fluorescent probe for the detection of fluorides [77]. Boronic-chalcone hybrid (**71**) exhibits not only fluorescent properties but also other biological activity. Compound **71** has been reported as an antitumor agent targeting MDM2 oncoprotein [78]. Compound **72** can induce antitumor activity against malignant glioma cell lines both in vitro and in vivo [79]. Compound **73** exhibits potent anticancer activity (HCT116 cells, IC_{50} = 3.9 μM) together with proteasome inhibitory activity [80].



69 (TUB092)

Figure 18.
*Chemical structure of TUB092 (**69**), which binds to tubulin at the colchicine site; this analogue is directly related to chalcones **58** and **59** (see* **Figure 16***) [68].*

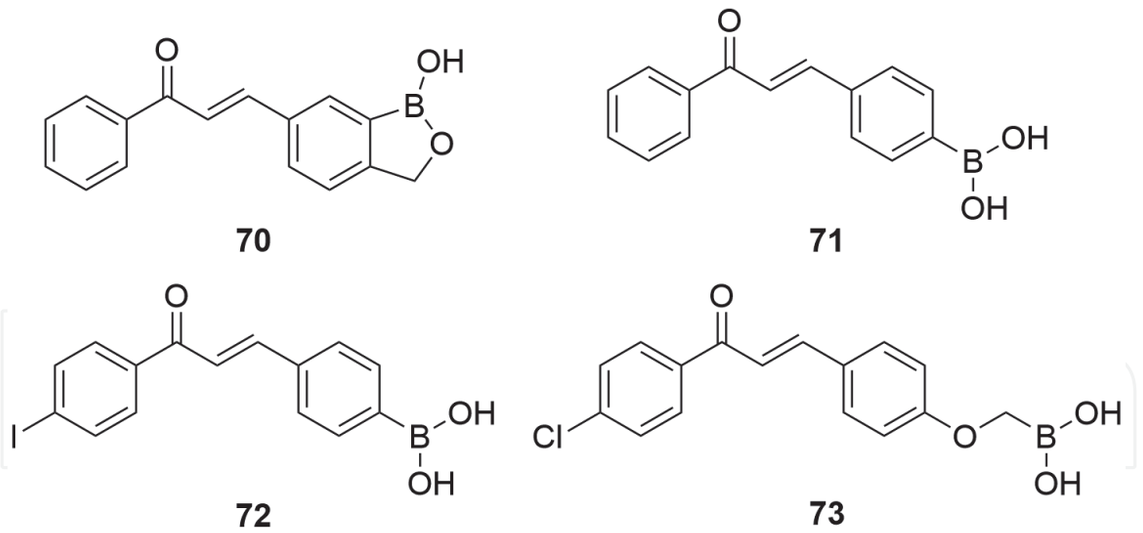


Figure 19.
Chemical structures of boron-containing chalcones [76–80].

2.4 Selected mechanism of action studies

Ducki et al. predicted tubulin to be the target of chalcone due to the similarity between chalcone and the β-tubulin inhibitor combretastatin A4 [81]. A 5D-QSAR model was used to conclude that the methyl group at the α-position made a sizable difference in the preferred conformation from s-cis (**74**) to s-trans (**75**) for tubulin binding. This theory explains the high potency of α-methyl chalcone (K562, IC_{50} = 0.21 nM; tubulin, IC_{50} = 0.46 μM) [81, 82]. In 1992, MDL-27048 (**76**) was the first chalcone found to have antimitotic activity [83]. This compound was bound to tubulin at the colchicine-binding site and inhibited tubulin polymerization. Based on a proposed binding model for MDL-27048 [83, 84], a virtual screening of 9720 natural compounds was carried out. Compound **77** has been found to show good inhibitory activity of tubulin polymerization [85]. Compounds **78**–**85** (**Figure 20**) have been designed and synthesized in later medicinal chemistry work. These compounds were originally predicted to bind to tubulin at the colchicine-binding site, which has been confirmed via in vitro competition binding assays [47, 75, 86–90]. Very recently, a series of novel indole-chalcone derivatives were synthesized and evaluated for their antiproliferative activity. Among these indole-chalcones, compound **81** has exhibited IC_{50} values of 3–9 nM against six cancer cell lines, with similar activities against resistant cancer cells, and low toxicity toward normal human cells. Molecular docking and mechanistic studies have demonstrated that this compound could bind to the colchicine-binding site, inhibit tubulin polymerization with an IC_{50} of 2.68 μM, arrest the cell cycle at the G2/M phase, induce apoptosis, and decrease the mitochondrial membrane potential (MMP). Moreover, this compound and its phosphate salt **82** with better water solubility have been shown to exhibit 66 and 70% in vivo antitumor inhibitory rates (ip, 30 mg/kg), respectively, without any apparent loss of body weight.

2.5 Targeting topoisomerase

Critically, the correct assembly of DNA is essential for cellular function. This assembly is in part governed by a series of topoisomerases (TOPOs), including TOPO-I and TOPO-II, which are responsible for the winding and unwinding of DNA. TOPO function is a critical process for DNA transcription and replication, and has been targeted as an anticancer strategy [91]. Several chalcones have shown

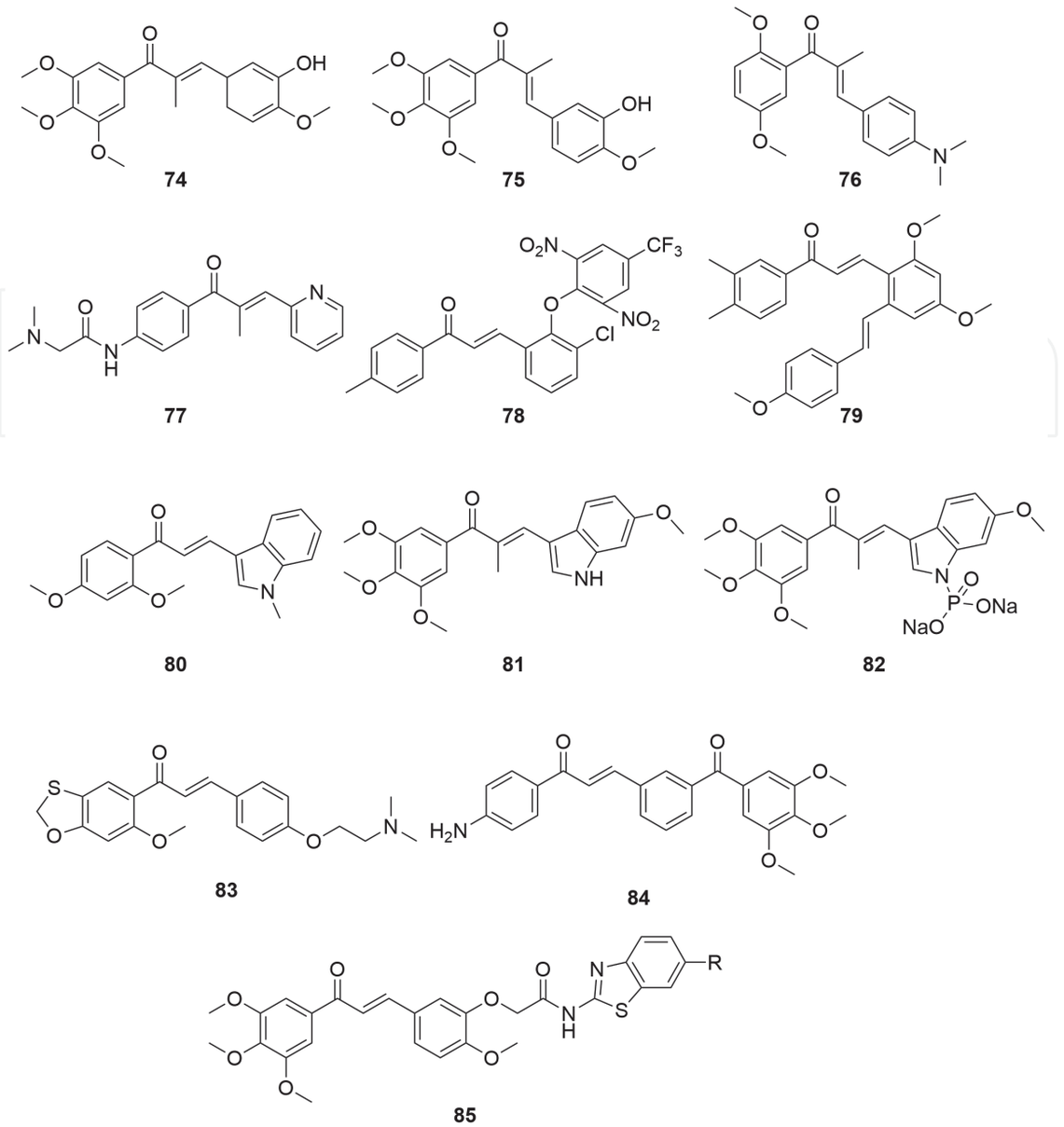


Figure 20.
Chalcones computationally predicted to target tubulin [81, 82].

TOPO inhibitory activity [92]. Chalcone **86** in addition to being a nonspecific inhibitor of TOPO-I/II also inhibits cathepsin. The observed cytotoxicity was comparable with camptothecin and etoposide. The correlations between TOPO inhibition and cytotoxicity was not reported [93]. Natural product-derived (isolated from *Angelica keiskei*) chalcone-based inhibitors of TOPO-II have also been reported by the Akihisa group with the most potent in the series, **87**, a more potent TOPO-II inhibitor than etoposide (a clinically used TOPO inhibitor) [93]. Other chalcone-based selective TOPO-II (versus TOPO-I) inhibitors such as **88** have been reported with high anticancer activity (**Figure 21**) [93, 94].

2.6 Estrogen receptor

In many cancers, the hormone receptor status is a governing factor in determining the treatment protocols. For example, in breast cancer key treatment drivers relate to the presence (or absence) of the estrogen, progesterone, and HER receptor subtypes. The first two receptors are sex-linked and act as transcription factors guiding the interplay between endogenous ligands such as 17β-estradiol. There are

multiple clinical drugs that target aberrant ER activity to alleviate the symptoms of menopause, inflammation, and cancer [95].

Chalcone isoliquiritigen in (**89**) was isolated from liquorish root and displayed a concentration-dependent differential activity against ER-positive breast cancer cells (T-47D) (**Figure 22**). At low concentrations, **89** stimulates cell growth, at high dose it displays inhibitory activity; intriguingly, the activity at high concentration was noted as being ER concentration independent. In agreement with other studies, **89** was found to directly bind to the ER with low micromolar affinity. The related candidachalcone (**90**) is also an ER-responsive chalcone; and while not exact data were presented, it is suggested to be a mid-micromolar ER ligand [96, 97].

Gan et al. reported that chalcones **91** and **92** showed cellular TrxR inhibitory activity in a panel of Michael acceptor-type pharmacophores (**Figure 23**). MS analysis demonstrated that the most potent chalcone derivative (**92**) covalently modified TrxR at the selenocysteine residue U498 [21]. In 2015, Zhang et al. reported a series of chalcone analogues based on xanthohumol (**4**). Among them, compound **93** displayed good cytotoxicity against HeLa cells (IC_{50} = 1.4 μM),

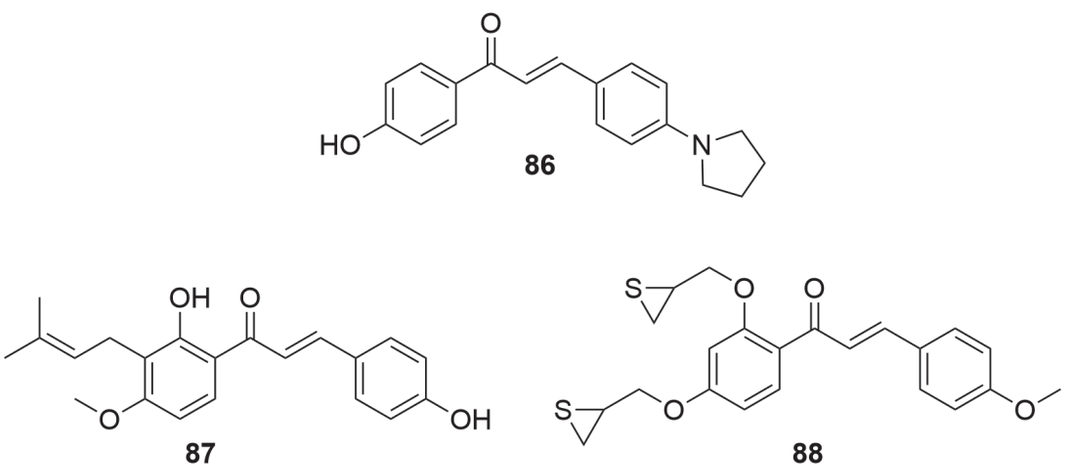


Figure 21.
Chemical structures of chalcones known to inhibit topoisomerases (TOPO) [93, 94].

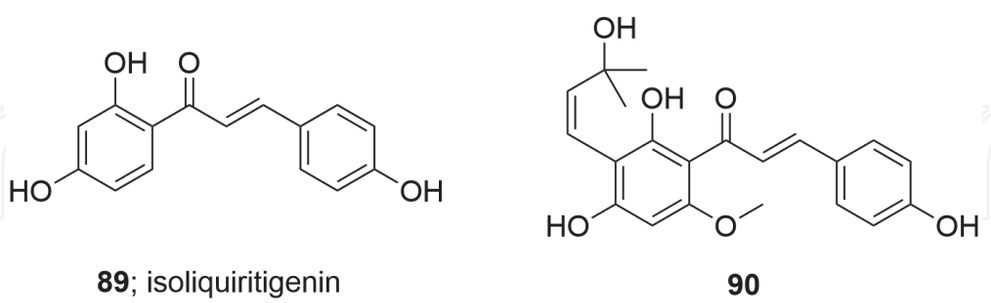


Figure 22.
*Chalcone isokiquiriten (**89**) and an analogue (**90**) with activity against the ER-positive breast cancer cell line (T-47D) [96, 97].*

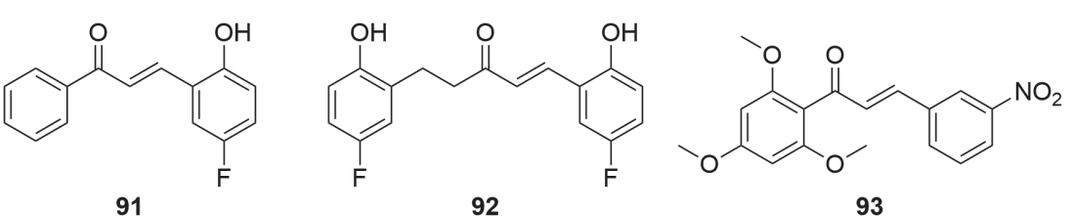
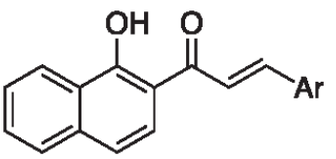


Figure 23.
Chemical structures of selected chalcones known as TrxR inhibitors [21].

selective inhibition of TrxR, and induction of cell apoptosis. Mechanistically, the U498C mutation of TrxR was performed to support the covalent mechanism. As a result, this compound could significantly decrease the cellular thiol level and induce the expression of reactive oxygen species (ROS) [98].

2.7 Targeting CYPs

The parent analogue, ANF (**94**), has long been recognized as displaying a broad range of pharmacological activities. Of particular note is its effects on CYP1. It has been shown that ANF (**94**) is capable of reversing CYP1B1-mediated drug resistance, increasing the efficacy of cytotoxic drugs. Simple ring opening of the flavone moiety releases a hydroxynaphthyl chalcone (**95**). Modification of the pendant phenyl ring was shown to impart modest CYP inhibition relative to ANF (**Table 3**). The parent phenyl (**96**) displays a CYP1B1 IC_{50} of 157.7 nM (c.f. ANF IC_{50} 5.9 nM). Introduction of ring substituents enhances activity with the 4-F (**97**; 48 nM),



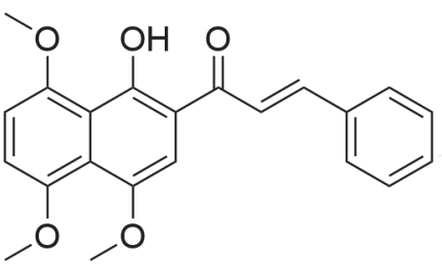
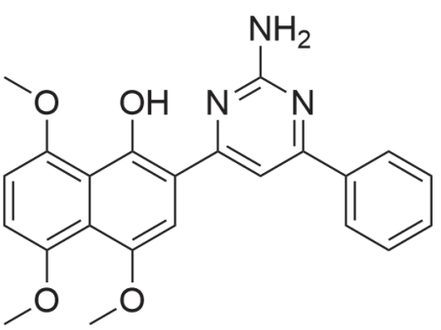
Ar	CYP IC_{50} values (nM)			IC_{50} ratio	
	1B1	**1A1**	**1A2**	**1A1/1B1**	**1A2/1B1**
ANF **94**	5.9 ± 1.3	80.3 ± 3.6	18.0 ± 3.6	13.6	3.1
Ph **95**	157.7 ± 18.5	>1000	>1000	>6.3	>6.3
4-FPh **96**	48.3 ± 8.2	>1000	>1000	>20.7	>20.7
2-OCH3Ph **97**	37.8 ± 5.8	556.6 ± 28.7	>1000	14.7	>26.5
3-Pyridyl **98**	16.7 ± 3.7	117.1 ± 18.9	227.0 ± 25.2	7.0	13.6
					
99	4.9 ± 0.6	161.3 ± 17.4	734.7 ± 31.2	32.9	149.9
					
100	4.8 ± 0.5	51.8 ± 9.7	>1000	10.8	>208.3

Table 3.
Inhibitor potency of modified chalcones against CYP1 enzymes.

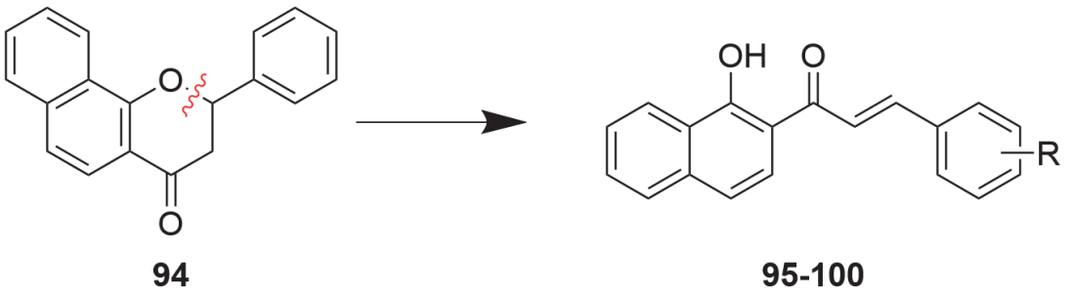


Figure 24.
Structural modification of ANF leading to a family of CYP1B1 inhibitors displaying high levels of cytotoxicity, see **Table 3** *for details of "R" [99].*

	IC_{50} values (μM)			
Compound	**MCF-7**	**MDA-MB-231**	**LCC6/P-gp**	**MCF-7/1B1**
ANF (**94**)	80.7 ± 7.6	>100	82.3 ± 8.5	>100
95	25.9 ± 3.2	46.2 ± 5.3	>100	32.3 ± 4.5
96	48.1 ± 3.7	79.6 ± 4.9	48.6 ± 2.9	>100
97	43.4 ± 1.6	75.3 ± 6.8	43.9 ± 4.4	>100
98	7.6 ± 0.7	19.8 ± 2.8	8.0 ± 2.3	12.7 ± 1.7
99	6.3 ± 1.4	15.8 ± 2.2	9.6 ± 1.3	15.1 ± 1.8
100	46.8 ± 4.7	48.9 ± 4.1	>100	20.4 ± 3.3

Table 4.
Cytotoxic activities of benzochalcones against MCF-7, MDA-MB-231, LCC6/P-GP, and MCF-7 cell lines.

2-OCH_3 (**98**; 37.7 nM), and 3-pyridyl (**99**; 16.7 nM), also with improved CYP1A2/B1 selectivity. Increasing the hydrophobicity of the phenyl moiety was detrimental to potency, but the introduction of a tetrasubstituted naphthyl moiety proved to be highly efficacious [99] (**Figure 24**).

Of the modified ANF analogues reported, **100** and **101** displayed CYP1B1 inhibition level equivalent to ANF but with significantly enhanced selectivities of 150- and >200-fold (ANF, CYP1A2/B1 = 3.1) (**Table 3**).

Examination of these analogues against MCF-7, MDA-MB-231, LCC6/P-gp, and MCF-7/1B1 revealed the 2-pyridyl chalcone analogue to be broad-spectrum active in both the wild type (MCF-7 and MDA-MB-231 cells) and also in the drug-resistant cells (LCC6/P-gp and MCF-7/1B1) (**Table 3**). Replacement of the phenyl moiety with a tetramethoxynaphthalene resulted in a drop in CYP1B1 activity (IC_{50} > 1000 nM), but the MCF-7 and LCC6/P-gp cytotoxicity increased. This presumably is a consequence of increased cellular uptake [99] (**Table 4**).

3. Conclusions

The medicinal chemistry landscape is a mobile one. Approaches that were viewed as unviable mere 5- to 10-years ago are now gaining traction. The introduction of PAINS filters has stymied some areas of medicinal chemistry development, correctly; but in other areas, the changing paradigms may necessitate a reexamination of the type of screening filters applied. This is especially relevant to the potential development of chalcones in the anticancer drug space. It has been

consistently shown that not only do these agents possess high levels of antiproliferative activity as single agents, they synergise well across a significant number of clinically used anticancer drugs.

As this field progresses, careful reevaluation of off-target effects, compound specificity, and promiscuity will remain key, but there is significant potential for transformation of chalcones into true clinical compounds. It is worth noting that it is the role of the medicinal chemist to modulate the unfavorable effects of lead compounds in the development of clinical candidates. This, perhaps though is best left in an academic environment until "compound cleaning" to a true development candidate can be achieved.

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Conflicts of interest

The authors declare no conflicts of interest.

Author details

Adam McCluskey and Cecilia Russell*
Chemistry, The University of Newcastle, University Drive, Callaghan, NSW, Australia

*Address all correspondence to: cecilia.russell@newcastle.edu.au

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