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Mini-review

A review of anti-infective and anti-inflammatory chalcones

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Abstract

Chalcones, considered as the precursors of flavonoids and isoflavonoids, are abundant in edible plants, and have also been shown to display a diverse array of pharmacological activities. The purpose of this review is to provide an overview of the pharmacological activity of synthetic and naturally occurring chalcones. This review is complementary to earlier reviews and covers more recent reports of antimicrobial activity of chalcones (antibacterial and antifungal), as well as antileishmanial, antimalarial, antiviral and anti-inflammatory activities of these compounds.

Keywords: Review; Chalcones; Antibacterial; Antifungal; Anti-inflammatory; Antileishmanial; Antimalarial; Antiviral; Activity

Chalcones – one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff have been recently subjects of great interest for their interesting pharmacological activities [1].

Chalcones, or 1,3-diaryl-2-propen-1-ones, belong to the flavonoid family. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. A vast number of naturally occurring chalcones are polyhydroxylated in the aryl rings. The radical quenching properties of the phenolic groups present in many chalcones have raised interest in using the compounds or chalcone rich plant extracts as drugs or food preservatives [2]. Chalcones have been reported to possess many useful properties, including anti-inflammatory, antimicrobial, antifungal, antioxidant, cytotoxic, antitumor and anticancer activities. For the reviews see Refs. [3,4].

Liquorice has been used in China for the treatment of gastric and duodenal ulcers, bronchial asthma, Addison's disease, food and drug poisoning and skin disease such as eczema and urticaria [5]. It still finds medical application because of its wide-ranging therapeutic properties including relief of rheumatic and other types of pain and healing effect on ulcers. The crude extract of liquorice has also found commercial use as a food additive in Japan since it contains the sweetening principle glycyrrhizin [6]. Isoliquiritigenin, a liquorice chalcone, is currently in use as a phosphodiesterase III inhibitor for the treatment of cardiovascular diseases [7]. In the far East countries such as Korea, Japan, and China butein has been traditionally used for treatment of pain, thrombotic disease gastritis, stomach cancer, and parasitic infections, as well as a food additive [8,9]. A number of chalcone derivatives, have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase [10], aldose reductase [11], epoxide hydrolase [12], protein tyrosine kinase [13,14] and quinone reductase [15].

This review is complementary to the earlier reviews and covers more recent and not adduced reports of naturally occurring and synthetic chalcones those demonstrate their significant activity.

1. Antibacterial properties

The antibacterial activity of chalcones is being increasingly documented. Many research groups either isolated and identified the structure of chalcones that possess antibacterial activity, or synthesized or modified natural chalcones. The

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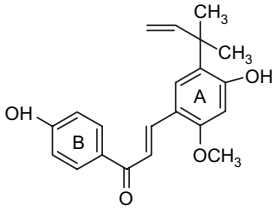
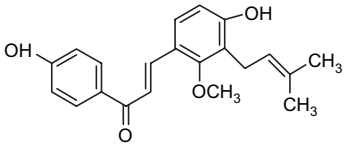
bactericidal effects have been related to the ability of the α,β-unsaturated ketone to undergo a conjugated addition to a nucleophilic group like a thiol group in an essential protein.

Liquorice (root and rhizome of *Glycyrrhiza* spp.) is currently used in the tobacco, confectionery, and pharmaceutical industries. Among the retrochalcones (chalcones which do not have an oxygen-function at the 2-position) isolated from *Glycyrrhiza inflata* licochalcone A **1A** and licochalcone C **1B** showed potent antibacterial activity especially to *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*.



1A licochalcone A



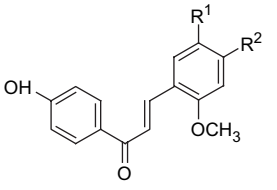
1B licochalcone C

The bacteriostatic effects of licochalcone A **1A** tested by Tsukiyama et al. [16] were shown by MICs of 2–15 μg ml^{-1} for Gram-positive bacteria including spore-forming bacteria, such as the genera *Bacillus coagulans*, *B. subtilis* and *Bacillus stearothermophilus* (MIC = 2 μg ml^{-1}) as well as *Clostridium sporogenes* (8 μg ml^{-1}), and toxin-producing bacteria such as *Bacillus cereus* (3 μg ml^{-1}) and *S. aureus* (3 μg ml^{-1}). Licochalcone A was also effective when tested against *Lactobacillus acidophilus* and *Lactobacillus plantarum* with MICs of 5 μg ml^{-1}, as well as for *Enterococcus faecalis* and *Enterococcus faecium* with MICs of 6 μg ml^{-1}, and active against *Streptococcus lactis* and *Staphylococcus mutans* with MICs of 8 and 5 μg ml^{-1}, respectively. The bacteriostatic activity of licochalcone A was resistant to heating at 80–121 °C for 15 min and stable from pH 5.0 to pH 7.0, although the antibacterial activity at an acidic pH was higher than that at a neutral or alkaline pH. The antibacterial activity of licochalcone A was stable even in the presence of 3% (wt/vol) NaCl, and this compound was resistant to protease digestion [16].

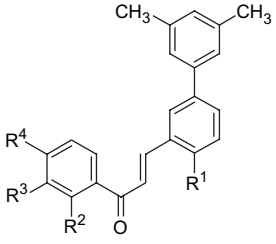
It was also reported by Friis-Möller and colleagues [17] that licochalcone A at a concentration of 1–2 mg l^{-1} inhibited the growth of *Legionella pneumophila*, *Legionella longbeacheae*, *Legionella wadsworthii*, *Legionella bozemanii*, *Legionella dumoffi* and *Legionella feelei*. Additionally, when Fukai et al. [18] examined licochalcone A, inhibitory activity was detected against the growth of *Helicobacter pyroli* in vitro (MIC = 25 μg ml^{-1}).

Kromann et al. [19] tested the analogues of licochalcone A against *S. aureus* and showed that the free hydroxyl group in 4′-position of ring B was necessary for the antibacterial activity. On the other hand, no change in its activity is observed when the hydroxyl group in the 4-position of ring A is removed, blocked by a methyl, or replaced by a chlorine (compound **2** MIC = 10 μM against *S. aureus*). Removal of both hydroxyl groups or blockage of both hydroxyl groups by methylation eliminates the activity completely. When the lipophilic prenyl group is removed a total loss of activity is also observed. If the prenyl group is exchanged by a propyl group a moderate antibacterial effect is observed. The introduction of the longer hexyl group results in chalcone **3** that is more potent than licochalcone A. When comparing ClogP with the antibacterial activity of the compounds studied, a clear positive correlation between lipophilicity and antibacterial activity is seen. The strong lipophilic character of the molecule plays an essential role in the antibacterial effect. These findings are in accordance with the results shown by Haraguchi et al. [20].

Antibacterial assays of liquorice phenolics for *S. aureus*, including a few strains of methicillin-resistant (MRSA), and methicillin-sensitive *S. aureus* (MSSA) were examined by Hatano et al. [21] and Fukai et al. [22]. Licochalcone A showed antibacterial effect on the MRSA strains (OM481, OM505) with MIC values of 16 μg ml^{-1} [21] and MRSA strains (K3 and ST28) with MIC = 6.25 μg ml^{-1} [22]. It exhibited antimicrobial activity (MIC = 16, 3.13, or 6.25 μg ml^{-1}) against MSSAs (strains FDA 209P, and Smith, respectively).



2 R^1= $C(CH_3)_2CH{=}CH_2$ R^2= Cl
3 R^1= C_6H_{13} R^2= OH



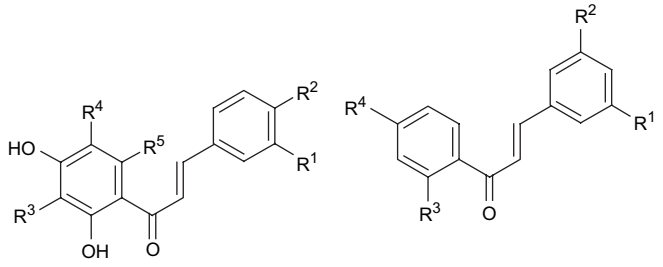
4 R^1= piperazine R^3= $NHCH_2CH_2N(CH_3)_2$ R^2R^4= H
5 R^1= 4-methylpiperazine R^3= $NHCH_2CH_2N(CH_3)_2$ R^2R^4= H
6 R^1= $OCH_2CH_2N(CH_3)_2$ R^2= F R^3= H R^4= OCH_3
7 R^1= $OCH_2CH_2N(CH_3)_2$ R^2= $CH_2N(CH_3)_2$ R^3= H R^4= OCH_3

In general, the substituent groups OH and OCH_3 exert the opposite effects on the anti-MRSA activity of chalcones, while the halogens (chlorine or fluorine) do not modify it significantly. The hydroxyl groups have the capacity to induce and enhance the activity, while the methoxy groups in the structure

of chalcones drastically reduce or eliminate the anti-MRSA activity [21]. A novel class of chalcones having aliphatic amino substituents **4–7** were studied by Nielsen et al. [23] and the SAR analysis was made. The position of the aliphatic amine in ring B only has a marginal effect on the activity. The distance between the aliphatic amino group and the aromatic ring is not important for the activity. The lipophilicity of ring A substituents appears to be the most important property with regard to modulating the activity. An increase in the size of the bulky substituent in the 5-position (ring A) gradually increases the activity. An aliphatic nitrogen atom spaced to ring A by a single methylene group reduces the activity, whereas an aliphatic nitrogen atom in a more distant position results in potent compounds independent of the linker. The most potent compound **4** in this study had a piperazine in the 2-position of ring A, and showed MIC values of 2 μM against methicillin-resistant *S. aureus*. This compound is also active against *E. faecium* and *Escherichia coli* with MIC = 5 μM.

A more recent paper [24] reported the antibacterial activity of isoliquiritigenine **8** against *S. aureus*, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* with MIC = 250 μg ml^{-1}, and pinocembrin chalcone **9** against *S. aureus* [25].

Chalcone **10** [26] isolated from *Dalea versicolor* exhibited individually and in synergy with known antibiotics (berberin, erythromycin and tetracycline) the activity towards the human pathogen *S. aureus* and the opportunistic pathogen *B. cereus*. The addition of this chalcone increased the activity of antibiotics in each case, but did so to a lesser extent in the case of the NorA *S. aureus* mutant, indicating a likely mode of action associated with the NorA efflux pump. This compound in the presence of berberine effected a dramatic 30-fold increase in activity against *B. cereus*.



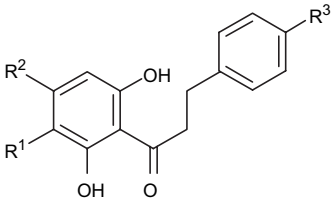
8 $R^1R^3R^4R^5$= H R^2= OH
9 $R^1R^3R^4R^5$= H R^5= OH
10 R^1R^2= H R^3R^4=CH_3 R^5= OCH_3

11 R^1R^2= CF_3 R^3= H R^4= COOH
12 R^1R^2= Br R^3= H R^4= COOH
13 R^1= Cl R^2R^4= H R^3= OH
14 R^1= I R^2R^4= H R^3= OH

Nielsen et al. [27] studied 4′-carboxy chalcones substituted in ring A and showed that many of these compounds were very interesting antibacterial compounds. The activity of these chalcones was correlated with the lipophilicity of the substituents in ring A. The lipophilicity of the substituent in ring A is essential for the activity. The lipophilic compounds were very potent and as the substituents become more polar the activity gradually decreased. Chalcones **11** and **12** had an inhibitory effect on bacterial growth but did not cause bacterial killing even at concentration of 16 times the MIC, thereby proving a bacteriostatic profile. The mechanism of the carboxy chalcones activity was different from that of hydroxychalcones, as the former were bacteriostatic, while the latter were bactericidal [27].

Biological screening of dihydrochalcones **15–18** [28–30] showed that these compounds demonstrated relatively good activity against the Gram-positive bacteria *S. aureus* and *B. subtilis*, and the Gram-negative bacterium *Pseudomonas aeruginosa*.

The asebogenin **18** [30] showed inhibitory activity (IC_{50} of 10 and 4.5 μg ml^{-1}, respectively) against *S. aureus* and methicillin-resistant *S. aureus* (MRSA).



15 R^1R^3= H R^2= OCH_3
16 R^1= CH_3 R^2= OH R^3= H
17 R^1= CHO R^2= OH R^3= H
18 R^1= H R^2= OCH_3 R^3= OH

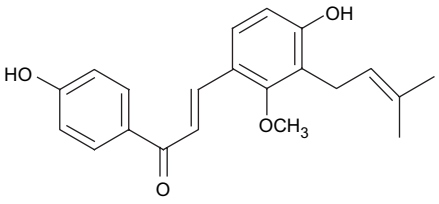
Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), a facultative intracellular bacillus, is the world's number one killer among infectious diseases and the leading cause of death among women of reproductive age. *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii*, *Mycobacterium xenophii* and *Mycobacterium marinum* were inhibited by licochalcone A **1A** (MIC ≤ 20 mg l^{-1}) [17].

The substitution of a halogen substituent on ring A of 2′-hydroxychalcone led to an increase in anti-TB activity. Compounds with a halogen at the 3-position demonstrated stronger anti-TB activity than those with a halogen substituent at the 2- or 4-position. Chalcones **13** and **14** [31] with a 2-hydroxyl group in ring B and a 3-chloro- or 3-iodo-group in ring A demonstrated the strongest activity, with 90–92% inhibition, against *Mtb* H37Rv at a drug concentration of 12.5 μg ml^{-1}. The activity of 2′-hydroxychalcone (61% inhibition) was enhanced by introducing a chloro (89%) or a methoxy group (78%) at the 4′-position of ring B. Introduction of an additional substituent, such as a methoxy, amino, bromo or carboxyl group on ring B of 2′-hydroxychalcone led to a dramatic decrease or a complete loss of activity [31].

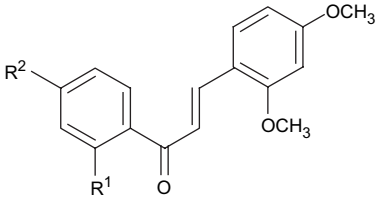
2. Antileishmanial properties

Leishmaniasis is a group of prevalent diseases caused by protozoan parasites belonging to the genus *Leishmania*.

Recently, a series of synthetic and naturally occurring chalcone derivatives have been reported to be potential agents against *Leishmania* in a number of in vitro and in vivo assays.



19 licochalcone C



20 R^1= H R^2= $OCH_2CH{=}CH_2$

21 R^1= H R^2= OC_4H_9

22 R^1= OH R^2= H

23 R^1R^2= H

24 R^1= H R^2= OH

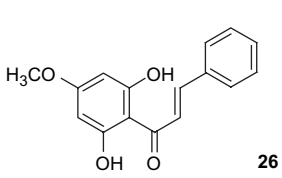
25 R^1= H R^2= $OCH_2CH{=}CH_2$

Though a large number of synthetic compounds have been tested, licochalcone A still remains one of the few naturally occurring chalcones under investigation. Various species of the protozoan parasite *Leishmania* cause a broad spectrum of diseases ranging from the cutaneous healing skin lesions caused by *Leishmania major* to a fatal visceral form called kala azar caused by *Leishmania donovani.*

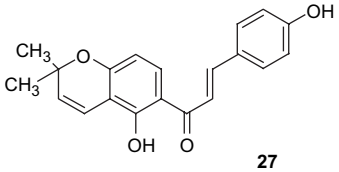
Chen et al. [32] demonstrated that licochalcone A **1A** inhibited the activities of both nicotinamide adenine dinucleotide reduced-fumarate reductase (NADH-FRD) and succinate dehydrogenase (SDH) in the permeabilized promastigotes in a concentration-dependent manner. This compound also inhibited the activities of SDH, NADH dehydrogenase (NDH), succinate-cytochrome *c* reductase (SCC), and NADH-cytochrome *c* reductase (NCC). The IC_{50}s of licochalcone A for SDH (593 μM), NDH (460 μM), SCC (1,519 μM), and NCC (1,985 μM) after 60 min of incubation were at least 33 times higher than the IC_{50} for fumarate reductase (FRD) (14 μM). The IC_{50}s of licochalcone A for SDH and NDH in the crude mitochondria of mammalian cells of human peripheral blood mononuclear cells (PBMC) were very high: both were 1.4 mM after 60 min of incubation. The IC_{50}s of licochalcone A for SDH in mammalian cells were more than 67 times higher than the IC_{50} for FRD in the parasite. Licochalcone A clearly exhibited concentration- and time-dependent inhibitory effects on soluble FRD in the parasite. The IC_{50} of licochalcone A after 60 min of incubation was 32 μM. Licochalcone A probably first inhibits FRD of the parasite, then influences the parasite respiratory chain and affects the function and ultrastructure of the parasite mitochondria, and finally kills the parasite. Licochalcone C **19** inhibited the growth of the *L. major* parasite to the same extent as licochalcone A [33]. Two other chalcones **20** and **21**, which showed potent activity against both extra- and intracellular forms of *Leishmania* parasites, also exhibited concentration- and time-dependent inhibitory effects on the activity of solubilized FRD in the parasite (IC_{50} = 153 and 118 μM, respectively).

The novel oxygenated chalcones **22**–**25** and **1A** tested by Zhai et al. [34] inhibited the in vitro growth of *L. major* promastigotes (IC_{50} in the range of 4.0–10.5 μM) measured by ^{3}H-thymidine incorporation and *L. donovani* amastigotes (IC_{50} in the range of 0.65–6.1 μM) in human monocyte-derived macrophages (MDM). These compounds also inhibited the respiration of the parasite and the activity of mitochondrial dehydrogenases. The antileishmanial activity of oxygenated chalcones might be a result of interference with the function of the parasite mitochondria. These results correlate well with those of Liu and colleagues [35] and Kayser and Kiderlen [36]. The chalcone and 4-chlorochalcone [37] clearly showed a concentration-dependent inhibitory effect on the in vitro growth of *Leishmania braziliensis* [IC_{50} = 13.7 and 21.9 μM, respectively] promastigotes and on *Trypanosoma cruzi* epimastigotes with no evidence of a cytotoxic effect on mouse peritoneal macrophages.

2′,6′-Dihydroxy-4′-methoxychalcone **26** [38] showed a significant activity in vitro against promastigotes and intracellular amastigotes of *Leishmania amazonensis*, with 50% effective doses of 0.5 and 24 μg ml^{-1}, respectively. Its inhibitory effect on amastigotes is apparently a direct effect on the parasites and is not due to activation of the nitrogen oxidative metabolism of macrophages, since the production of nitric oxide by both unstimulated and recombinant gamma interferon-stimulated macrophages was decreased rather than increased with **26**. Ultrastructural studies also showed that in the presence of **26**, the mitochondria of promastigotes were enlarged and disorganized. Despite destruction of intracellular amastigotes, no disarrangement of macrophage organelles was observed, even at 80 μg of **26** per milliliter. These observations suggest that 2′,6′-dihydroxy-4′-methoxychalcone is selectively toxic to the parasites. A few analogues containing nitro, fluoro or bromine groups at the *para*-position of ring A displayed increased selective activity against the parasites as compared with **26** [39].

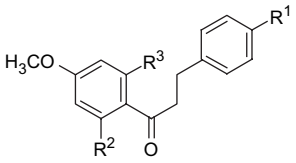


26



27

Chromeno chalcones and dihydrochalcones, containing 2′,2′-dimethyl benzopyran system were tested against extracellular promastigotes of *L. donovani* and intracellular amastigotes residing within murine macrophages [40,41]. The most potent chromeno chalcone (crotaramosmin) **27** had hydroxyl group on 4-position in ring A, and showed 84% inhibition against promastigotes and 74% inhibition against amastigotes at 50 μg ml^{-1} dose.



28 R^1= H R^2R^3= $OCOCH_3$
29 R^1R^3= $OCOCH_3$ R^2= OH
30 $R^1R^2R^3$= $OCOCH_3$

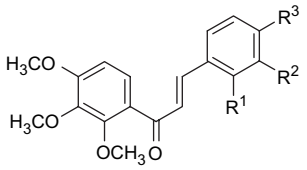
Hermoso et al. [42] prepared dihydrochalcone derivatives **28–30** in order to study the effect of these groups on the activity against *L. braziliensis*, *Leishmania tropica* and *Leishmania infantum*. The substitution of the methoxy group at C-4′ (ring B) by an acetate group increased both the activity and toxicity. However, a replacement of the methoxy group at C-4′ by an *O*-tetra-acetyl-β-D-glucosyl group increased the activity by almost 3-fold without increasing cytotoxicity. In addition, Hermoso et al. results, in accordance with modelling studies and the effects of chalcones on promastigotes of *L. major* reported by Nielsen et al. [43] support the fact that the pharmacophore contains two aromatic rings, and the propanone chain just functions as a spacer, and that the ability to inhibit parasite growth apparently depends on the presence and ratio of lipophilic/hydrophilic substituents at both aromatic rings, as concluded by Kayser and Kiderlen [36]. Analysis of the in vitro antileishmanial activity of chalcone derivatives [43] indicates also that ring B substitution did not play a major role for the antileishmanial activity, but substitution of hydroxyl groups for acetate groups not only increased the activity but also decreased the cytotoxicity to murine macrophages J774.

3. Antimalarial properties

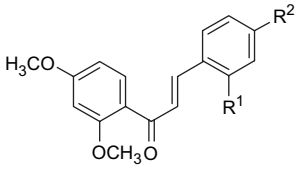
Plasmodium falciparum and *Plasmodium vivax* are the two major human malaria parasites. *P. falciparum* is responsible for most deaths, and it has developed resistance to nearly all available drugs. No wonder that the antimalarial activity of chalcones has generated great interest. Many chalcones have been described for their high antimalarial activity, probably as a result of a Michael addition of nucleophilic species to the double bond of the enone [44,45].

Licochalcone A **1A** isolated from Chinese liquorice roots, has been reported [46,47] as highly effective in an in vitro screen against chloroquine-susceptible (3D7) and chloroquine-resistant (Dd2) *P. falciparum* strains in a [^{3}H]hypoxanthine uptake assay. Licochalcone A administered either intraperitoneally or orally for 3–6 days protected the mice from the otherwise lethal *Plasmodium yoelii* infection.

In a detailed study Liu et al. [48] and Go et al. [49] showed that in vitro antimalarial activity of chalcones against a strain of chloroquine-resistant human malarial parasite, *P. falciparum* (K1) was mainly determined by the properties of ring B. The size and hydrophobicity of substituents were identified as critical parameters. Hydroxylated chalcones were less active than the corresponding alkoxylated analogues. A few of the alkoxylated chalcones **31–36** had IC_{50} values below 6.5 μM.



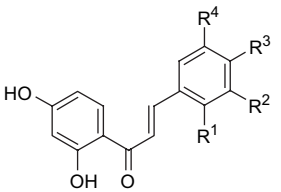
31 R^1R^2= H R^3= CF_3
32 R^1R^3= Cl R^2= H



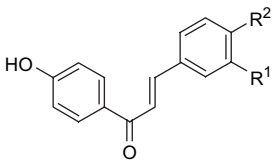
33 R^1R^2= OCH_3
34 R^1= H R^2= C_2H_5
35 R^1= H R^2= CF_3
36 R^1R^2= F

Among the hydroxylated chalcones, the most active compound was 4-chloro-2′,4′-dihydroxychalcone **37** with an IC_{50} of 12.3 μM against a strain of chloroquine-resistant human malarial parasite, *P. falciparum* (K1) in a [^{3}H]hypoxanthine uptake assay. A few other hydroxylated chalcones **38–41** have IC_{50} values below 20 μM.

The alkoxylated and hydroxylated chalcones were found to inhibit sorbitol-induced hemolysis of parasitized erythrocytes to a significant extent (≤40% of control values) at a concentration of 10 μM [49]. Most of the good inhibitors of sorbitol-induced hemolysis appear also as active antiplasmodial agents, but not all active antiplasmodial chalcones inhibit sorbitol-induced hemolysis. There is a possibility that the metabolites of chalcones act synergistically with the parent substance.

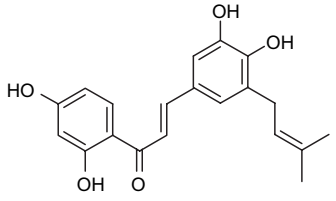


37 $R^1R^2R^4$= H R^3= Cl
38 R^1R^3= F R^2R^4= H
39 R^1= pyridinyl $R^2R^3R^4$= H

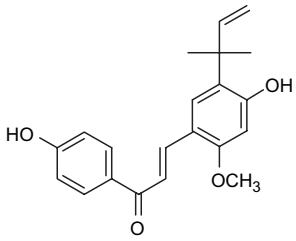


40 R^1R^2= Cl
41 R^1= H R^2= $N(CH_3)_2$

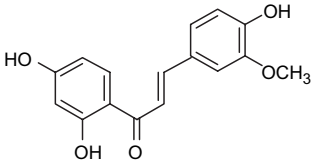
5-Prenylbutein **42**, licoagrochalcone A **43** and homobutein **44** [50] showed in vitro antiplasmodial activity against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) strains of *P. falciparum* with IC_{50} values in the range 10.3–16.1 μM.



42 5-prenylbutein

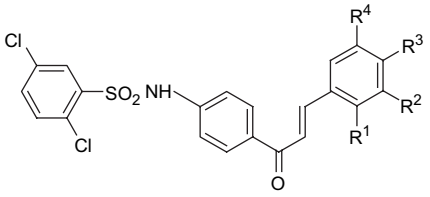


43 licoagrochalcone A



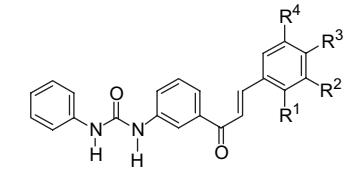
44 homobutein

Most of the sulfonamide chalcones [51] possessing di- and trimethoxy-substituted groups in the aromatic ring A showed measurable levels of inhibition of β-hematin formation with $IC_{50} = 0.48$ μM for **45** and 0.67 μM for **46**. The most active compound **47** was also effective as antimalarial by the inhibition of cultured *P. falciparum* strain W2 parasites (1 μM). Having only one Cl, F, CH_3 or OCH_3 substituent in the aromatic ring did not improve the activity of the sulfonamide chalcones compared with that of the corresponding disubstituted or trisubstituted analogues. The compounds that are not strongly basic, a property required for accumulation in the malaria parasite acidic food vacuole in which hemozoin formation takes place, have poor antimalarial activities.

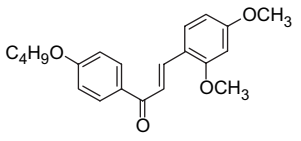


45 R^1= H $R^2R^3R^4$= OCH_3
46 R^1R^3= F R^2R^4= H
47 $R^1R^2R^4$= H R^3= CH_3

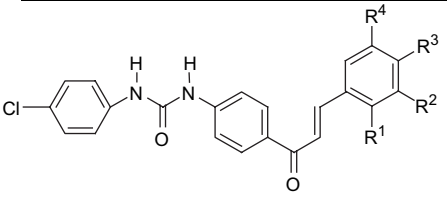
Domínguez et al. [52] reported a series of phenylurenyl chalcone derivatives with substitution in ring A. Their data suggest that the activity in most cases was governed to a large extent by groups attached to the substituted aromatic ring A (difluoro, dichloro, trimethoxy). The phenylurenyl chalcone derivatives were excellent inhibitors against cultured *P. falciparum* parasites ($IC_{50} = 1.76$–10 μM), with a good correlation with activity in the murine malaria model. In the series of 4′-phenylurenyl chalcone derivatives **48**–**50** the *para*-position in the urenyl ring plays an important role in their antimalarial activity. The compounds with chloro substituent in this position showed better activity. However, 3′-phenylurenyl chalcone derivatives **51**–**54** were much better active than corresponding 4′-phenylurenyl chalcone derivatives.



51 $R^2R^3R^4$= OCH_3 R^1= H
52 R^1R^3= Cl R^2R^4= H
53 $R^1R^2R^4$= H R^3= CH_3
54 R^1R^4= H R^2R^3= OCH_3



55

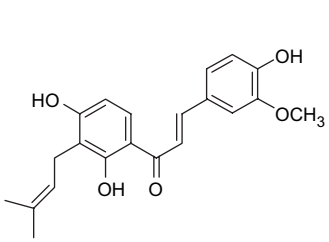


48 R^1R^3= Cl R^2R^4= H
49 R^1R^3= F R^2R^4= H
50 R^1= H $R^2R^3R^4$= OCH_3

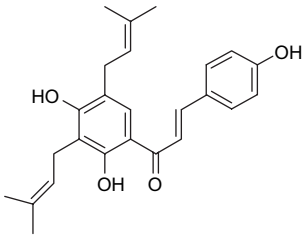
Domínguez et al. [52] reported a series of phenylurenyl chalcone derivatives with substitution in ring A. Their data suggest that the activity in most cases was governed to a large extent by groups attached to the substituted aromatic ring A (difluoro, dichloro, trimethoxy). The phenylurenyl chalcone derivatives were excellent inhibitors against cultured *P. falciparum* parasites ($IC_{50} = 1.76$–10 μM), with a good correlation

2,4-Dimethoxy-4′-butoxychalcone **55** [53] is a novel compound which has outstanding antimalarial activities against both human (in vitro) and rodent (in vivo) parasites with no observable signs of toxicity. Compound **55** exhibited a concentration-dependent inhibitory effect on the [^{3}H]hypoxanthine uptake of the chloroquine-susceptible (IC_{50} of 3D7 was 8.9 μM) and chloroquine-resistant (IC_{50} of Dd2 was 14.8 μM)

strains of *P. falciparum*. This chalcone completely inhibited the parasitemia of mice infected with *Plasmodium berghei* K173 and protected the mice from the lethal infection at a dose of 50 mg/kg/day given for 5 days by oral and intraperitoneal routes and 20 mg/kg/day for 5 days by the subcutaneous route.

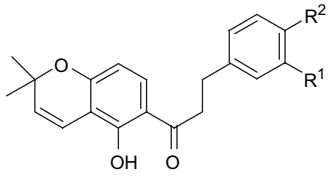


56 crotaorixin



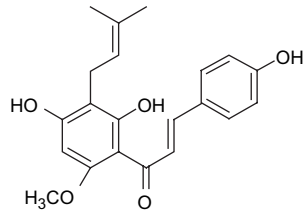
57 medicagenin

Crotaorixin **56** [54] isolated from the aerial parts of the *Crotalaria orixensis* exhibited 100% inhibition of maturation of *P. falciparum* (strain NF-54) parasites from ring stage to schizont stage both at 50 and 10 $\mu g\,ml^{-1}$ concentrations. The diprenylated compound medicagenin **57** which was isolated from the roots of *Crotalaria medicagenia* inhibited the parasites 100% at 2 $\mu g\,ml^{-1}$ concentration while the chromenodihydrochalcones (crotaramosmin **27**, crotaramin **58** and crotin **59**) from *Crotalaria ramosissima* showed lower activity. The diprenylation with a free 4,4′-dihydroxy system led to improved activity.



58 R^1= H R^2= OCH_3

59 R^1R^2= OH



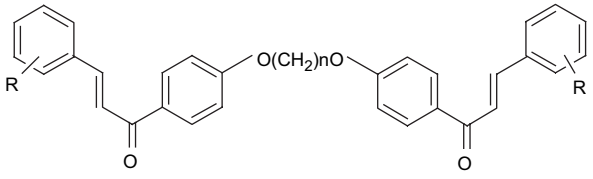
60 xanthohumol

In vitro evaluation of 4′-*tert*-butylo-4-bromochalcone [55] showed that it had the ability to inhibit recombinant *P. falciparum* iron superoxide dismutase (*Pf* SOD) (83% at 50 μM) and showed significant antimalarial activities against chloroquine-sensitive *P. falciparum* strain HB3 ($IC_{50} = 37$ μM) and the chloroquine-resistant strains Dd2 ($IC_{50} = 27$ μM). The chalcone-induced decrease of SOD activity could be attributable to covalent modification occurring at positively charged residues responsible for the electrostatic control of substrate diffusion, located at the entrance of the channel conducting towards the active metal ion.

Following from the in vitro antimalarial screening reported by Li et al. [56] the chalcones with chloro and fluoro substitutions at ring A and with electron-donating substituent (methoxy) at ring B have better antimalarial activities than when the ring A was exchanged with the ring B. The α,β-unsaturated ketone linker provides conjugation between aromatic groups on both ends and it seems to be critical for good activity.

Larsen et al. [57] summarised the antiplasmodial activity of two series of *E*- and *Z*-conformationally restricted analogues and their parent chalcones against *P. falciparum* 3D7. The analogues with the double bond in the *Z*-conformation were nearly inactive, whereas the corresponding analogues being locked in the *E*-conformation were equipotent to the parent chalcones.

Frölich et al. [58] tested in vitro antiplasmodial activity of prenylated chalcone derivatives from hops (*Humulus lupulus*) against the chloroquine-sensitive strain poW and the multiresistant clone Dd2 using a [^{3}H]hypoxanthine-incorporation assay. The main hop chalcone, xanthohumol **60**, was the most active with IC_{50} values of 8.2 ± 0.3 (poW) and 24.0 ± 0.8 μM (Dd2). The results have demonstrated for the first time the ability of chalcone derivatives to interfere with the haemin-degradation process of *P. falciparum*.



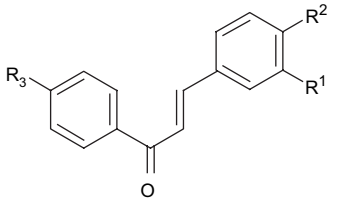
n= 1,3,4,6 R= 3,4-$(OCH_3)_2$ or 3-OCH_3 or 2,4-$(OCH_3)_2$

The in vivo antimalarial activity of bischalcones [45,59] against chloroquine-sensitive and resistant strains of *P. berghei* in mice revealed that the site of oxygenated substituents in the phenyl ring A greatly influences the activity profile. In general, chalcones with 3-methoxy and 3,4-dimethoxy substituents displayed a significant activity compared to 2,4-dimethoxy substituents. The compound with three-methylene-group chain contributes significantly more to the activity than those with the four- and six-methylene-group chain.

4. Antifungal properties

Since dermatophytes are a group of fungi which characteristically infect the keratinized areas of the body and dermatomycoses are very difficult to eradicate, it is very interesting to note that chalcone derivatives showed activity against dermatophytes and not against other types of fungi. Lopez et al. [60] tested chalcones **61**–**64** against a panel of human opportunistic pathogenic fungi, using the agar dilution method. Regarding the influence of the substituents on ring A, an interesting structure–activity correlation can be observed. (a) Electron-donating groups tended to weaken the antifungal activity. (b) Electron-withdrawing groups in the *para*-position increased the potency. Nevertheless, when the NO_2 or Cl group is in 2-position, a decrease in the activity is observed, suggesting that the presence of these group in the *ortho*-position of ring A could introduce important steric effects (effects that result from the size of substituents and the repulsion between them). Regarding the correlation of the antifungal activity of substituted chalcones with the planarity of their molecules,

a decrease of activity was observed for these compounds with a bulky *ortho* substituent on ring A (which affect the planarity confirming the steric hindrance) respective to the non-substituted chalcone. The additional presence of *ortho* substituents on ring B leads to a total loss of activity. (c) The presence of an enone linkage would be a structural requirement necessary but not by itself sufficient for the antifungal activity. Unexpected, the most active compound **64** does not have electron-withdrawing group in the *para*-position of ring A, but also does not have substituent in *ortho*-position. This compound showed strong antifungal activities against *Microsporum canis* (MIC = 25 μg ml^{-1}), *Microsporum gypseum* (1.5 μg ml^{-1}), *Trichophyton mentagrophytes* (3 μg ml^{-1}), *Trichophyton rubrum* (3 μg ml^{-1}) and *Epidermophyton floccosum* (0.5 μg ml^{-1}). 2-Chloro-2′-hydroxy-4′,6′-dimethoxychalcone [61] showed the lowest MIC against *T. rubrum* (12.5 μg ml^{-1}).



61 R^1R^3= H R^2= NO_2

62 R^1= OCH_3 R^2R^3= H

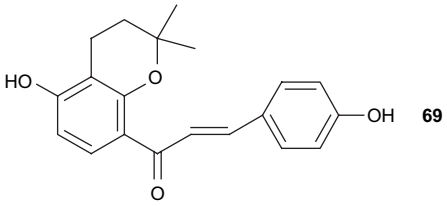
63 R^1R^3= H R^2= CH_3

64 R^1= OCH_3 R^2= H R^3= Br

The prenylated chalcones (isobavachalcone **65**) and **66** [62] isolated from the leaves of *Maclura tinctoria* were active against both fungal pathogens *Candida albicans* (IC_{50} of 3 and 15 μg ml^{-1}, respectively) and *Cryptococcus neoformans* (IC_{50} of 7 μg ml^{-1}).

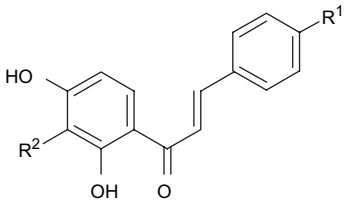
The methanolic extract of *Zuccagnia punctata* [63] consisting of 2′,4′-dihydroxy-3′-methoxychalcone **67** and 2′,4′-dihydroxychalcone **68** displayed very good activities (MIC = 6.25 and 3.12 μg ml^{-1}) against *Phomopsis longicolla* Hobbs CE117, and (MIC = 6.25 μg ml^{-1}) against *Colletotrichum truncatum* CE175, respectively. *P. longicolla* is a primary agent of seed decay, a highly severe pathology that affects soybean seed quality and yield, and it is present in almost every region of soybean production in the world. *C. truncatum* is among the most common soybean pathogens. It is the causal agent of soybean anthracnose, a disease acquired mainly in the last growing step that affects stems and pods diminishing the number of seeds and their weight.

Suman et al. [64] tested some substituted chalcones for their antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Colletotrichum capsicum* strains of phytopathogenic fungi. The study showed that α,β-dibromo-3,3′-dinitrochalcone had the activity against all three fungi with MIC = 6.25 μg ml^{-1}, and 4,4′-dimethylchalcone showed activity against *C. capsicum* (MIC = 6.25 μg ml^{-1}).



69

Crotmadine **69** isolated from the leaves and stems of *Crotalaria madurensis* [65] exhibited antifungal activity against *T. mentagrophytes* at a concentration of 62.5 μg ml^{-1}. Geranyl chalcone derivatives isolated from *Artocarpus nobilis* by Jayasinghe et al. [66] showed good fungicidal activity against *Cladosporium cladosporioides* with the MIC values in the range of 2–15 μg/spot.



65 R^1= OH R^2= $CH_2CH{=}C(CH_3)_2$

66 R^1= OH R^2= $CH_2CH(OH)C(CH_3){=}CH_2$

67 R^1= H R^2= OCH_3

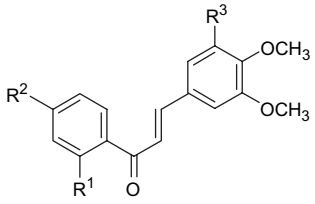
68 R^1R^2= H

Biological screening of dihydrochalcone derivatives by Okunade et al. [28] showed that **15** demonstrated relatively good activity against the two leading AIDS-related fungal pathogens *C. albicans* and *C. neoformans*, and marginal activity against the acid-fast bacterium *Mycobacteria intracellulare*. This is the first report of evaluation of this species for activity against these important AIDS-related pathogens. Additionally, 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone **16** [29] showed promising bioactivity in antimicrobial assays. It showed activity against *B. subtilis* and *T. mentagrophytes* at 60 μg/disk. Extract from the leaves of the Peruvian plant *Psidium acutangulum* [67] containing dihydrochalcone **17** revealed potent antifungal activity against *Rhizoctonia solani* and *Helminthosporium teres*, and antibacterial activity against *Xanthomonas campestris*.

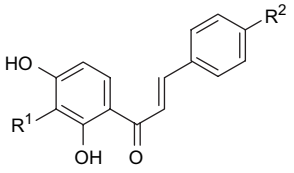
5. Antiviral properties

Antiviral properties of chalcones were discovered in studies on inhibition of plant viruses and human rhinoviruses. The variable antiviral activity of chalcones suggests that the activity of each chalcone depends on specific substitution patterns. A hydroxy and methoxy substituted chalcone derivatives were investigated by Onyilagha et al. [68,69] for activity against tomato ringspot nepovirus (ToRSV) infectivity. Hydroxylation

of ring B at 2′,3′,4′, and ring A at C-4-positions activates chalcones against ToRSV, but when C-5′ is also hydroxylated, all antiviral activity are lost. The ability of chalcones to induce resistance against ToRSV was tested in time course experiments. The activities of chalcones were apparently lost when they were applied before or after ToRSV infection. The most effective inhibitory compounds were **68** and **70**–**73**.



70 R^1R^3= H R^2= OCH_3
71 R^1R^2= OH R^3= OCH_3

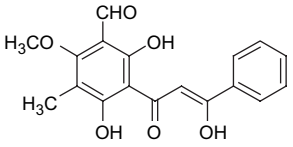


72 R^1R^2= OH
73 R^1= H R^2= OCH_3

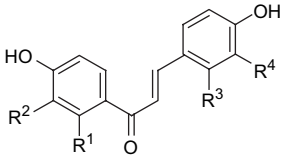
Acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), has been a life-threatening health problem since 1980, and flavonoids have been investigated for anti-HIV activity. Wang et al. [70] reported that xanthohumol **60** was a selective inhibitor of HIV-1 and may represent a novel therapeutic agent for HIV-1 infection. The target of xanthohumol on HIV-1 may lie on the steps post reverse transcription. The EC_{50}'s of **60** on inhibiting HIV-1 p24 antigen and RT production were 1.28 μg ml^{-1} and 0.50 μg ml^{-1}, respectively. Xanthohumol also inhibited HIV-1 replication in peripheral blood mononuclear cells (PBMC) infected with HIV-1_{IIIB}, the EC_{50} of **60** for inhibiting p24 antigen production was 20.74 μg ml^{-1}. However, when Buckwold et al. [71] examined the xanthohumol-enriched hop extract, no anti-HIV-1 activity was detected, but weak to moderate antiviral activity against bovine viral diarrhea virus (BVDV), herpes simplex virus type 2 (HSV-2), rhinovirus (Rhino) and HSV-1 with IC_{50} values in the low μg ml^{-1} range was observed. Xanthohumol alone also showed activity against BVDV, HSV-2, and HSV-1, as well as additionally against cytomegalovirus (CMV).

Interestingly, a recent report by Wu and colleagues [72] demonstrated that chalcone **74** from the genus *Desmos* showed potent anti-HIV activity (EC_{50} 0.022 μg ml^{-1}) with a good therapeutic index (TI) (489). A C-4 methoxy group in the chalcone skeleton may be critical for anti-HIV activity. On the other hand, Ru(II)/Ru(III) polypyridyl complexes containing 2,6-(2′-benzimidazolyl)-pyridine/chalcone as co-ligand [73] inhibited HIV replication by 50% with a concentration of $<$0.1 μg ml^{-1}.

The inhibitory activity of butein and phloretin on HIV-1 protease was evaluated by Xu et al. [74] using fluorescence and HPLC assays. The results demonstrated that butein **75** applied at a concentration of 50 μg ml^{-1} caused more than 50% inhibition of HIV-1 protease, whereas phloretin showed only 27% inhibition.

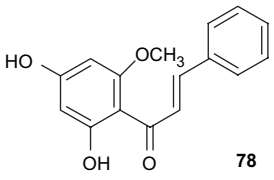


74

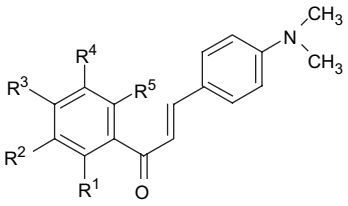


75 R^1R^4= OH R^2R^3= H
76 R^1R^2= H R^3= OCH_3 R^4= OH
77 R^1= H R^2R^4= OH R^3= OCH_3

Licochalcones A **1A** and B **76** as well as 3,3′,4,4′-tetrahydroxy-2-methoxychalcone **77** [75], suppressed the TPA-induced HIV promoter, whereas they did not cause an apparent reduction in the Luc activity in pCMVLuc transfected cells. These chalcones had a negative effect on HIV transcription, possibly because they bind to some specific protein factors. Additionally, cardamonin **78** exhibited an appreciable anti-HIV-1 PR activity (75.1% inhibition) with an IC_{50} value of 31 μg ml^{-1} [76–78].



78



79 $R^1R^4R^5$= H R^2R^3= OCH_3
80 R^1R^5= OCH_3 $R^2R^3R^4$= H
81 R^1R^4= OCH_3 $R^2R^3R^5$= H

6. Anti-inflammatory properties

The inhibition of prostaglandin E_2 (PGE_2) and nitric oxide (NO) production has been proposed as a potential therapy for different inflammatory disorders. Large amounts of NO may lead to tissue damage. In inflammatory diseases such as rheumatoid arthritis, excessive NO production by activated macrophages has been observed. Therefore, it would be interesting to develop potent and selective inhibitors of NO for potential therapeutic use.

Herencia et al. [79–82] tested a series of chalcone derivatives for possible anti-inflammatory effect. Chalcone **79** was significantly active as a scavenger of superoxide anion generated by stimulated human neutrophils or by the hypoxanthine/xanthine oxidase system (HX/XO), with IC_{50} values of 0.1 and 0.3 μM, respectively. It inhibited also the inducible NO synthase (iNOS) expression through a superoxide-dependent mechanism in stimulated mouse peritoneal macrophages and protected cells against oxidant stress. Within its anti-inflammatory features, it is noteworthy that **79** reduced leukocyte migration into the air pouch, an effect probably dependent on scavenging of superoxide as oxygen free radicals and other

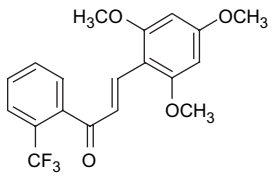
oxidants, can trigger neutrophil activation and adhesion to endothelium. Additionally, it significantly reduced tumor necrosis factor-α (TNF-α) levels, a crucial mediator of the inflammatory process, especially in chronic inflammatory conditions.

Dimethylamino-chalcones [83] were also studied in vitro for their inhibitory activity on the production of NO and PGE_2 mediators, produced by lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Chalcones **80** and **81** caused a concentration-dependent inhibition of the production of NO with IC_{50} values of 0.6 and 0.7 μM, respectively, whereas 2′,4′ dimethoxylation, trimethoxylation, the lack of methoxylation, as well as dichlorination led to less active or inactive compounds. The active compounds could display their inhibitory profile against NO and PGE_2 production by acting as inhibitors of inducible NO synthase and cyclooxygenase-2 expression. In addition, oral administration of **81** at 25 mg/kg inhibited significantly the formation of oedema in the carrageenan-induced model of inflammation in mice.

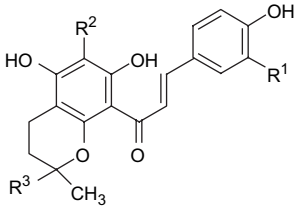
Trimethoxychalcone derivatives, with various patterns of fluorination, were evaluated by Rojas et al. [84] for their influence on nitric oxide production. 2,4,6-Trimethoxy-2′-trifluoromethylchalcone **82** inhibited the production of NO and prostaglandin E_2 in lipopolysaccharaide-stimulated RAW 264.7 macrophage cells. The inhibition (76.3% inhibition at the concentration of 10 μM) was dose-dependent without any evidence of a cytotoxic effect. It can be suggested that NO reduction was a consequence of inhibition of the expression of iNOS, whereas PGE_2 reduction was not due to a direct inhibitory action on cyclooxygenase-2 activity or expression. In vivo inhibitory effects on nitrite and prostaglandin E_2 levels were also observed for **82**. This compound exhibited an inhibitory behaviour similar to its in vitro results on nitrite and PGE_2 accumulation. In the 24-h zymosan-stimulated mouse air pouch, **82** reduced nitrite and prostaglandin E_2 levels as well as in the rat adjuvant arthritis.

Some chalcones with prenyl or geranyl groups have been identified in hops and beers. These compounds have the same backbone structure but differ in the prenyl side chain [85]. They were shown to significantly inhibit NO production without showing cytotoxicity at concentrations lower than 10 μM (cell viability > 95%). Their inhibitory activity was almost the same (IC values were in the range from 5.6 to 9.4 μM), which indicated that the prenyl chain may not be necessary for the NO production inhibitory activities. On the other hand, the compound lacking the double bond between the α- and β-positions, exhibited a much weaker inhibitory activity than other chalcones, suggesting that the double bond is important for the inhibitory activity of chalcones.

The novel chalcone derivatives isolated from the fruits of *Mallotus philippinensis* called mallotophilippens C **83**, D **84** and E **85** [86] or from hops (*H. lupulus*) called xanthohumol **60**, xanthohumol B **86**, D **87** and dihydroxanthohumol **88** [87] inhibited the production of NO induced by LPS and INF-γ in murine macrophage-like cell line, RAW 264.7. Furthermore, mallotophilippens inhibited inducible NO synthase (iNOS), cyclooxygenase 2 (COX-2), interleukin 6 (IL-6) and IL-1β mRNA gene expression. Daikonya and co-workers hypothesized that the main inhibitory mechanism of these compounds may be the inactivation of the nuclear factor κB (NF-κB) [86].



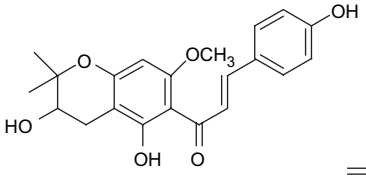
82



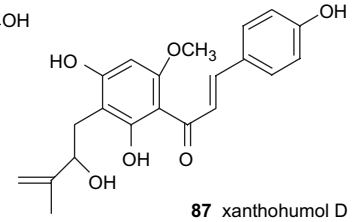
83 R^1= H R^2= $CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$ R^3= CH_3

84 R^1= OH R^2= $CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$ R^3= CH_3

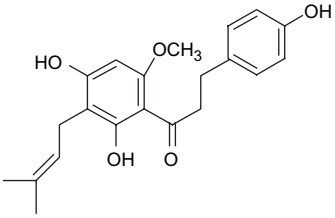
85 R^1= OH R^2= $CH_2CH{=}C(CH_3)_2$ R^3= $CH_2CH_2CH{=}C(CH_3)_2$



86 xanthohumol B



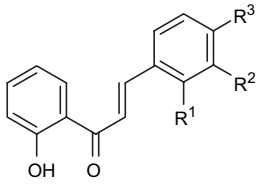
87 xanthohumol D



88 dihydroxanthohumol

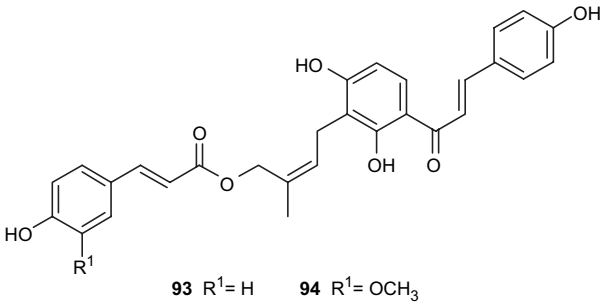
2′-Hydroxychalcone **89** [88] can be used to control cell trafficking by blocking the expression of cell adhesion molecules. It blocked the adhesion of peripheral neutrophils to endothelial cells. It was equally effective in inhibiting either TNF-α- or LPS-induced expression of leukocyte adhesion molecules.

Compound **89** inhibited TNF-α-induced NF-κB levels with the use of gel retardation assays and Western blot analyses. Because NF-κB is essential for the induced expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, these results confirm that 2′-hydroxychalcone inhibits the NF-κB dependent transcription of cell adhesion molecule genes.



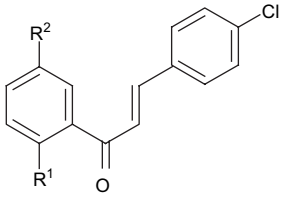
89 $R^1R^2R^3$= H
90 R^1R^2= H R^3= OH
91 R^1= H R^2R^3= Cl
92 R^1= OH R^2R^3= H

The interaction between ICAM-1, and leukocyte function associated antigen-1 (LFA-1) is involved in a number of inflammatory diseases, such as allergic asthma, arthritis, nephritis and pneumonia. Tanaka et al. [93] showed that isoliquiritigenin **8** and butein **75** as well as other 2′ or 4′ hydroxy substituted 4-hydroxychalcones significantly downregulated the cell surface expression of either ICAM-1 or VCAM-1, or of both, suggesting that these compounds may be effective in inhibiting allergic inflammation or rejective reactions by transplants.



93 R^1= H **94** R^1= OCH_3

Almost all of hydroxychalcones and alkoxychalcones tested **90–98** [89–92] exhibited potent inhibitory effects on the release of β-glucuronidase and lysozyme from rat neutrophils stimulated with formyl-Met-Leu-Phe/cytochalasin B (fMLP/CB). Of the hydroxychalcones, compound **92** was the most potent inhibitor of the release of β-glucuronidase ($IC_{50} = 1.6$ μM) and lysozyme ($IC_{50} = 1.4$ μM) from rat neutrophils stimulated with fMLP/CB. Almost all 2′,5′-dialkoxychalcones exhibited potent inhibitory effects on NO formation from murine microglial cell lines N9 stimulated with LPS. Compound **96** showed the greatest effect ($IC_{50} = 0.7$ μM). The inhibitory effects of dialkoxychalcones, **96–98** on inflammation are probably not due to the inhibition of mast cells and neutrophil degranulation, but are mediated through the suppression of NO formation from N9 cells. Chalcone **98** [92] caused strong and dose-dependent inhibitory effects on the release of β-glucuronidase and histamine from rat peritoneal mast cell stimulated with compound **48/80** (10 μg ml^{-1}). The hydroxylation, methoxylation and *O*-methylation of 4-hydroxychalcone at C-2′, or C-3′, C-3 and C-4, respectively, enhanced the inhibitory effects on mast cells caused by compound **48/80**. The inhibitory effects of the compounds tested on inflammation are not due to the release of steroid hormones from adrenal gland, but are partly mediated through the suppression of chemical mediators released from mast cells and neutrophils.



95 R^1R^2= OC_3H_7 **97** R^1R^2= OC_2H_5
96 R^1R^2= OC_4H_9 **98** R^1R^2= OH

7. Conclusions

Chalcone is a unique template that is associated with several biological activities. The radical quenching properties of the phenolic groups present in many chalcones have raised interest in using the compounds or chalcone rich plant extracts as drugs or food preservatives. The anti-infective and anti-inflammatory activities of a variety of chalcones have been presented in this review article. The literature is analysed to provide a meaningful overview of the structural requirements for activity, wherever possible.

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