Send Orders for Reprints to reprints@benthamscience.net

Current Medicinal Chemistry, **2015,** *Volume*

Chalcones as Promising Lead Compounds on Cancer Therapy

Antonio J. León-González[a, b], Nuria Acero[c], Dolores Muñoz-Mingarro[c], Inmaculada Navarro[d], Carmen Martín-Cordero[*a]

[a] *Departamento de Farmacología, Facultad de Farmacia, Universidad de Sevilla, Prof. García González 2, 41012 Sevilla (Spain);* [b] *UMR 7213 CNRS, Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, Université de Strasbourg, Illkirch, France;* [c] *Departamento de Biología, Facultad de Farmacia y Medicina, Universidad San Pablo-CEU, Urbanización Montepríncipe, Boadilla del Monte, 28668 Madrid, Spain;* [d] *Departamento de Química Física, Facultad de Farmacia, Universidad de Sevilla, Prof. García González 2, 41012 Sevilla (Spain).*

Abstract: Chalcones constitute a group of phenolic compounds that command an increasing interest on cancer research. Natural chalcones are widespread through the plant kingdom. The most abundant and investigated chalcones are isoliquiritigenin, flavokawain and xanthohumol, which are present in the Fabaceae, Piperaceae, Cannabaceae, and Moraceae families. These chalcones have been shown to be promising lead antitumor-chemopreventive drugs by three different activities: antioxidants, cytotoxic and apoptosis inducers. In recent years, SAR (structure-activity relationship) has contributed towards the improvement of anticancer properties of chalcones by substituting aryl rings and introducing heterocyclic moieties. This review summarizes the anticancer activities shown by natural chalcones and the SAR and describes how different chemical moiety modifications could lead them to be therapeutically useful in the treatment of cancer.

Keywords: Apoptosis; cancer; chalcone; chemoprevention; flavonoid; synthesis; tubulin.

1. INTRODUCTION

Chalcones constitute an important group of phenolic compounds, biosynthesized through the shikimate-acetate pathway. Their chemical structure is constituted by an α,β-unsaturated ketone, and they are also known as 1,3-diaryl-2-propen-1-ones. Different hydroxy, methoxy or prenyl substitutions on their aryl rings [1] provide a wide structural diversity on the chalcone family.

Chalcones are widely occurring in plants, as precursors of flavonols and other flavonoids synthesis. They are commonly present as yellow pigments in flowers and other parts of the plant: roots, rhizomes, heartwood, barks, buds, leaves, fruits and seeds. Chalcones are biosynthesized mainly by plant species from Fabaceae, Asteraceae and Moraceae families. Noteworthy, a few chalcones have also been identified in the Annonaceae, Lauraceae, Myrtaceae, Piperaceae, Pteridaceae, Empetraceae and Cannabaceae families. Chalcone-containing plants such as Angelica, Glycyrrhiza, Humulus, Sophora, Ficus, Dorstenia, Morus, Artocarpus, Corema, and Piper plants are commonly used as spices, edible fruits and vegetables and as traditional herbal medicine [2-5].

*Departamento de Farmacología, Facultad de Farmacia, Universidad de Sevilla, Prof. García González 2, 41012 Sevilla (Spain) Tel. 0034 954 55 67 23; E-mail: carmenmc@us.es

Chalcones have attracted growing attention during the last decade due to their chemopreventive, anti-inflammatory, antioxidant, cytotoxic, antimicrobial, antiprotozoal, antihistaminic, analgesic, anti-diabetic, and immune-modulator properties [6-11].

There are several ongoing projects to find more effective synthetic chalcone analogues based on the above known mechanisms of natural chalcones [10, 12]. This review summarizes the anticancer and chemopreventive properties of natural chalcones and the chemical modifications in their structures that could lead to potential therapeutic cancer agents.

2. MECHANISMS INVOLVED IN THE ANTICANCER ACTIVITY OF CHALCONES

Chalcones are α,β-unsaturated ketones that could act as Michael acceptors in reactions with good nucleophiles, especially with thiol groups, such as those of cysteine residues in proteins. These direct interactions with intracellular targets allow chalcones to modulate some pathways involved in carcinogenesis. For example, butein inhibits the NF-κB pathway (further described in section 2.8) by directly inhibiting IKK-β on Cys-179, leading to a pro-apoptotic effect [13]. In addition, the preferential reactivity with thiols in contrast to amino and hydroxyl groups, makes these molecules less toxic than other therapeutic cancer drugs [14]. These compounds are structurally diverse, easily available, and inexpensive. Moreover, it is relatively easy to

XXX-XXX/14 $58.00+.00

synthesize the chalcones and to modify their chemical structure to obtain synthetic derivatives, thereby enhancing their bioactivity [15].

Chalcones have chemopreventive activities, that is, they are able to arrest, reverse or delay the carcinogenic process towards invasive cancer [2]. The steps of carcinogenesis include tumor initiation, promotion, and progression. Chalcones have shown to interfere with each of these three steps:

[1] Tumor initiation: Anti-initiation properties of chalcones are linked to their antioxidant activity, by scavenging reactive oxygen species (ROS) (superoxide radical, hydroxyl radical, hydrogen peroxide) and reactive nitrogen species (RNS) such as nitric oxide (NO). Furthermore, chalcones are able to inhibit cytochrome p450 (CYP1A1), among others phase I enzymes involved in the oxidation or reduction of xenobiotics. P450 generates even more free radicals during xenobiotics metabolism, thereby increasing the risks of carcinogenesis. Chalcones induce phase II detoxification enzymes that facilitate the excretion of xenobiotics, including glutathione-S-transferase (GST), NAD(P)H quinone oxidoreductase 1 (QR1), heme oxygenase-1 (HO-1), thioredoxin (Trx), and thioredoxin reductase (TrxR), exerting a protective effect against chemical-induced carcinogenesis [16, 17].

[2] Tumor promotion: Chalcones inhibit the generation and activation of endogenous tumor promoters such as prostaglandins (by inhibition of the main enzymes involved in the prostaglandin synthesis, cyclooxygenase-1 and 2, COX-1 and COX-2, respectively) and 17β-estradiol (by inhibition of aromatase). When tumor promoters are activated, they alter signal transduction pathways as well as DNA structure and transduction. Chalcones are able to inhibit tumor promoters, and therefore, stop the carcinogenesis process.

[3] Tumor progression: Chalcones arrest the tumor progression thanks to their cytotoxic, antiproliferative, and apoptotic properties. Chalcones have shown to inhibit invasion, metastasis and angiogenesis of cancer cells [18-22]. Chalcones reduce cancer cell viability, invasiveness, metastasis and angiogenesis by interacting with a variety of cell signaling proteins, such as the tumor suppressor protein p53 [23], the pro-apoptotic protein B-cell lymphoma 2 (Bcl-2) [24] and TNF-related apoptosis-inducing ligand (TRAIL) [25]. The main mechanisms of action of these molecules as anticancer drugs are summarized below.

2.1. Chemoprotective and antimutagenic effect

DNA damage by ROS, has been widely accepted as a key event in carcinogenesis. Free radicals damage DNA, thereby generating mutations, double strand breaks and genomic instability. Chalcones interfere with free radicals by three different mechanisms: a) blocking ROS synthesis; b) scavenging ROS once generated; c) protecting their targets, including DNA, against ROS attack; and d) repairing ROS damage. For example, xanthohumol acts as a chemopreventive agent against the toxicity induced by two types of ROS, hydroxyl, superoxide and peroxyl radicals [12, 26, 27]. Isoliquiritigenin also exhibited significant scavenging activity against peroxynitrite and its precursor nitric oxide (NO), thereby preventing cellular oxidative damage [28]. Certain chalcones have been recently shown as *in vitro* activators of the non-homologous end-joining DNA break repair pathway.[29]. This pathway repairs DNA breaks once DNA has been damaged by ROS damage.

As mentioned above, an alternative mechanism to protect against ROS attack is to eliminate them by detoxification. This occurs thanks to phase II enzymes, mainly present in the liver. The nuclear transcription factor (erythroid-derived 2)-like 2 (Nrf2) regulates the inducible expression of numerous detoxifying and antioxidant genes by binding to a specific DNA sequence known as ARE (Antioxidant Response Element), and hence is regarded as a promising target in the search of new chemopreventive agents. The cytosolic protein Keap1constutively binds Nrf2, promoting its permanent degradation by the proteasome [30]. This pathway is considered a key regulator of the antioxidant systems in response to both endogenous and exogenous ROS induced stresses. Chalcones with α,β-unsaturated ketone structure, such as xanthohumol, can modify the cysteine residues of the inhibitory protein Keap1, thereby causing its dissociation of Nrf2, which is subsequently translocated into the nucleus, binding to the ARE and promoting the transcription of phase II protein-encoding genes, including quinone reductase (QR1), heme oxygenase (HO-1) and thioredoxin 1 (TRX1) [31]. Wang et al. also reported that chalcones isolated from *Millettia pulchra* Kurz var-laxior (Dunn) Z. Wei (Yulangsan) have QR1 inducing activity and thus chemopreventive ability [32].

In vivo studies have shown that dietary feeding flavokawain A also induces QR1 and glutathione in its reduced form in liver, lung, prostate and bladder murine tissues without showing adverse effects on major organ function and homeostasis [16].

Chronic inflammation, characterized by overexpression of cyclooxygenase-2 (COX-2) and overproduction of prostaglandin E2 (PGE2), plays a key role in colorectal carcinogenesis. The chemopreventive effect of isoliquiritigenin on an *in vivo* azoxymethane-induced model of colon carcinogenesis was mediated by the decrease of PGE2 and NO production through the down-regulation of COX-2 and inducible nitric oxide synthase (iNOS) expression, respectively [33].

The initiation and proliferation of hormone-dependent cancers, including breast cancer, has been related to the estrogen exposure. In this sense, butein is able to prevent breast cancer by acting as an aromatase inhibitor, the enzyme that synthesizes estrogen [34]. Recently Monteiro et al. reported that flavonoids from hop, mainly xanthohumol, also exert an inhibitory effect on the aromatase activity [35].

2.2. p53 pathway

p53 is a tumor suppressor protein also known as “the guardian of the genome” [36] that acts in response to DNA damage, oncogene activation and other stress signals, repairing DNA, arresting cell cycle and inducing cell apoptosis or senescence [37]. In consequence, p53 prevents mutations of the genome and cancer formation. The p53

tumor suppressor pathway is frequently altered in human cancers [38].

MDM2 is the main cellular antagonist of p53 [39], by inhibiting its transcriptional activity, promoting its nuclear export and its ubiquitination, by targeting p53 for proteasomal degradation [40] (Figure 1).

Chalcones, due to their diverse chemical structure, can increase levels of p53 by either increasing its stability or blocking its degradation. Isoliquiritigenin [23], could induce p53 expression [41]; activate p53 phosphorylation and acetylation which releases p53 from MDM2 [42]; reduce p53 ubiquitination [43]; and inhibit p53 proteasome degradation [44] (Figure 1). Certain carboxylic acid groups of chalcones [45], and most efficiently certain series of boronic chalcones [46] disrupt p53/MDM2 interaction by binding to the p53-transactivation domain of MDM2 [45]. By preventing MDM2 from binding to p53, p53 stability is increased and therefore, it can promote the expression of certain proteins involved in cell cycle checkpoints regulation and apoptosis induction [47].

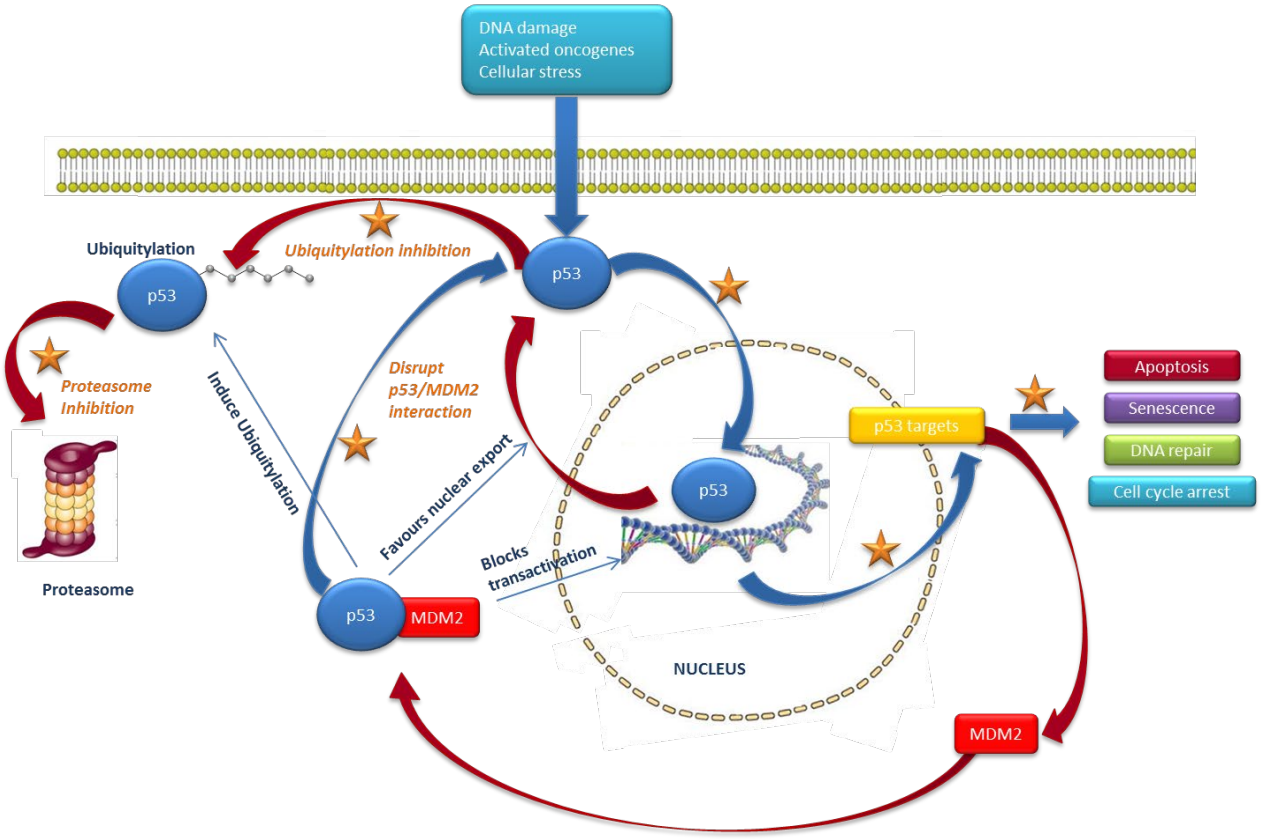


Figure 1. (From Chène, 2003 [48] modified). **Chalcones affecting p53 pathway.** p53 induces MDM2 expression. MDM2 blocks p53 transactivation, and promotes its nuclear export and its ubiquitination, thereby targeting p53 for proteasome degradation. Chalcones (represented as a yellow star) could disrupt p53/MDM2 interaction, induce p53 expression, reduce its ubiquitination or inhibit proteasome degradation. As a consequence of these mechanisms p53 stability increase and transcriptionally activates a number of targets genes involved in tumor suppressor activity.

2.3. Cell cycle arrest

Cyclins and cyclin-dependent kinases (CDKs) are key regulators of cell cycle progression, whereas inhibitors of cyclin-dependent kinases (CDKI) induce cell cycle arrest. Another protein, named retinoblastoma (Rb), is a tumor suppressor protein responsible for G1 checkpoint, blocking S-phase and arresting cell growth [49]. The tumor suppressor p53 also regulates cell cycle progression by arresting cells at G1 or G_2/M checkpoints, depending on the type of cell lines. Chalcones have shown to modulate cell cycle progression by different mechanisms: a) down-regulation of CDK2, 4 or 6, which induces S and G_2/M cell cycle arrest (isoliquiritigenin [50], panduratin A [51] or licochalcone [52]; b) induction of CDKI p21 and p27; c) maintainance of Rb active, by protecting against its phosphorylation that would trigger deactivation [12]; and d) p53 activation [53, 54].

2.4. Proteasomal inhibition

Chalcones have provoked major interest as inhibitors of the ubiquitin-proteasome pathway. The proteasome activity regulation leads to reduce degradation of proteins involved in cell cycle or apoptosis, not only p53, as has been described above, but also other proteins such as Bax, Bid (pro-apoptotic proteins) or CDKs [44, 55, 56]. In this case, the accumulation of poly-ubiquitinated proteins is accompanied by a compensatory increase in lysosomal

protein degradation, which allows the co-treatment with lysosome inhibitors that synergistically reduce cancer cell viability [7, 57].

In addition, chalcones could also inhibit the 20S proteasomal catalytic subunit. Certain cancer types that are critically dependent of proteolytic degradation may be particularly sensitive to proteasomal inhibitors. The presence of an α,β-unsaturated carbonyl system might interact with the catalytic proteasome site by acting as covalent binders. On the other hand, boronic substitutions are not mandatory for this inhibitory activity, whereas aromatic amino acid substitution allows the inhibition of the three catalytic proteasome activities [57].

2.5. Microtubular network disruption

The mechanism of action of numerous anticancer agents is mediated by G_2/M cell cycle arrest by altering the dynamics of microtubular cytoskeleton. Some chalcones have shown to bind strongly to β-tubulin, altering the polymerization, and in consequence disrupting cellular division and leading to apoptosis. Structure-activity studies point that chalcone scaffold could fit into the colchicine-binding site of tubulin, thereby preventing the conformational changes needed for tubulin to be functional. Chalcone binding to this tubulin active site could result in cellular microtubule disassembly, failure in mitotic spindle formation, and cell death [58]. In particular, chalcones will arrest mitosis at prometaphase or metaphase-anaphase [59], showing an antitumor activity comparable to vinblastine [60], one of the antitumor agents currently used in clinics for the treatment of different lymphomas and solid tumors. A structure-activity relationship study has shown that the microtubule disruption activity could be highly correlated with chalcones methoxylation [61, 62], although a 6-alkoxybenzoxathiole ring could be considered as structural equivalent of trimethoxyphenyl units [63]. Vitorović-Todorović et al. results showed that bulkier substituents on the aroyl moieties of the molecules impose steric hindrance for the binding of compounds to tubulin [62]. Mesenzani et al. suggested that the double bond in the chalcone scaffold is not essential for the interaction with tubulin, and that the introduction of a chemically inert heterocyclic ring gives metabolic stability advantages to the molecules [64]. The cytoskeleton of eukaryotic cells plays a vital role not only in mitosis, but also in cell functions such as intracellular transport, cell shape, cell signaling and polarity. Therefore microtubule dynamics constitutes a major target for the development of anticancer drugs [65].

2.6. Cell apoptosis pathways

Apoptosis could be induced by two intimately connected pathways: the mitochondrial pathway and the stimulation of death receptors [12]. Chalcones, could affect both pathways thanks to their diverse chemical structure.

The mitochondrial pathway of apoptosis is regulated by the Bcl-2 family proteins. Pro-apoptotic proteins include Bak, Bad or Bax. They regulate mitochondrial membrane permeability, thereby causing membrane destabilization, cytochrome c release, production of ROS, caspase cascade activation, and cell death [66]. This process is inhibited by anti-apoptotic proteins including Bcl-2, Bcl-xL, Bcl-W, XIAP and survivin.

Some chalcones have shown to have a dual effect by inducing pro-apoptotic proteins and down-regulation of anti-apoptotic proteins. Examples include: Isoliquiritigenin induced apoptosis on DU145 and MAT-LyLu rat prostate cancer cells [67] by promoting the mitochondrial membrane depolarization and upregulation of cleaved caspase-9, -7 and -3 and poly (ADP-ribose) polymerase (PARP). Flavokawain A induced apoptosis through upregulation of the pro-apoptotic Bax-dependent mitochondrial pathway and the down-regulation of antiapoptotic proteins [68]. Isobavachalcone is believed to increase cleaved caspase-3 and caspase-9 levels, thereby inducing apoptosis and being an efficient and harmless drug for neuroblastoma treatment [10].

Death receptor-mediated pathway is the other pathway involved in the apoptosis induction. A cytokine named TRAIL (TNF-related apoptosis inducing ligand) binds to specific death receptors and activate the apoptosis process. Death receptors, including DR-4 and DR-5, are members of the TNF receptor superfamily. They are characterized by an intracellular death domain that recruits adapter proteins like TRADD (Tumor necrosis factor receptor type 1-associated DEATH domain protein), and FADD (Fas-Associated protein with Death Domain). TRADD and FADD will bind to the initiator caspase-8 [12] and induce the programmed cell death. Targeting TRAIL receptors is considered a promising anticancer therapeutic strategy because promotes selectively the apoptosis in cancer cells with low toxic effects in normal cells [69]. The chalcones flavokawain B and pandaturin A have shown to induce apoptosis by up-regulation of death receptors as DR-5, and DR-4, TRIAL or Fas expression on prostate, leukemia, and colon cancer cells lines [7]. Tumor resistance and recurrence are among the current challenges in Oncology. Unfortunately, more than half of tumors become resistant to TRAIL. Combined TRAIL treatment with chalcones exhibited significant synergism sensitizing TRAIL-resistant cells to apoptosis. Isobavachalcone, xanthohumol, and licochalcone A are examples of chalcones that markedly enhance TRAIL-mediated apoptosis in prostate (LNCaP) and cervical (HeLa) cancer cells [25]. Cardamonin enhanced the expressions of DR-5 and DR-4 and decreased the Bcl-xL level in TRAIL-resistant DLD1 colorectal adenocarcinoma cells. The combination of this compound and TRAIL synergistically enhanced TRAIL-induced apoptosis against TRAIL-resistant cells. On its behalf, isoliquiritigenin and TRAIL treatment enhance apoptosis in HT-29 colorectal adenocarcinoma cells synergistically trough activation of caspase-8, -10, -9 and -3 [70]. In addition, cardamonin increased CCAAT/enhancer-binding protein-homologous protein (CHOP) [71].

Another chalcone obtained from kava extract, flavokawain B, acted through ROS generation and GADD153 (growth arrest- and DNA damage-inducible gene 153) up-regulation altering the expression of Bcl-2 family members, thus reducing mitochondrial membrane potential and leading to apoptosis in HCT116 cells [24]. In contrast, the same compound on DU145 and PC-3 prostate cancer cell lines, activated the Bax-initiated mitochondria pathway as

the result of an upregulation of DR-5, Bim and Puma expression and a decrease of XIAP and surviving levels. Among them, up-regulation of Bim played a critical role in this compound activity, as well as a synergistic apoptotic effect with TRAIL [72]. Other studies have been conducted showing an anti-neoplasic activity of flavokawain B on non-small cell lung cancer (H460 cell line) [73], breast cancer 4T1 [74], lymphoblastic leukemia [75] or squamous carcinoma cells [76]. These results demonstrate the therapeutic potential of flavokawain B, that has also shown an anti-metastatic activity [74].

2.7. ATP-Binding Cassette transporters

One of the main obstacles for cancer treatment is the acquired resistance to chemotherapeutics through the over-expression of ATP-binding Cassette (ABC) transporters. These transporters use the energy driven from ATP hydrolysis to transport cytotoxic drugs from the inside of cancer cells to the outside (i.e. pumping drugs out of the cells), preventing their cell-killing effects. There are two main types of ABC multidrug resistant transporters: ABCB1/MDR1, also known as P-glycoprotein (P-gp) and breast cancer resistance protein, BCRP. Chalcones have shown to inhibit both types of these transporters. Thanks to this property, chalcones may become good candidates as chemotherapy adjuvants [77, 78]. Chalcones with 3′,4′,5′-trimethoxy substitution in ring A has shown the ability to inhibit P-gp efflux pump. Chalcones activity is improved with increase of their hydrophobicity [79]. Parveen et al. performed 2D- and 3D-QSAR studies indicating the importance of H-bond acceptors, methoxy groups, hydrophobic groups as well as the number of rotatable bonds as pharmacophoric features influencing P-gp inhibitory activity of chalcones [80].

On the other hand, 2,4-dimethoxy groups or 2,4-dihydroxyl groups on ring A were found to be potent inhibitors of BCRP[81]. In addition, potent chalcones with P-gp inhibitory activity were poor inhibitors of other ABC transporters as BCRP, and vice versa. Such selectivity is highly desirable in clinical use because the specific protein inhibitor would not interfere with other transporters and cause unwanted drug-drug interactions [10]. The multidrug resistance protein 1 (MRP1) mediates drug efflux out of cells often in co-transport with glutathione (GSH). GSH efflux mediated by MRP1 can be stimulated by chalcones. Since GSH is an important antioxidant defense, a huge depletion would trigger apoptosis of cells [82].

2.8. NF-κB pathway

Chalcones modulate several inflammatory pathways involved in different steps of carcinogenesis, inducing the down-regulation of cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes. Chalcones have also shown to inhibit the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) [83, 84] by inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) via suppression of IKK (IκB kinase) and subsequent IκBα (inhibitor of kappa B) degradation.

NF-κB is a transcription factor that plays an important role in carcinogenesis, and is therefore a potential target for cancer therapy. NF-κB remains inactive in the cytosol while bound to IκBα. In response to cellular stress signals, IKK phosphorylates the complex NF-κB/IκBα. This phosphorylation will trigger the dissociation of NF-κB from IκBα. Phosphorylated IκBα is degraded by proteasome and NF-κB is translocated into the nucleus. NF-κB triggers proliferation and anti-apoptotic gene expression (Figure 2).

Chalcones could inhibit this pathway from acting at different stages (Figure 2): inhibition of IKK activation, thereby protecting the complex NF-κB/IκBα and preventing NF-κB dissociation; and blocking the nuclear translocation of NF-κB, thereby inhibiting it DNA binding. Several examples are now detailed for each of the different mechanisms. Butein has been shown to inhibit IKK activation, which blocks IκBα phosphorylation and degradation. As consequence, NF-κB remains in its inactive form, and therefore, anti-apoptosis, proliferation and invasion gene expression is suppressed [13]. In the same way, xanthohumol potentiates the apoptotic response in leukemia cells through the modification of cysteine residues in IKK and p65 NF-κB, thereby inhibiting the phosphorylation and degradation of IκBα and down-regulating the nuclear translocation of p65,the NF-κB subunit with the transcriptional activity. Xanthohumol [85]. Isoliquiritigenin, flavokawain A and B or pandaturin A, among others, were also found to prevent the IκBα degradation [7]. Other chalcones have been shown to prevent NF-κB from DNA binding and to activate the transcription of anti-apoptotic genes. In this sense, dibenzoylpropane, a curcumin analogue, has shown the ability to inhibit the direct DNA binding ability of p65 [86]. Other chalcones, such as 4′-hydroxychalcone, were able to prolong the stability of IκBα by inhibiting its proteosomal degradation. This effect was specific for IκBα and not for IKK [87]. Finally, bichalcone analogues significantly block the nuclear translocation of NF-κB p65 [88].

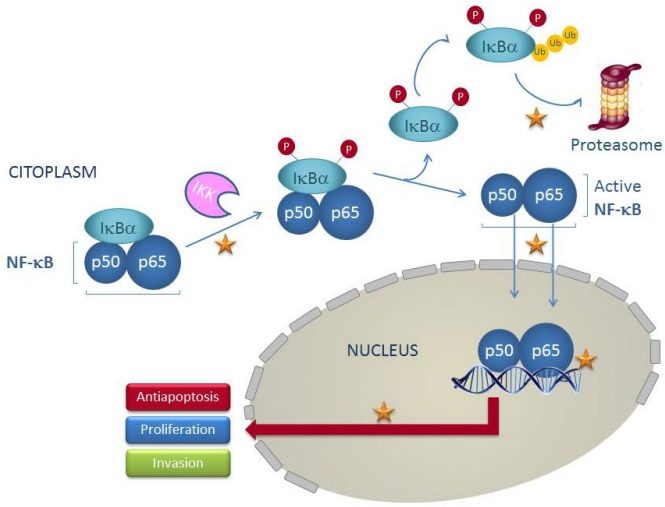


Figure 2: Chalcones (represented as yellow stars) target different components of the NF-κB pathway. NF-κB is composed of p50/p65 heterodimeric complex. Chalcones are able to inhibit the phosphorylation and the consequent degradation of IκBα, thereby suppressing p65 nuclear translocation, p65 direct DNA binding activity and NF-κB-dependent reporter gene transcription.

2.9. Anti-angiogenesis, anti-invasion and anti-metastasis

Tumors grow by forming new blood vessels which then provide oxygen and nutrients. This process of generating new blood vessels is known as angiogenesis.

Tumor cells develop invasive properties against other tissues and cellular structures. Angiogenesis and invasion are the two main steps for a primary tumor to become metastatic.

The angiogenesis process is highly correlated and interconnected with other cellular pathways such as the aforementioned anti-apoptotic process. The angiogenesis process starts with the secretion of different angiogenic factors. The Vascular Endothelial Growth Factor (VEGF) is the principal factor. Another important pathway involved in cell growth, and cell migration is the Akt pathway (Protein kinase B). Over 50% of carcinomas have activated this pathway [89]. VEGF production correlates with Akt activation. Therefore, anti-angiogenic compounds that inhibit new blood vessels formation, anti-vascular agents that destroy existing tumor blood vessels, and anti-growth and anti-invasive agents have emerged as an interesting therapeutic approach against cancer and to block metastasis.

Since all these pathways are highly correlated, chalcones with apoptotic properties are also expected to be active against cell invasion and angiogenesis.

In endothelial cells, xanthohumol presented antiangiogenic effects that were correlated to a blockade of IκBα phosphorylation, with a consequent lack of NF-κB activation. Xanthohumol also repressed phosphorylation of endothelial Akt in response to growth factor stimulation, thereby interfering in the cell survival and invasion pathways [90]. Xanthohumol decreased VEGF production in leukemia cells as well.[91]. Furthermore, xanthohumol blocked invasion by an additional different mechanism, by inducing the expression of E-cadherin. This protein plays an essential role in cell adhesion. It has been found that down-regulation of E-cadherin expression is related with cellular dedifferentiation and invasiveness in cancer, mainly in breast cancer [92]. Therefore, xanthohumol shows promise as a dual anti-angiogenic and anti-invasive coadjutant in cancer treatment [93].

Administration of isoliquiritigenin to a murine breast cancer xenograft model showed an inhibition of breast cancer growth and neo-angiogenesis with exerting minor toxic effects. Isoliquiritigenin inhibited VEGFR-2 (receptor for VEGF) activity by stably binding to its ATP binding site [94].Circulating endothelial progenitor cells (EPCs) play a main role in the maintenance of vascular integrity and in tissue repair. EPCs also facilitate the tumor growth and metastasis by promoting the generation of new vessels. Butein suppressed the AKT/mTOR pathway on EPCs reducing their survival and invasiveness [95]. Butein also suppressed cancer metastasis by down-regulating cellular adhesion molecules, and by repressing the expression and activity of matrix metalloproteinase-9 (MMP-9) and urokinase plasminogen activator. Moreover, this chalcone may partly exert its anticancer activity via inhibition the oxidative and glycolytic synthesis of ATP [96].

Xanthoangelol, is another chalcone that has shown antiangiogenic properties [12].

Anti-vascular properties of chalcones are related to their ability to interact with tubulin. These compounds disrupt the endothelial cell layer that surrounds blood vessels. Once this occur, tumor vessels are no longer able to provide oxygen and nutrients to the tumor, inducing necrosis [60].

3. CHALCONES DERIVATIVES WITH ANTICANCER ACTIVITY

Chalcones have a simple structure and numerous pharmacological activities. These two advantages have driven to numerous design, synthesis, and biological evaluation studies of natural and synthetic chalcones with the objective of seeking potential lead compounds.

3.1. Synthesis of novel chalcones

Chalcones can be obtained synthetically through condensation of an aromatic aldehyde with an acetophenone by different methods, thereby permitting a large variety of structures [97]. The main synthetic method is the Claisen-Schmidt reaction, which consistis of the one-step condensation of an acetophenone and a benzaldehyde derivative, by using commonly hydro-alcoholic solvent and basic conditions to obtain diphenyl-2-propen-1-ones [98]. This kind of aldol condensation can also be performed in acidic conditions, by aldol formation followed by dehydration, using acyclic acidic ionic liquids, among others, as catalyzer [99]. One of the most promising methods is the

Suzuki reaction between cinnamoyl chlorides and phenyl boronic acids, which is catalyzed by palladium derivatives in alkaline and anhydrous conditions [100]. Fig. 3 shows the schema of the two most common synthetic methods. Chalcones have also been obtained by Witting reaction, Friedel-Crafts acylation of a phenol with a cinnamoyl chloride, or the carbonylative Heck coupling among aryl halides and styrenes in the presence of carbon monoxide. Ultrasound, microwave and catalyzers such as lithium nitrate, amino grafted zeolites, and silicasulfuric acid, have been used to improve the yield of chalcones synthesis and to reduce of the use of solvents [97].

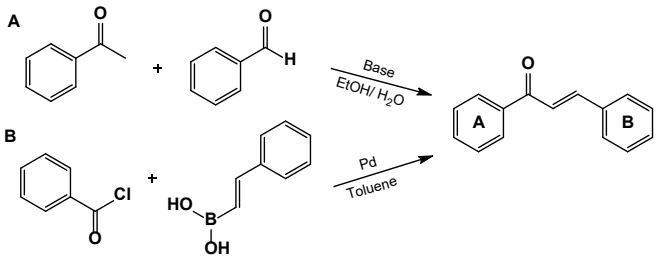


Figure 3. Synthesis of chalcones. A. Claisen-Schmidt condensation. B. Suzuki cross-coupling.

3.2. Evaluation of potential anticancer activity of chalcone derivatives

Once the chalcones are obtained, they are recrystallized and their chemical structure is generally determined by a combination of analytical techniques, such as melting point, infrared spectrometry, nuclear magnetic resonance and elemental analysis. In order to determine the most cytotoxic chalcones derivatives among a new series, the most common screening assays are colorimetric assays, which include the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium salt and sulphorhodamine B (SRB), for the assessment of cell viability, expressed as half maximal inhibitory concentration (IC50) after incubation during a determined time [101, 102]. Other studies performed the protocol of the Drug Evaluation Branch of the National Cancer Institute (NCI 60), using 60 cell lines derived from leukemia, melanoma, lung, colon, central nervous system, ovarian, renal, prostate, and breast cancer [103].

In some cases, the cited studies also investigate the potential mechanism of action of selected chalcones after screening by determining cell apoptosis rate, cell cycle distribution and protein expression [101]. Molecular docking is presented as a promising approach in the research of novel chalcone derivatives, by determining the possible interactions between the structures of different chalcones derivatives and a biological target (e.g., the colchicine binding site of β-tubulin) [104].

3.3. Recent advances in chalcones derivatives with anticancer activity

Different strategies were followed to design chalcones in order to increase their anticancer activity. These include: substitution of aryl rings, such as hydroxylation, methoxylation and prenylation; replacement of aryl rings by heteroaryl, alicyclic or steroidal groups; and molecular hybridization through conjugation with other pharmacologically active molecules. Aldol condensation enabled chalcone derivatives to be obtained with hydroxylation patterns in different positions to those of natural chalcones. In this sense, Haddad et al. selected 2,2'-dihydroxychalcone (2,2'-DHC) after a cytotoxicity screening in LNCaP and PC3 prostate cancer cell lines and showed alteration of 100 cell cycle genes, including down-regulation of key regulators in G_2/M phase [105, 106]. 2,2'-DHC also sensitized human colon adenocarcinoma cells to chlorambucil and mephalan by disrupting the cellular antioxidant mechanism via inhibition of glutathione transferase activity [107]. Similarly, Saydam et al. showed the pro-apoptotic effect of 4,4'-dihydroxychalcone on HL-60 myeloid leukemia cells and suggested that the mechanism was probably related to the ability of the α,β-unsaturated carbonyl system to alkylate the DNA bases [108].

Nam et al. showed that the cytotoxicity of chalcones could be enhanced both by introducing electron-withdrawing substituents on the B ring, or by replacing this ring by extended or heteroaromatic rings [109]. Among them, 2-chloro-2',5'-dihydroxychalcone, exerted a marked cytotoxic effect against HUVECs cells both *in vivo* and *in vitro*, suggesting a potential interest as anti-angiogenic agent.

Xanthohumol, present in hop flowers (*Humulus lupulus*, Cannabaceae), and other natural prenylated polyphenols have interesting properties for the food industry and pharmacognosy, moreover, they have been shown to have *in vivo* and *in vitro* cancer cytotoxic activity [110]. Unfortunately, it is difficult to obtain prenylated chalcones from natural sources due to the low content present in the plants and to the fact that they constitute part of complex mixtures in the plant extracts [111]. The conditions of the Suzuki coupling reaction allow the use of methoxymethylether (MOM) as a protector of substituents, thereby permitting the synthesis of more complex derivatives, such as isoprenyl moieties. The introduction of one prenyl or geranyl group in the 5' or 6' positions improves the cytotoxic activity of chalcones derivatives against human leukemia K562 cells by inducing apoptosis [112, 113].

Oxyprenylated chalcones have also been presented as anti-cancer, anti-inflammatory, and anti-bacterial agents [114, 115]. 4-chloro-2'-hydroxy-4'-isoprenyloxychalcone can be obtained by Claisen-Schmidt condensation followed by a substitution reaction with prenyl bromide in acetone. This oxyprenylated chalcone showed cytotoxicity against HCT116, HeLa, and A549 cancel cell lines and was active against a colon carcinoma colo205 xenograft model *in vivo.* The molecular mechanism behind these effects could be due to the inhibition of Cell Division Cycle 25 (CDC25) B protein activity, shown *in vitro*[116].

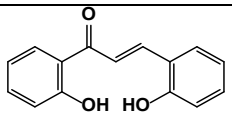
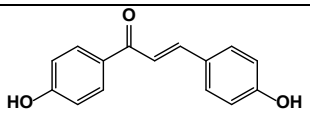
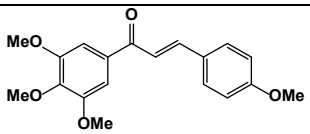
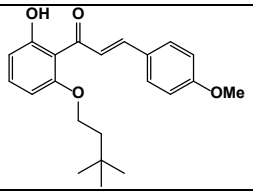
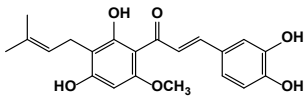
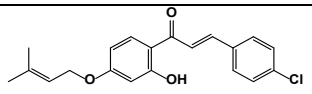
Several natural anticancer drugs currently used in oncology, such as vincristine and vinblastine, and other under investigation in clinical trials, such as indibulin, are known to be inhibitors of tubulin polymerization. These

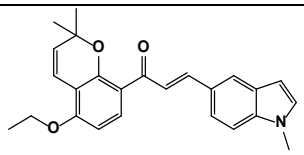
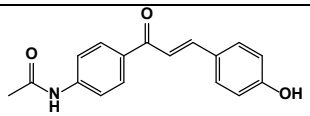
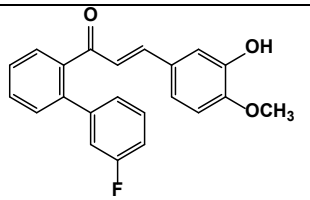
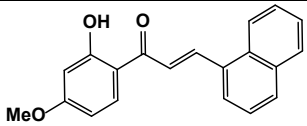
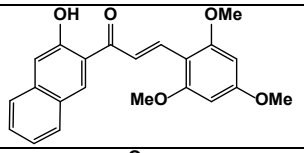
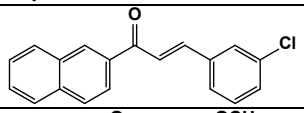
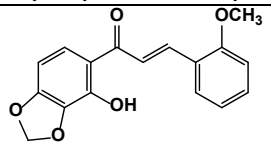
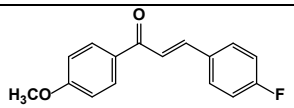
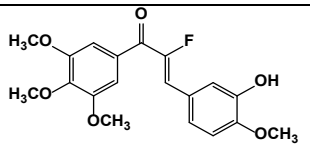
compounds have a structural component in common; all of them contain an indole moiety in their structures [117, 118]. A group of chalcones, known as pyranochalcones, contains this indole moiety as well, and as expected, they also inhibit tubulin polymerization by interacting the colchicine binding site of tubulin. Millepachine isolated from *Millettia pachycarpa*, [119], and a series of pyrano chalcones derivatives containing an indole moiety shown this activity [118].

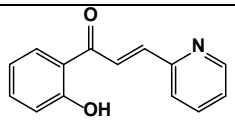
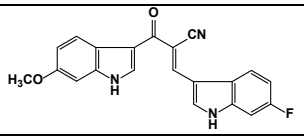
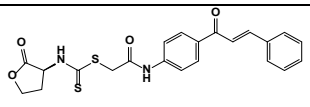
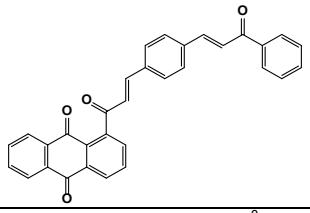
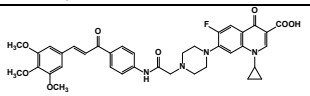
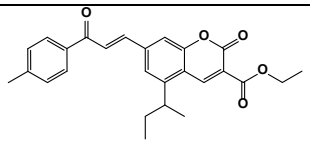
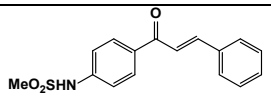
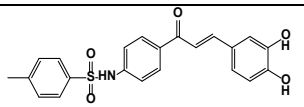
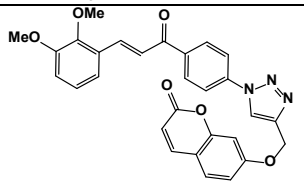
The aryl ring can be substituted by a heterocyclic aromatic ring such as pyridine, indole or azole, leading heteroaryl chalcones [120]. A series of α-cyano bis(indolyl)chalcones was synthetized by Knoevenagel condensation under microwave irradiation in presence of ethylene glycol using piperidine as a base catalyst. It was shown that methoxy and fluoro substituents on indole rings enhanced the cytotoxic activity against A549 lung cancer cells (IC_{50} = 0.8 μM) [121].

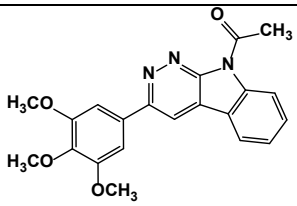
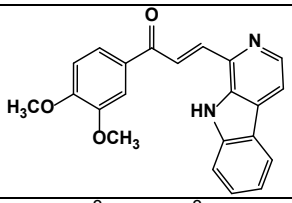
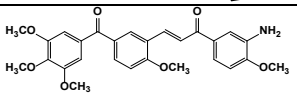
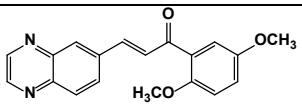
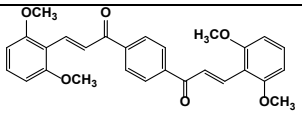
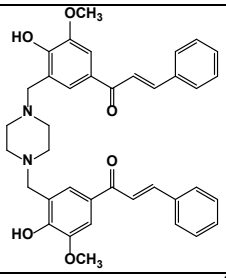
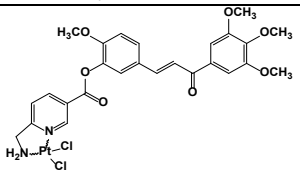
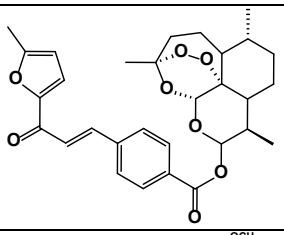
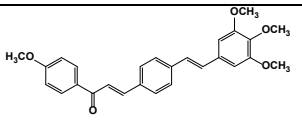
Molecular hybridization provides a promising strategy of drug design, consisting of incorporating two pharmacophores in a single molecule in order to amplify a pharmacological effect, interact with multiple targets or counterbalance the side effects. Due to the structural diversity of chalcone derivatives, molecular hybridization could become an important tool in the design of anticancer hybrids, mainly as microtubule inhibitors [122]. Chalcones can be hybridized with other natural bioactive molecules, such as anthraquinones, resveratrol, and dihydroartemisin, with other chalcones and even with totally synthetic drugs (e.g. ciprofloxacin).

The following table shows examples of the different classes of synthetic chalcones, the technique employed for screening their cytotoxicity, and the observed anticancer mechanisms.

Class	Main chalcone derivative	Synthesis	Screening	Mechanism	Ref.
PHENYL RINGS **SUBSTITUTED**					
Hydroxylated		Claisen-Schmidt condensation	IC_{50} 72h CyQuant cell proliferation assay (Molecular Probes, USA) <20 μM: LNCaP and PC3 (prostate)	Alteration of 100 cell cycle genes, including down-regulation of 27 genes with key functions in G_2/M phase	[105, 106]
Hydroxylated		Claisen-Schmidt condensation	IC_{50} 96h trypan blue 2 μM: HL-60 (myeloid leukemia)	Inhibition of cell proliferation and induction of apoptosis	[108]
Methoxylated		Claisen-Schmidt condensation	IC_{50} 24h MTT <10 μM: A2780 (p53 wild-type ovary), A2780/CDDP (cisplatin resistant, p53 mutant) and SKOV3 (p53 null) IC_{50} 24h MTT>100 μM: T29 (pre-neoplastic ovarian epithelial cells)	Induction of G_0/G_1 cell cycle arrest via ↓cyclin D1 and CDK4, ↑p16, p21 and p27. Induction of apoptosis through ↓Bcl-2 and Bcl-xL, and ↑Bax and cleaved PARP-1. Reduction of the invasiveness of A2780 cells via ↓STAT3 signaling pathway and ↑the tumor suppressor PTEN	[101]
Isopentyloxylated		Claisen-Schmidt condensation	IC_{50} 48h SRB <5 μM: ACHN (kidney), NCI-H23 (lung), MDA-MB-231 (breast), HCT-15 (colon), NUGC-3 (stomach) and PC-3 (prostate)	Cytotoxicity and NFκB inhibition	[123]
Prenylated		Aldol coupling of methoxymethyl acetal (MOM)-protected and prenylated acetophenone with benzaldehyde	IC_{50} 72h MTT <5 μM: HeLa (cervix)	Cytotoxicity	[110]
O-Prenylated		Claisen-Schmidt condensation and substitution reaction with prenyl bromide in acetone	IC_{50} 72h MTT <5 μM: HCT116 (colon), A549 (lung), and HeLa (cervix)	*In vitro* inhibition of cycle 25B phosphatase *In vivo* antitumor activity on colo205 (colon cancer) xenograft model	[116]

Pyrano chalcone derivatives containing indole moiety		Claisen–Schmidt condensation with N-alkyl indole aldehydes	IC_{50} 48h MTT <2 μM: SMMC-7221, HepG2 (liver), PC-3 (prostate), A549 (lung), K562 (leukemia), SKOV3 (ovary), MCF-7 (breast), HCT116, (colon), HCT-8/T, HCT-8/V (drug resistant), IC_{50} 48h MTT >10 μM: LO2 (normal human cells)	Induction of G_2/M cell cycle arrest by inhibition of microtubule polymerization, interacting at the colchicine binding site of tubulin. *In vivo* antitumor activity in hepatocarcinoma HepG2 xenograft model.	[118]
Acetamide		Claisen-Schmidt condensation	IC_{50} 24h MTT>50 μM: U87MG, U373MG, and T98G (glioma).	Reduction of glioma cell invasion, migration, and colony formation. *In vivo* antitumor activity on glioblastoma U87MG xenograft model	[124]
Ortho-aryl		Aldol condensation followed by Suzuki coupling	IC_{50} 48h SRB < 0.1 μM : A549 (lung), CNE2 (nasopharyngeal), SW480 (colon), MCF7 (breast), and HepG2 (hepatocarcinoma)	*In vitro* and cellular inhibition of tubulin polymerization, G_2/M cell cycle arrest and apoptosis induction. *In vivo* antitumor activity on A549 lung cancer xenograft model	[102]
Benzochalcone		Claisen–Schmidt condensation of substituted acetophenone or 1-hydroxy-2-aceto-naphthone with methoxy benzaldehydes under basic conditions in ethanol Claisen-Schmidt condensation	IC_{50} 7 days clonogenic survival assay <10 μM : Capan-1 (pancreas)	Disruption of microtubule assembly, leading to mitotic arrest and activation of the caspase pathway, resulting in apoptosis.	[125, 126]
Naphthochalcone		Claisen-Schmidt condensation	7 days clonogenic survival assay SW620 (colon)	Inhibition of tubulin polymerization and DNA damage triggered mitotic arrest, p53-dependent and caspase-2-mediated apoptosis.	[59]
Naphthochalcone		Claisen-Schmidt condensation	IC_{50} 24h MTT <50 μM : L1210 (Murine lymphoblastic leukemia)	Induction of apoptosis by intrinsic and extrinsic pathways. Endoplasmic reticulum stress triggered by changes in intracellular calcium concentration.	[127]
Methylenedioxy		Claisen-Schmidt condensation	IC_{50} 72h ATP Promega's CellTiter-Glo® <50 μM : K562, Jurkat, U937 (leukemia) IC_{50} 72h ATP Promega's CellTiter-Glo® >50 μM: PBMCs (peripheral blood mononuclear cells)	Induction of apoptosis, via cleavage of caspase 3 and 7.	[128]
Fluorochalcone		Aldol condensation	IC_{50} 48h MTT < 10 μM : A375 (melanoma)	Induction of cell cycle arrest and apoptosis via caspase activation.	[129]
α-SUBSTITUTED					
α-fluorochalcone		Knoevenagel condensation	IC_{50} 120h MTT < 0.01 μM : K562 (chronic myelogenous leukaemia)	Inhibition of tubulin polymerization	[130]
HETEROARYL					

Pyridinyl		Claisen-Schmidt condensation	IC_{50} 48h SRB <20 μM : TK-10 (kidney), MCF-7 (breast), HT-29 (colon)	Cytotoxic with a good drug likeness value	[131]
α-cyano bis(indolyl)		Knoevenagel condensation under microwave irradiation	IC_{50} 48h bythe cell Counting Kit-8 < 1 μM : A549 (lung)	Inhibition of tubulin polymerization	[121]
HYBRIDS					
N-Acyl homoserine lactones (AHLs).		Claisen-Schmidt condensation. analogs with the chalcone and homoserine lactone scaffold linked by the dithiocarbamate group	IC_{50} 72h MTT < 10 μM : MCF-7 (breast)	Induction of G_2/M phases arrest and induction of apoptosis in a dose-dependent manner	[132]
Anthraquinone –chalcone hybrids		Claisen–Schmidt condensation	IC_{50} 72h MTT <5 μM: HeLa (cervix), LS174 (colon), A549 (lung) IC_{50} 72h MTT >30 μM: non-cancerous cell line MRC-5	Induction of S and G_2/M phases arrest in a dose-dependent manner and induction of caspase-dependent apoptosis. DNA-binding activity	[133]
N-4-piperazinyl-ciprofloxacin-chalcone		Claisen–Schmidt condensation	Protocol of the Drug Evaluation Branch of the National Cancer Institute, Bethesda, USA for *in vitro* anticancer screening, five-dose full NCI 60 cell panel assay. GI_{50} range from 0.21 to 57.3 μM	Inhibition of Topo I and topo II activity	[134]
Chalcone-coumarin		Claisen–Schmidt condensation	IC_{50} 48h SRB <10 μM: C33A and HeLa (cervix)	Induction of intrinsic apoptotic pathway and G_2/M phases arrest by generating ROS, up regulation of p53and modulating expression of Bcl-2 family proteins, leading to apoptosome mediated activation of caspase cascades. *In vivo* antitumor activity on HeLa cervical cancer xenograft model in *in nod* SCID mice.	[135]
Methanesulfonamide		Claisen-Schmidt condensation	IC_{50} 72h SRB <25 μM: HepG2 (hepatocarcinoma), A549 (lung), and MCF-7 (breast)	*In vitro* inhibition of COX-1 and COX-2	[136]
Toluenesulfonamide		Claisen-Schmidt condensation	IC_{50} 48h SRB < 30 μM : NCI-H460 and NCI-H1299 (lung)	c-Myc-dependent ROS production	[137]
Chalcone-triazole-coumarin		Claisen–Schmidt condensation of chalcones followed by azide/alkyne dipolar cycloaddition	IC_{50} 48h MTT <10 μM: HuCCA-1 (cholangiocarcinoma), HepG2 (hepatocarcinoma), A549 (lung) IC_{50} 48h XTT <10 μM: MOLT-3 (lymphoblastic leukemia). IC_{50} 48h GFP >50 μM: Vero (Non-cancerous cell line)	Dual inhibition of α- and β-tubulin	[104]

Azacarboline analogue		Claisen–Schmidt condensation of isatin with an acetophenones followed by dehydration, acetylation and cyclation	IC_{50} 48h SRB <10 μM: THP-1 (leukemia), COLO-205 and HCT-116 (colon).	Molecular docking performed to investigate the binding the colchicine-binding site of tubulin	[138]
β-carboline based chalcones		Claisen–Schmidt condensation of substituted 1-phenylethanone and β-carboline-1-carbaldehyde.	IC_{50} 48h SRB <5 μM: MCF-7 (breast)	Induction of DNA fragmentation and apoptosis	[139]
Phenstatin–chalcone		Claisen–Schmidt condensation	IC_{50} 48h MTT <1 μM: MOLT-4 (leukemia), HT29 (colon), NCI/ADR-RES (ovary) and MCF7 (breast)	Significant antiproliferative activity against a panel of sixty human cancer cell lines of the NCI. Induction of G_2/M cell cycle arrest and apoptosis by inhibition of microtubule polymerization, interacting at the colchicine binding site of tubulin.	[140]
Chalcone-quinoxaline hybrid		Claisen–Schmidt condensation	IC_{50} 48h MTT <2 μM: A549 (lung), B16F10 (mouse melanoma), C6 (rat glioma), DU145 (prostate) and K562 (erythroleukemia)	Inhibits *in vitro* U87-MG tumor growth through modulation of Bax, p53, p21 and caspase-9. Also, N9 treatment decreases the MDM2 protein levels. *In vivo* prevented the growth of U87-MG xenograft tumor in nude mice.	[141]
Bis-chalcones		Claisen–Schmidt condensation	EC_{50} Ability to inhibit mitoxantrone efflux < 1 μM: ABCG2-transfected HEK293 cells	ABCG2 and BCRP inhibitor	[142]
Bichalcones linked with a 1,4-dimethylenepiperazine moiety		Mannich reaction and Clasien–Schmidt condensation.	IC_{50} 48h MTT <2 μM: GBM-8401, MO59K (glioblastoma), NPC 039, NPC 076 (nasopharynge) FaDu (pharynge), CAL-27, SAS (tongue), MDA-MB-231 (breast), A375 (melanoma), and HeLa (cervix)	Cytotoxicity	[143]
Pt(II) complex		Claisen–Schmidt condensation, Williamson etherification, Yamagouchi esterification and formation of Pt(II) with K_2PtCl_4	IC_{50} 48h MTT <1 μM: U87 (glioma)	Induction of G_1 and G_2/M cell cycle arrest and apoptosis by triggering DNA damage	[144, 145]
Dihydroartemisinyl-chalcone ester		Claisen–Schmidt condensation of chalcones followed by esterification with dihydroartemisinin	IC_{50} 48h SRB < 5 μM: TK-10 (kidney), UACC-62 (melanoma) and MCF-7 (breast)	Cytotoxicity	[146]
Resveratrol ester		Claisen–Schmidt condensation of chalcones followed by reaction with N-	Protocol of the Drug Evaluation Branch of the National Cancer Institute, Bethesda, USA for *in vitro* anticancer screening, five-dose full NCI 60 cell panel	Cytotoxicity by binding tubulin	[121]

		bromosuccinimide and with triphenylphosphine to introduce the stilbene moiety	assay. GI_{50} range from 1.98 to 38.4 μM		

Table 1. Recent advances in chalcones derivatives with anticancer activity

CONCLUSION

Chalcones constitute a diverse group of molecules that have been shown to exert anticancer properties through different mechanisms of action, and are presented as a promising tool for treatment and prevention of cancer. Chalcones could easily be chemically modified, and this characteristic could suppose an advantage in the optimization of known chalcones in order to give better pharmacokinetics and pharmacodynamic properties that increase their therapeutic value, and to generate interesting new molecules with different biological properties.

CONFLICT OF INTEREST

The authors declare no conflict of interest. AJLG was founded with a postdoctoral fellowship by Alfonso Martin Escudero Foundation.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Marina Galvez-Peralta for helpful discussions and advice during the preparation of this manuscript.

REFERENCES

[1] Bruneton, J. *Pharmacognosy, Phytochemistry, Medicinal Plants*. 3rd ed. Intercept Ltd: Hampshire, **1999**.

[2] Orlikova, B.; Tasdemir, D.; Golais, F.; Dicato, M.; Diederich, M., Dietary chalcones with chemopreventive and chemotherapeutic potential. *Genes Nutr,* **2011**, *6*, (2), 125-147.

[3] Leon-Gonzalez, A.J.; Manson, M.M.; Lopez-Lazaro, M.; Navarro, I.; Martin-Cordero, C., Induction of Apoptosis and Cell Cycle Arrest in Human Colon Carcinoma Cells by Corema album Leaves. *Natural Product Communications,* **2014**, *9*, (1), 55-56.

[4] Rozmer, Z.; Perjési, P., Naturally occurring chalcones and their biological activities. *Phytochemistry Reviews,* **2014**, 1-34.

[5] Ninomiya, M.K., M. In *Natural Products*. Ramawat, K.G., Merillon, J.M., Ed.; Springer: Heidelberg, **2013**, pp 1867–1900.

[6] Yadav, V.R.; Prasad, S.; Sung, B.; Aggarwal, B.B., The role of chalcones in suppression of NF-kappaB-mediated inflammation and cancer. *Int Immunopharmacol,* **2011**, *11*, (3), 295-309.

[7] Jandial, D.D.; Blair, C.A.; Zhang, S.; Krill, L.S.; Zhang, Y.B.; Zi, X., Molecular targeted approaches to cancer therapy and prevention using chalcones. *Curr Cancer Drug Targets,* **2014**, *14*, (2), 181-200.

[8] Go, M.L.; Wu, X.; Liu, X.L., Chalcones: an update on cytotoxic and chemoprotective properties. *Curr Med Chem,* **2005**, *12*, (4), 481-499.

[9] Nowakowska, Z., A review of anti-infective and anti-inflammatory chalcones. *Eur J Med Chem,* **2007**, *42*, (2), 125-137.

[10] Batovska, D.I.; Todorova, I.T., Trends in utilization of the pharmacological potential of chalcones. *Curr Clin Pharmacol,* **2010**, *5*, (1), 1-29.

[11] Gupta, D.J., D. K.; Trivedi, P., Recent advances in chalcones as antiinfective agents. *Int. J. Chem. Sci.,* **2010**, *8*, 649–654.

[12] Zhang, E.H.; Wang, R.F.; Guo, S.Z.; Liu, B., An update on antitumor activity of naturally occurring chalcones. *Evid Based Complement Alternat Med,* **2013**, *2013*, 815621.

[13] Pandey, M.K.; Sandur, S.K.; Sung, B.; Sethi, G.; Kunnumakkara, A.B.; Aggarwal, B.B., Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-kappaB and NF-kappaB-regulated gene expression through direct inhibition of IkappaBalpha kinase beta on cysteine 179 residue. *Journal of Biological Chemistry,* **2007**, *282*, (24), 17340-17350.

[14] Dimmock, J.R.E., D.W.; Beazely, M.A.; Kandepu. N.M. In *Curr. Med. Chem.*, **1999**; Vol. *8*, pp 1125-1149.

[15] Nagaraju, M.; Gnana Deepthi, E.; Ashwini, C.; Vishnuvardhan, M.V.P.S.; Lakshma Nayak, V.; Chandra, R.; Ramakrishna, S.; Gawali, B.B., Synthesis and selective cytotoxic activity of novel hybrid chalcones against prostate cancer cells. *Bioorg Med Chem Lett,* **2012**, *22*, (13), 4314-4317.

[16] Li, X.; Xu, X.; Ji, T.; Liu, Z.; Gu, M.; Hoang, B.H.; Zi, X., Dietary feeding of Flavokawain A, a Kava chalcone, exhibits a satisfactory safety profile and its association with enhancement of phase II enzymes in mice. *Toxicol Rep,* **2014**, *1*, 2-11.

[17] Lahsasni, S.A.; Al Korbi, F.H.; Aljaber, N.A.A., Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues. *Chemistry Central Journal,* **2014**, *8*.

[18] Cuendet, M.; Guo, J.; Luo, Y.; Chen, S.; Oteham, C.P.; Moon, R.C.; van Breemen, R.B.; Marler, L.E.; Pezzuto, J.M., Cancer chemopreventive activity and metabolism of

isoliquiritigenin, a compound found in licorice. *Cancer Prev Res (Phila),* **2010**, *3*, (2), 221-232.

[19] Gerhauser, C., Beer constituents as potential cancer chemopreventive agents. *Eur J Cancer,* **2005**, *41*, (13), 1941-1954.

[20] Gerhauser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauft, J.; Neumann, I.; Scherf, H.R.; Frank, N.; Bartsch, H.; Becker, H., Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. *Mol Cancer Ther,* **2002**, *1*, (11), 959-969.

[21] Maiti, A.; Cuendet, M.; Croy, V.L.; Endringer, D.C.; Pezzuto, J.M.; Cushman, M., Synthesis and biological evaluation of (+/-)-abyssinone II and its analogues as aromatase inhibitors for chemoprevention of breast cancer. *J Med Chem,* **2007**, *50*, (12), 2799-2806.

[22] Cheng, Z.J.; Lin, C.N.; Hwang, T.L.; Teng, C.M., Broussochalcone A, a potent antioxidant and effective suppressor of inducible nitric oxide synthase in lipopolysaccharide-activated macrophages. *Biochem Pharmacol,* **2001**, *61*, (8), 939-946.

[23] Hsu, Y.L.; Kuo, P.L.; Lin, C.C., Isoliquiritigenin induces apoptosis and cell cycle arrest through p53-dependent pathway in Hep G2 cells. *Life Sci,* **2005**, *77*, (3), 279-292.

[24] Kuo, Y.F.; Su, Y.Z.; Tseng, Y.H.; Wang, S.Y.; Wang, H.M.; Chueh, P.J., Flavokawain B, a novel chalcone from Alpinia pricei Hayata with potent apoptotic activity: Involvement of ROS and GADD153 upstream of mitochondria-dependent apoptosis in HCT116 cells. *Free Radic Biol Med,* **2010**, *49*, (2), 214-226.

[25] Szliszka, E.; Krol, W., The role of dietary polyphenols in tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis for cancer chemoprevention. *Eur J Cancer Prev,* **2011**, *20*, (1), 63-69.

[26] Zhao, F.; Nozawa, H.; Daikonnya, A.; Kondo, K.; Kitanaka, S., Inhibitors of nitric oxide production from hops (Humulus lupulus L.). *Biol Pharm Bull,* **2003**, *26*, (1), 61-65.

[27] Zhao, F.; Watanabe, Y.; Nozawa, H.; Daikonnya, A.; Kondo, K.; Kitanaka, S., Prenylflavonoids and phloroglucinol derivatives from hops (Humulus lupulus). *J Nat Prod,* **2005**, *68*, (1), 43-49.

[28] Yokozawa, T.; Cho, E.J.; Rhyu, D.Y.; Shibahara, N.; Aoyagi, K., Glycyrrhizae Radix attenuates peroxynitrite-induced renal oxidative damage through inhibition of protein nitration. *Free Radic Res,* **2005**, *39*, (2), 203-211.

[29] Charles, C.; Nachtergael, A.; Ouedraogo, M.; Belayew, A.; Duez, P., Effects of chemopreventive natural products on non-homologous end-joining DNA double-strand break repair. *Mutat Res Genet Toxicol Environ Mutagen,* **2014**, *768*, 33-41.

[30] Geismann, C.; Arlt, A.; Sebens, S.; Schafer, H., Cytoprotection "gone astray": Nrf2 and its role in cancer. *Onco Targets Ther,* **2014**, *7*, 1497-1518.

[31] Yao, J.; Zhang, B.; Ge, C.; Peng, S.; Fang, J., Xanthohumol, a polyphenol chalcone present in hops, activating Nrf2 enzymes to confer protection against oxidative damage in PC12 cells. *J Agric Food Chem,* **2015**, *63*, (5), 1521-1531.

[32] Wang, W.L.; Wang, J.; Li, N.; Zhang, X.R.; Zhao, W.H.; Li, J.Y.; Si, Y.Y., Chemopreventive flavonoids from Millettia pulchra Kurz var-laxior (Dunn) Z. Wei (Yulangsan) function as Michael reaction acceptors. *Bioorg Med Chem Lett,* **2015**, *25*, (5), 1078-1081.

[33] Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T., Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci,* **2004**, *95*, (5), 448-453.

[34] Wang, Y.; Chan, F.L.; Chen, S.; Leung, L.K., The plant polyphenol butein inhibits testosterone-induced proliferation in breast cancer cells expressing aromatase. *Life Sci,* **2005**, *77*, (1), 39-51.

[35] Monteiro, R.; Faria, A.; Azevedo, I.; Calhau, C., Modulation of breast cancer cell survival by aromatase inhibiting hop (Humulus lupulus L.) flavonoids. *Journal of Steroid Biochemistry and Molecular Biology,* **2007**, *105*, (1-5), 124-130.

[36] Lane, D.P., Cancer. p53, guardian of the genome. *Nature,* **1992**, *358*, (6381), 15-16.

[37] Kruse, J.P.; Gu, W., Modes of p53 Regulation. *Cell,* **2009**, *137*, (4), 609-622.

[38] Hollstein, M.; Rice, K.; Greenblatt, M.S.; Soussi, T.; Fuchs, R.; Sorlie, T.; Hovig, E.; Smithsorensen, B.; Montesano, R.; Harris, C.C., Database of P53 Gene Somatic Mutations in Human Tumors and Cell-Lines. *Nucleic Acids Research,* **1994**, *22*, (17), 3551-3555.

[39] Moll, U.M.; Petrenko, O., The MDM2-p53 interaction. *Molecular Cancer Research,* **2003**, *1*, (14), 1001-1008.

[40] Devine, T.; Dai, M.S., Targeting the ubiquitin-mediated proteasome degradation of p53 for cancer therapy. *Curr Pharm Des,* **2013**, *19*, (18), 3248-3262.

[41] Kamal, A.; Dastagiri, D.; Ramaiah, M.J.; Reddy, J.S.; Bharathi, E.V.; Srinivas, C.; Pushpavalli, S.N.; Pal, D.; Pal-Bhadra, M., Synthesis of imidazothiazole-chalcone derivatives as anticancer and apoptosis inducing agents. *ChemMedChem,* **2010**, *5*, (11), 1937-1947.

[42] Lai, C.K.; Rao, Y.K.; Chang, K.R.; Lin, C.W.; Su, H.L.; Chang, C.S.; Lai, C.H.; Tzeng, Y.M., 3,3',4', 5'-Tetramethoxychalcone inhibits human oral cancer cell proliferation and migration via p53-mediated mitochondrial-dependent apoptosis. *Anticancer Res,* **2014**, *34*, (4), 1811-1819.

[43] Chen, Z.P.; Knutson, E.; Wang, S.; Martinez, L.A.; Albrecht, T., Stabilization of p53 in human cytomegalovirus-initiated cells is associated with sequestration of HDM2 and decreased p53 ubiquitination. *Journal of Biological Chemistry,* **2007**, *282*, (40), 29284-29295.

[44] Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S.R.; Huang, P., A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. *Mol Pharmacol,* **2006**, *70*, (1), 426-433.

[45] Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Muhlhahn, P.; Schumacher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R.A.; Holak, T.A., Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53. *Biochemistry,* **2001**, *40*, (2), 336-344.

[46] Kumar, S.K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N.E.; Khan, S.R., Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents. *J Med Chem,* **2003**, *46*, (14), 2813-2815.

[47] Yee, K.S.; Vousden, K.H., Complicating the complexity of p53. *Carcinogenesis,* **2005**, *26*, (8), 1317-1322.

[48] Chene, P., Inhibiting the p53-MDM2 interaction: An important target for cancer therapy. *Nature Reviews Cancer,* **2003**, *3*, (2), 102-109.

[49] Giacinti, C.; Giordano, A., RB and cell cycle progression. *Oncogene,* **2006**, *25*, (38), 5220-5227.

[50] Kanazawa, M.; Satomi, Y.; Mizutani, Y.; Ukimura, O.; Kawauchi, A.; Sakai, T.; Baba, M.; Okuyama, T.; Nishino, H.; Miki, T., Isoliquiritigenin inhibits the growth of prostate cancer. *European Urology,* **2003**, *43*, (5), 580-586.

[51] Yun, J.M.; Kweon, M.H.; Kwon, H.; Hwang, J.K.; Mukhtar, H., Induction of apoptosis and cell cycle arrest by a chalcone panduratin A isolated from Kaempferia pandurata in androgen-independent human prostate cancer cells PC3 and DU145. *Carcinogenesis,* **2006**, *27*, (7), 1454-1464.

[52] Fu, Y.; Hsieh, T.C.; Guo, J.Q.; Kunicki, J.; Lee, M.Y.W.T.; Darzynkiewicz, Z.; Wu, J.M., Licochalcone-A, a novel flavonoid isolated from licorice root (Glycyrrhiza glabra), causes G2 and late-G1 arrests in androgen-independent PC-3 prostate cancer cells. *Biochem Biophys Res Commun,* **2004**, *322*, (1), 263-270.

[53] Agarwal, M.L.; Agarwal, A.; Taylor, W.R.; Stark, G.R., P53 Controls Both the G(2)/M and the G(1) Cell-Cycle Checkpoints and Mediates Reversible Growth Arrest in Human Fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America,* **1995**, *92*, (18), 8493-8497.

[54] Zhang, Y.; Srinivasan, B.; Xing, C.G.; Lu, J.X., A New Chalcone Derivative (E)-3-(4-Methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one Suppresses Prostate Cancer Involving p53-mediated Cell Cycle Arrests and Apoptosis. *Anticancer Res,* **2012**, *32*, (9), 3689-3698.

[55] Breitschopf, K.; Zeiher, A.M.; Dimmeler, S., Ubiquitin-mediated degradation of the proapoptotic active form of bid - A functional consequence on apoptosis induction. *Journal of Biological Chemistry,* **2000**, *275*, (28), 21648-21652.

[56] Li, B.Y.; Dou, Q.P., Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proceedings of the National Academy of Sciences of the United States of America,* **2000**, *97*, (8), 3850-3855.

[57] Anchoori, R.K.; Khan, S.R.; Sueblinvong, T.; Felthauser, A.; Iizuka, Y.; Gavioli, R.; Destro, F.; Vogel, R.I.; Peng, S.W.; Roden, R.B.S.; Bazzaro, M., Stressing the Ubiquitin-Proteasome System without 20S Proteolytic Inhibition Selectively Kills Cervical Cancer Cells. *PLoS One,* **2011**, *6*, (8).

[58] Salum, L.B.; Altei, W.F.; Chiaradia, L.D.; Cordeiro, M.N.S.; Canevarolo, R.R.; Melo, C.P.S.; Winter, E.; Mattei, B.; Daghestani, H.N.; Santos-Silva, M.C.; Creczynski-Pasa, T.B.; Yunes, R.A.; Yunes, J.A.; Andricopulo, A.D.; Day, B.W.; Nunes, R.J.; Vogt, A., Cytotoxic 3,4,5-trimethoxychalcones as mitotic arresters and cell migration inhibitors. *Eur J Med Chem,* **2013**, *63*, 501-510.

[59] Lee, J.M.; Lee, M.S.; Koh, D.; Lee, Y.H.; Lim, Y.; Shin, S.Y., A new synthetic 2'-hydroxy-2,4,6-trimethoxy-5',6'-naphthochalcone induces G2/M cell cycle arrest and apoptosis by disrupting the microtubular network of human colon cancer cells. *Cancer Lett,* **2014**, *354*, (2), 348-354.

[60] Ducki, S., The development of chalcones as promising anticancer agents. *Idrugs,* **2007**, *10*, (1), 42-46.

[61] Ducki, S.; Mackenzie, G.; Greedy, B.; Armitage, S.; Chabert, J.F.D.; Bennett, E.; Nettles, J.; Snyder, J.P.; Lawrence, N.J., Combretastatin-like chalcones as inhibitors of microtubule polymerisation. Part 2: Structure-based discovery of alpha-aryl chalcones. *Bioorg Med Chem,* **2009**, *17*, (22), 7711-7722.

[62] Vitorovic-Todorovic, M.D.; Eric-Nikolic, A.; Kolundzija, B.; Hamel, E.; Ristic, S.; Juranic, I.O.; Drakulic, B.J., (E)-4-aryl-4-oxo-2-butenoic acid amides, chalcone-aroylacrylic acid chimeras: design, antiproliferative activity and inhibition of tubulin polymerization. *Eur J Med Chem,* **2013**, *62*, 40-50.

[63] Konieczny, M.T.; Bulakowska, A.; Pirska, D.; Konieczny, W.; Skladanowski, A.; Sabisz, M.; Wojciechowski, M.; Lemke, K.; Pieczykolan, A.; Strozek, W., Structural factors affecting affinity of cytotoxic oxathiole-fused chalcones toward tubulin. *Eur J Med Chem,* **2015**, *89*, 733-742.

[64] Mesenzani, O.; Massarotti, A.; Giustiniano, M.; Pirali, T.; Bevilacqua, V.; Caldarelli, A.; Canonico, P.; Sorba, G.; Novellino, E.; Genazzani, A.A.; Tron, G.C., Replacement of the double bond of antitubulin chalcones with triazoles and tetrazoles: Synthesis and biological evaluation. *Bioorg Med Chem Lett,* **2011**, *21*, (2), 764-768.

[65] Kamal, A.; Balakrishna, M.; Nayak, V.L.; Shaik, T.B.; Faazil, S.; Nimbarte, V.D., Design and synthesis of imidazo[2,1-b]thiazole-chalcone conjugates: microtubule-destabilizing agents. *ChemMedChem,* **2014**, *9*, (12), 2766-2780.

[66] Hengartner, M.O., The biochemistry of apoptosis. *Nature,* **2000**, *407*, (6805), 770-776.

[67] Jung, J.I.; Lim, S.S.; Choi, H.J.; Cho, H.J.; Shin, H.K.; Kim, E.J.; Chung, W.Y.; Park, K.K.; Park, J.H.Y., Isoliquiritigenin induces apoptosis by depolarizing mitochondrial membranes in prostate cancer cells. *Journal of Nutritional Biochemistry,* **2006**, *17*, (10), 689-696.

[68] Zi, X.L.; Simoneau, A.R., Flavokawain A, a novel chalcone from kava extract, induces apoptosis in bladder cancer cells by involvement of Bax protein-dependent and mitochondria-dependent apoptotic pathway and suppresses tumor growth in mice. *Cancer Research,* **2005**, *65*, (8), 3479-3486.

[69] Thorburn, A.; Behbakht, K.; Ford, H., TRAIL receptor-targeted therapeutics: resistance mechanisms and strategies to avoid them. *Drug Resist Updat,* **2008**, *11*, (1-2), 17-24.

[70] Yoshida, T.; Horinaka, M.; Takara, M.; Tsuchihashi, M.; Mukai, N.; Wakada, M.; Sakai, T., Combination of isoliquiritigenin and tumor necrosis factor-related apoptosis-inducing ligand induces apoptosis in colon cancer HT29 cells. *Environ Health Prev Med,* **2008**, *13*, (5), 281-287.

[71] Ohtsuki, T.; Kikuchi, H.; Koyano, T.; Kowithayakorn, T.; Sakai, T.; Ishibashi, M., Death receptor 5 promoter-enhancing compounds isolated from Catimbium speciosum and their enhancement effect on TRAIL-induced apoptosis. *Bioorg Med Chem,* **2009**, *17*, (18), 6748-6754.

[72] Tang, Y.X.; Li, X.B.; Liu, Z.B.; Simoneau, A.R.; Xie, J.; Zi, X.L., Flavokawain B, a kava chalcone, induces apoptosis via up-regulation of death-receptor 5 and Bim expression in androgen receptor negative, hormonal refractory prostate cancer cell lines and reduces tumor growth. *International Journal of Cancer,* **2010**, *127*, (8), 1758-1768.

[73] An, J.X.; Gao, Y.R.; Wang, J.; Zhu, Q.Y.; Ma, Y.L.; Wu, J.; Sun, J.; Tang, Y.X., Flavokawain B induces apoptosis of non-small cell lung cancer H460 cells via Bax-initiated mitochondrial and JNK pathway. *Biotechnology Letters,* **2012**, *34*, (10), 1781-1788.

[74] Abu, N.; Mohamed, N.E.; Yeap, S.K.; Lim, K.L.; Akhtar, M.N.; Zulfadli, A.J.; Kee, B.B.; Abdullah, M.P.; Omar, A.R.; Alitheen, N.B., In vivo antitumor and antimetastatic effects of flavokawain B in 4T1 breast cancer cell-challenged mice. *Drug Des Devel Ther,* **2015**, *9*, 1401-1417.

[75] Tang, Y.L.; Huang, L.B.; Tian, Y.; Wang, L.N.; Zhang, X.L.; Ke, Z.Y.; Deng, W.G.; Luo, X.Q., Flavokawain B inhibits the growth of acute lymphoblastic leukemia cells via p53 and caspase-dependent mechanisms. *Leuk Lymphoma,* **2015**, 1-10.

[76] Lin, E.; Lin, W.H.; Wang, S.Y.; Chen, C.S.; Liao, J.W.; Chang, H.W.; Chen, S.C.; Lin, K.Y.; Wang, L.; Yang, H.L.; Hseu, Y.C., Flavokawain B inhibits growth of human squamous carcinoma cells: Involvement of apoptosis and cell cycle dysregulation in vitro and in vivo. *Journal of Nutritional Biochemistry,* **2012**, *23*, (4), 368-378.

[77] Lecerf-Schmidt, F.; Peres, B.; Valdameri, G.; Gauthier, C.; Winter, E.; Payen, L.; Di Pietro, A.; Boumendjel, A., ABCG2: recent discovery of potent and highly selective inhibitors. *Future Medicinal Chemistry,* **2013**, *5*, (9), 1037-1045.

[78] Martin-Cordero, C.; Sanchez-Pico, A.; Leon-Gonzalez, A.J.; Perez-Pulido, A.J.; Daga, R.R., Yeast as a Biosensor of Detoxification: A Tool for Identifying New Compounds that Revert Multidrug Resistance. *Current Drug Targets,* **2013**, *14*, (9), 964-985.

[79] Ivanova, A.; Batovska, D.; Engi, H.; Parushev, S.; Ocsovszki, I.; Kostova, I.; Molnar, J., MDR-reversal activity of chalcones. *In Vivo,* **2008**, *22*, (3), 379-384.

[80] Parveen, Z.; Brunhofer, G.; Jabeen, I.; Erker, T.; Chiba, P.; Ecker, G.F., Synthesis, biological evaluation and 3D-QSAR studies of new chalcone derivatives as inhibitors of human P-glycoprotein. *Bioorg Med Chem,* **2014**, *22*, (7), 2311-2319.

[81] Han, Y.; Riwanto, M.; Go, M.L.; Ee, P.L.R., Modulation of breast cancer resistance protein (BCRP/ABCG2) by non-basic chalcone analogues. *European Journal of Pharmaceutical Sciences,* **2008**, *35*, (1-2), 30-41.

[82] Lorendeau, D.; Dury, L.; Genoux-Bastide, E.; Lecerf-Schmidt, F.; Simoes-Pires, C.; Carrupt, P.A.; Terreux, R.; Magnard, S.; Di Pietro, A.; Boumendjel, A.; Baubichon-Cortay, H., Collateral sensitivity of resistant MRP1-overexpressing cells to flavonoids and derivatives through GSH efflux. *Biochem Pharmacol,* **2014**, *90*, (3), 235-245.

[83] Kim, J.Y.; Park, S.J.; Yun, K.J.; Cho, Y.W.; Park, H.J.; Lee, K.T., Isoliquiritigenin isolated from the roots of Glycyrrhiza uralensis inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. *Eur J Pharmacol,* **2008**, *584*, (1), 175-184.

[84] Funakoshi-Tago, M.; Tanabe, S.; Tago, K.; Itoh, H.; Mashino, T.; Sonoda, Y.; Kasahara, T., Licochalcone A potently inhibits tumor necrosis factor alpha-induced nuclear factor-kappaB activation through the direct inhibition of IkappaB kinase complex activation. *Mol Pharmacol,* **2009**, *76*, (4), 745-753.

[85] Harikumar, K.B.; Kunnumakkara, A.B.; Ahn, K.S.; Anand, P.; Krishnan, S.; Guha, S.; Aggarwal, B.B., Modification of the cysteine residues in I kappa B alpha kinase and NF-kappa B (p65) by xanthohumol leads to suppression of NF-kappa B-regulated gene products and potentiation of apoptosis in leukemia cells. *Blood,* **2009**, *113*, (9), 2003-2013.

[86] Anand, P.; Sung, B.; Kunnumakkara, A.B.; Rajasekharan, K.N.; Aggarwal, B.B., Suppression of pro-inflammatory and proliferative pathways by diferuloylmethane (curcumin) and its analogues dibenzoylmethane, dibenzoylpropane, and dibenzylideneacetone: Role of Michael acceptors and Michael donors. *Biochem Pharmacol,* **2011**, *82*, (12), 1901-1909.

[87] Orlikova, B.; Tasdemir, D.; Golais, F.; Dicato, M.; Diederich, M., The aromatic ketone 4'-hydroxychalcone inhibits TNFalpha-induced NF-kappaB activation via

proteasome inhibition. *Biochem Pharmacol,* **2011**, *82*, (6), 620-631.

[88] Reddy, M.V.B.; Shen, Y.C.; Yang, J.S.; Hwang, T.L.; Bastow, K.F.; Qian, K.D.; Lee, K.H.; Wu, T.S., New bichalcone analogs as NF-kappa B inhibitors and as cytotoxic agents inducing Fas/CD95-dependent apoptosis. *Bioorg Med Chem,* **2011**, *19*, (6), 1895-1906.

[89] Galvez-Peralta, M.; Flatten, K.S.; Loegering, D.A.; Peterson, K.L.; Schneider, P.A.; Erlichman, C.; Kaufmann, S.H., Context-dependent antagonism between Akt inhibitors and topoisomerase poisons. *Mol Pharmacol,* **2014**, *85*, (5), 723-734.

[90] Albini, A.; Dell'Eva, R.; Vene, R.; Ferrari, N.; Buhler, D.R.; Noonan, D.M.; Fassina, G., Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF-kappa B and Akt as targets. *Faseb Journal,* **2005**, *19*, (14), 527-+.

[91] Dell'Eva, R.; Ambrosini, C.; Vannini, N.; Piaggio, G.; Albini, A.; Ferrari, N., AKT/NF-kappaB inhibitor xanthohumol targets cell growth and angiogenesis in hematologic malignancies. *Cancer,* **2007**, *110*, (9), 2007-2011.

[92] Singhai, R.; Patil, V.W.; Jaiswal, S.R.; Patil, S.D.; Tayade, M.B.; Patil, A.V., E-Cadherin as a diagnostic biomarker in breast cancer. *N Am J Med Sci,* **2011**, *3*, (5), 227-233.

[93] Bracke, M.E.; Vanhoecke, B.W.A.; Derycke, L.; Bolca, S.; Possemiers, S.; Heyerick, A.; Stevens, C.V.; De Keukeleire, D.; Depypere, H.T.; Verstraete, W.; Williams, C.A.; McKenna, S.T.; Tomar, S.; Sharma, D.; Prasad, A.K.; DePass, A.L.; Parmar, V.S., Plant polyphenolics as anti-invasive cancer agents. *Anti-Cancer Agents in Medicinal Chemistry,* **2008**, *8*, (2), 171-185.

[94] Wang, Z.Y.; Wang, N.; Han, S.W.; Wang, D.M.; Mo, S.L.; Yu, L.Z.; Huang, H.; Tsui, K.; Shen, J.G.; Chen, J.P., Dietary Compound Isoliquiritigenin Inhibits Breast Cancer Neoangiogenesis via VEGF/VEGFR-2 Signaling Pathway. *PLoS One,* **2013**, *8*, (7).

[95] Chung, C.H.; Chang, C.H.; Chen, S.S.; Wang, H.H.; Yen, J.Y.; Hsiao, C.J.; Wu, N.L.; Chen, Y.L.; Huang, T.F.; Wang, P.C.; Yeh, H.I.; Wang, S.W., Butein Inhibits Angiogenesis of Human Endothelial Progenitor Cells via the Translation Dependent Signaling Pathway. *Evid Based Complement Alternat Med,* **2013**, *2013*, 943187.

[96] Liu, S.C.; Chen, C.; Chung, C.H.; Wang, P.C.; Wu, N.L.; Cheng, J.K.; Lai, Y.W.; Sun, H.L.; Peng, C.Y.; Tang, C.H.; Wang, S.W., Inhibitory Effects of Butein on Cancer Metastasis and Bioenergetic Modulation. *J Agric Food Chem,* **2014**, *62*, (37), 9109-9117.

[97] Bukhari, S.N.A.; Jasamai, M.; Jantan, I.; Ahmad, W., Review of Methods and Various Catalysts Used for Chalcone Synthesis. *Mini-Reviews in Organic Chemistry,* **2013**, *10*, (1), 73-83.

[98] Won, S.J.; Liu, C.T.; Tsao, L.T.; Weng, J.R.; Ko, H.H.; Wang, J.P.; Lin, C.N., Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. *Eur J Med Chem,* **2005**, *40*, (1), 103-112.

[99] Dong, F.; Jian, C.; Fei, Z.H.; Kai, G.; Liu, Z.L., Synthesis of chalcones via Claisen-Schmidt condensation reaction catalyzed by acyclic acidic ionic liquids. *Catalysis Communications,* **2008**, *9*, (9), 1924-1927.

[100] Eddarir, S.; Cotelle, N.; Bakkour, Y.; Rolando, C., An efficient synthesis of chalcones based on the Suzuki reaction. *Tetrahedron Letters,* **2003**, *44*, (28), 5359-5363.

[101] Qi, Z.; Liu, M.; Liu, Y.; Zhang, M.; Yang, G., Tetramethoxychalcone, a chalcone derivative, suppresses proliferation, blocks cell cycle progression, and induces apoptosis of human ovarian cancer cells. *PLoS One,* **2014**, *9*, (9), e106206.

[102] Zhu, C.; Zuo, Y.; Wang, R.; Liang, B.; Yue, X.; Wen, G.; Shang, N.; Huang, L.; Chen, Y.; Du, J.; Bu, X., Discovery of potent cytotoxic ortho-aryl chalcones as new scaffold targeting tubulin and mitosis with affinity-based fluorescence. *J Med Chem,* **2014**, *57*, (15), 6364-6382.

[103] Shoemaker, R.H., The NCI60 human tumour cell line anticancer drug screen. *Nature Reviews Cancer,* **2006**, *6*, (10), 813-823.

[104] Pingaew, R.; Saekee, A.; Mandi, P.; Nantasenamat, C.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V., Synthesis, biological evaluation and molecular docking of novel chalcone-coumarin hybrids as anticancer and antimalarial agents. *Eur J Med Chem,* **2014**, *85*, 65-76.

[105] Haddad, A.Q.; Fleshner, N.; Nelson, C.; Saour, B.; Musquera, M.; Venkateswaran, V.; Klotz, L., Antiproliferative mechanisms of the flavonoids 2,2'-dihydroxychalcone and fisetin in human prostate cancer cells. *Nutr Cancer,* **2010**, *62*, (5), 668-681.

[106] Haddad, A.Q.; Venkateswaran, V.; Viswanathan, L.; Teahan, S.J.; Fleshner, N.E.; Klotz, L.H., Novel antiproliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. *Prostate Cancer Prostatic Dis,* **2006**, *9*, (1), 68-76.

[107] Goh, K.; Chen, Y.; Zheng, L.; Ong, L.; Jin, Y.; Chow, P.; Zhang, K., 2,2'-Dihydroxychalcone, a glutathione transferase inhibitor, sensitises human colon adenocarcinoma cells to chlorambucil and melphalan, but not to actinomycin D. *Mol Med Rep,* **2008**, *1*, (4), 575-579.

[108] Saydam, G.; Aydin, H.H.; Sahin, F.; Kucukoglu, O.; Erciyas, E.; Terzioglu, E.; Buyukkececi, F.; Omay, S.B., Cytotoxic and inhibitory effects of 4,4'-dihydroxy chalcone (RVC-588) on proliferation of human leukemic HL-60 cells. *Leuk Res,* **2003**, *27*, (1), 57-64.

[109] Nam, N.H.; Kim, Y.; You, Y.J.; Hong, D.H.; Kim, H.M.; Ahn, B.Z., Cytotoxic 2',5'-dihydroxychalcones with unexpected antiangiogenic activity. *Eur J Med Chem,* **2003**, *38*, (2), 179-187.

[110] Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J., Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. *Bioorg Med Chem,* **2008**, *16*, (8), 4286-4293.

[111] Yazaki, K.; Sasaki, K.; Tsurumaru, Y., Prenylation of aromatic compounds, a key diversification of plant secondary metabolites. *Phytochemistry,* **2009**, *70*, (15-16), 1739-1745.

[112] Wang, H.M.; Zhang, L.; Liu, J.; Yang, Z.L.; Zhao, H.Y.; Yang, Y.; Shen, D.; Lu, K.; Fan, Z.C.; Yao, Q.W.; Zhang, Y.M.; Teng, Y.O.; Peng, Y., Synthesis and anti-cancer activity evaluation of novel prenylated and geranylated chalcone natural products and their analogs. *Eur J Med Chem,* **2015**, *92*, 439-448.

[113] Wang, H.M.; Yan, Z.H.; Lei, Y.N.; Sheng, K.; Yao, Q.W.; Lu, K.; Yu, P., Concise synthesis of prenylated and geranylated chalcone natural products by regiospecific iodination and Suzuki coupling reactions. *Tetrahedron Letters,* **2014**, *55*, (4), 897-899.

[114] Bonifait, L.; Zhao, L.; Azelmat, J.; Genovese, S.; Epifano, F.; Grenier, D., Synthesis and Biological Activities of 2,6-Dihydroxy-4-Isopentenyloxychalcone as an Antimicrobial and Anti-Inflammatory Compound. *Medicinal Chemistry,* **2014**, *10*, (3), 300-303.

[115] Epifano, F.; Genovese, S.; Menghini, L.; Curini, M., Chemistry and pharmacology of oxyprenylated secondary plant metabolites. *Phytochemistry,* **2007**, *68*, (7), 939-953.

[116] Zhang, J.; Ji, F.J.; Gu, Y.; Zhang, X.Y.; Qiao, S.X., Chalcones derivatives as potent Cell division cycle 25B phosphatase inhibitors. *Pharmacol Rep,* **2014**, *66*, (3), 515-519.

[117] Kuppens, I.E.L.M.; Witteveen, P.O.; Schot, M.; Schuessler, V.M.; Daehling, A.; Beijnen, J.H.; Voest, E.E.; Schellens, J.H.M., Phase I dose-finding and pharmacokinetic trial of orally administered indibulin (D-24851) to patients with solid tumors. *Investigational New Drugs,* **2007**, *25*, (3), 227-235.

[118] Wang, G.; Li, C.; He, L.; Lei, K.; Wang, F.; Pu, Y.; Yang, Z.; Cao, D.; Ma, L.; Chen, J.; Sang, Y.; Liang, X.; Xiang, M.; Peng, A.; Wei, Y.; Chen, L., Design, synthesis and biological evaluation of a series of pyrano chalcone derivatives containing indole moiety as novel anti-tubulin agents. *Bioorg Med Chem,* **2014**, *22*, (7), 2060-2079.

[119] Ye, H.Y.; Fu, A.; Wu, W.S.; Li, Y.F.; Wang, G.C.; Tang, M.H.; Li, S.C.; He, S.C.; Zhong, S.J.; Lai, H.J.; Yang, J.H.; Xiang, M.L.; Peng, A.H.; Chen, L.J., Cytotoxic and apoptotic effects of constituents from Millettia pachycarpa Benth. *Fitoterapia,* **2012**, *83*, (8), 1402-1408.

[120] Mathew, B.; Suresh, J.; Anbazghagan, S.; Paulraj, J.; Krishnan, G.K., Heteroaryl chalcones: Mini review about their therapeutic voyage. *Biomedicine & Preventive Nutrition,* **2014**, *4*, (3), 451-458.

[121] Kumar, D.; Maruthi Kumar, N.; Tantak, M.P.; Ogura, M.; Kusaka, E.; Ito, T., Synthesis and identification of alpha-cyano bis(indolyl)chalcones as novel anticancer agents. *Bioorg Med Chem Lett,* **2014**, *24*, (22), 5170-5174.

[122] Nepali, K.; Sharma, S.; Sharma, M.; Bedi, P.M.S.; Dhar, K.L., Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids. *Eur J Med Chem,* **2014**, *77*, 422-487.

[123] Venkateswararao, E.; Sharma, V.K.; Yun, J.; Kim, Y.; Jung, S.H., Anti-proliferative effect of chalcone derivatives through inactivation of NF-kappaB in human cancer cells. *Bioorg Med Chem,* **2014**, *22*, (13), 3386-3392.

[124] Ku, B.M.; Ryu, H.W.; Lee, Y.K.; Ryu, J.; Jeong, J.Y.; Choi, J.; Cho, H.J.; Park, K.H.; Kang, S.S., 4'-Acetoamido-4-hydroxychalcone, a chalcone derivative, inhibits glioma growth and invasion through regulation of the tropomyosin 1 gene. *Biochem Biophys Res Commun,* **2010**, *402*, (3), 525-530.

[125] Hwang, D.; Hyun, J.; Jo, G.; Koh, D.; Lim, Y., Synthesis and complete assignment of NMR data of 20 chalcones. *Magn Reson Chem,* **2011**, *49*, (1), 41-45.

[126] Shin, S.Y.; Kim, J.H.; Yoon, H.; Choi, Y.K.; Koh, D.; Lim, Y.; Lee, Y.H., Novel antimitotic activity of 2-hydroxy-4-methoxy-2',3'-benzochalcone (HymnPro) through the inhibition of tubulin polymerization. *J Agric Food Chem,* **2013**, *61*, (51), 12588-12597.

[127] Winter, E.; Chiaradia, L.D.; Silva, A.H.; Nunes, R.J.; Yunes, R.A.; Creczynski-Pasa, T.B., Involvement of extrinsic and intrinsic apoptotic pathways together with endoplasmic reticulum stress in cell death induced by naphthylchalcones in a leukemic cell line: advantages of multi-target action. *Toxicol In Vitro,* **2014**, *28*, (5), 769-777.

[128] Orlikova, B.; Menezes, J.C.; Ji, S.; Kamat, S.P.; Cavaleiro, J.A.; Diederich, M., Methylenedioxy flavonoids: assessment of cytotoxic and anti-cancer potential in human leukemia cells. *Eur J Med Chem,* **2014**, *84*, 173-180.

[129] Henmi, K.; Hiwatashi, Y.; Hikita, E.; Toyama, N.; Hirano, T., Methoxy- and fluoro-chalcone derivatives arrest cell cycle progression and induce apoptosis in human melanoma cell A375. *Biol Pharm Bull,* **2009**, *32*, (6), 1109-1113.

[130] Lawrence, N.J.; Patterson, R.P.; Ooi, L.L.; Cook, D.; Ducki, S., Effects of alpha-substitutions on structure and biological activity of anticancer chalcones. *Bioorg Med Chem Lett,* **2006**, *16*, (22), 5844-5848.

[131] Cabrera, M.; Simoens, M.; Falchi, G.; Lavaggi, M.L.; Piro, O.E.; Castellano, E.E.; Vidal, A.; Azqueta, A.; Monge, A.; de Cerain, A.L.; Sagrera, G.; Seoane, G.; Cerecetto, H.; Gonzalez, M., Synthetic chalcones, flavanones, and flavones as antitumoral agents: biological evaluation and structure-activity relationships. *Bioorg Med Chem,* **2007**, *15*, (10), 3356-3367.

[132] Ren, J.L.; Zhang, X.Y.; Yu, B.; Wang, X.X.; Shao, K.P.; Zhu, X.G.; Liu, H.M., Discovery of novel AHLs as potent antiproliferative agents. *Eur J Med Chem,* **2015**, *93C*, 321-329.

[133] Markovic, V.; Debeljak, N.; Stanojkovic, T.; Kolundzija, B.; Sladic, D.; Vujcic, M.; Janovic, B.; Tanic, N.; Perovic, M.; Tesic, V.; Antic, J.; Joksovic, M.D., Anthraquinone-chalcone hybrids: synthesis, preliminary antiproliferative evaluation and DNA-interaction studies. *Eur J Med Chem,* **2015**, *89*, 401-410.

[134] Abdel-Aziz, M.; Park, S.E.; Abuo-Rahma Gel, D.; Sayed, M.A.; Kwon, Y., Novel N-4-piperazinyl-ciprofloxacin-chalcone hybrids: synthesis, physicochemical properties, anticancer and topoisomerase I and II inhibitory activity. *Eur J Med Chem,* **2013**, *69*, 427-438.

[135] Singh, N.; Sarkar, J.; Sashidhara, K.V.; Ali, S.; Sinha, S., Anti-tumour activity of a novel coumarin-chalcone hybrid is mediated through intrinsic apoptotic pathway by inducing PUMA and altering Bax/Bcl-2 ratio. *Apoptosis,* **2014**, *19*, (6), 1017-1028.

[136] Nakhjavani, M.; Zarghi, A.; F, H.S., Cytotoxicity of selected novel chalcone derivatives on human breast, lung and hepatic carcinoma cell lines. *Iran J Pharm Res,* **2014**, *13*, (3), 953-958.

[137] Kim, T.H.; Seo, W.D.; Ryu, H.W.; Seo, H.R.; Jin, Y.B.; Lee, M.; Ji, Y.H.; Park, K.H.; Lee, Y.S., Anti-tumor effects by a synthetic chalcone compound is mediated by c-Myc-mediated reactive oxygen species production. *Chem Biol Interact,* **2010**, *188*, (1), 111-118.

[138] Sharma, S.; Kaur, C.; Budhiraja, A.; Nepali, K.; Gupta, M.K.; Saxena, A.K.; Bedi, P.M., Chalcone based azacarboline analogues as novel antitubulin agents: design, synthesis, biological evaluation and molecular modelling studies. *Eur J Med Chem,* **2014**, *85*, 648-660.

[139] Chauhan, S.S.; Singh, A.K.; Meena, S.; Lohani, M.; Singh, A.; Arya, R.K.; Cheruvu, S.H.; Sarkar, J.; Gayen, J.R.; Datta, D.; Chauhan, P.M., Synthesis of novel beta-carboline based chalcones with high cytotoxic activity against breast cancer cells. *Bioorg Med Chem Lett,* **2014**, *24*, (13), 2820-2824.

[140] Kamal, A.; Kumar, G.B.; Vishnuvardhan, M.V.; Shaik, A.B.; Reddy, V.S.; Mahesh, R.; Sayeeda, I.B.; Kapure, J.S., Synthesis of phenstatin/isocombretastatin-chalcone conjugates as potent tubulin polymerization inhibitors and mitochondrial apoptotic inducers. *Org Biomol Chem,* **2015**.

[141] Loch-Neckel, G.; Bicca, M.A.; Leal, P.C.; Mascarello, A.; Siqueira, J.M.; Calixto, J.B., In vitro and in vivo anti-glioma activity of a chalcone-quinoxaline hybrid. *Eur J Med Chem,* **2015**, *90*, 93-100.

[142] Winter, E.; Devantier Neuenfeldt, P.; Chiaradia-Delatorre, L.D.; Gauthier, C.; Yunes, R.A.; Nunes, R.J.; Creczynski-Pasa, T.B.; Di Pietro, A., Symmetric bis-chalcones as a new type of breast cancer resistance protein inhibitors with a mechanism different from that of chromones. *J Med Chem,* **2014**, *57*, (7), 2930-2941.

[143] Reddy, M.V.; Chen, S.S.; Lin, M.L.; Chan, H.H.; Kuo, P.C.; Wu, T.S., Preparation of a series of novel bichalcones linked with a 1,4-dimethylenepiperazine moiety and examination of their cytotoxicity. *Chem Pharm Bull (Tokyo),* **2011**, *59*, (12), 1549-1554.

[144] Zoldakova, M.; Kornyei, Z.; Brown, A.; Biersack, B.; Madarasz, E.; Schobert, R., Effects of a combretastatin A4 analogous chalcone and its Pt-complex on cancer cells: A comparative study of uptake, cell cycle and damage to cellular compartments. *Biochem Pharmacol,* **2010**, *80*, (10), 1487-1496.

[145] Schobert, R.; Biersack, B.; Dietrich, A.; Knauer, S.; Zoldakova, M.; Fruehauf, A.; Mueller, T., Pt(II) complexes of a combretastatin A-4 analogous chalcone: effects of conjugation on cytotoxicity, tumor specificity, and long-term tumor growth suppression. *J Med Chem,* **2009**, *52*, (2), 241-246.

[146] Smit, F.J.; van Biljon, R.A.; Birkholtz, L.M.; N'Da, D.D., Synthesis and in vitro biological evaluation of dihydroartemisinyl-chalcone esters. *Eur J Med Chem,* **2015**, *90*, 33-44.