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Modifications of the α,β-Double Bond in Chalcones only Marginally Affect the Antiprotozoal Activities

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Abstract—Methods for selective alkylation of chalcones in the α- or β-position and for selective reduction of the α,β-double bond have been developed. The antiparasitic potencies of the α,β-double bond modified chalcones only differ marginally from the potencies of the parent chalcones indicating that the propenone residue only functions as a spacer between the two benzene rings, which are the true pharmacophore.

Introduction

Chalcones are a group of natural products characterised by the presence of a 1,3-diphenylprop-2-en-1-one skeleton.[1] The radical quenching properties of the phenolic groups present in many of the naturally occurring chalcones have raised interest for using the compounds or chalcone rich plant extracts as drugs or food preservatives.[2,3] In addition chalcones possess a broad spectrum of biological activities including antibacterial,[4–6] anthelmintic,[7] amoebicidal,[8] antiulcer,[9–11] antimitotic effects,[12] and immunosuppressive activity,[13,14] including inhibition of lipoxygenase,[15–17] cyclooxygenase,[15] and interleukin biosynthesis.[16] The 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 thiolo group in an essential protein.[5] We have shown that a number of naturally occurring licorice chalcones in vitro inhibits the growth of parasites belonging the species *Leishmania*[18–20] and *Plasmodium*,[20,21] causing the diseases leishmaniasis and malaria, respectively. In vivo licochalcone A (**1**, Scheme 1) efficiently clears leishmania infections in mice and hamsters[22] and plasmodium infections in mice[21] without showing any toxic side effects, indicating that the broad spectrum of activities of the chalcones is not a general cytotoxic effect. Additional in vitro studies have revealed that structural modifications of chalcones afford compounds efficiently inhibiting the growth of parasites in concentrations that has little effect on the proliferation of phytohaemagglutinin A stimulated lymphocytes.[23] A later medicinal chemical study based on the hypothesis that chalcones are plasmodium protease inhibitors revealed that appropriate substitution of the chalcone skeleton results in derivatives with nanomolar IC_{50} values.[24] This study, however, did not encompass the selectivity of the prepared chalcones. Together the performed studies have created a major interest for chalcones as potential drugs against the diseases malaria and leishmaniasis. A spreading resistance against the drugs of first choice have created an alarming need for new drugs against both of these diseases.[25]

In spite of the increased interest for chalcones only very few studies have been performed on the importance of the double bond. Some dihydrochalcone glycosides have been found to possess antimalarial activity, most likely by blocking pores induced by the parasite in the host cell membrane.[26] Substitution on the α- or β-carbon also affords less interesting compound.[12,24] In contrast, conversion of the double bond into a triple bond has given interesting structures,[1,27] some of which can be used for inhibition of calcium uptake into lymphocytes[27] or as antimitotic agents.[12]

A limiting factor for studying the consequences of alkylating chalcones in the α- or β-position is that only few methods for their preparation are known and all of

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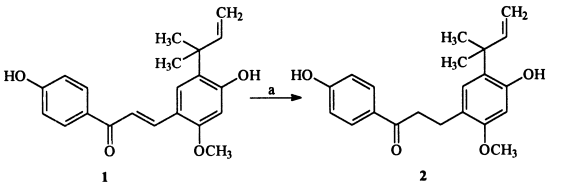


Scheme 1. (a) CF_3COOH, $(CH_3CH_2)_3SiH$, CH_2Cl_2, rt.

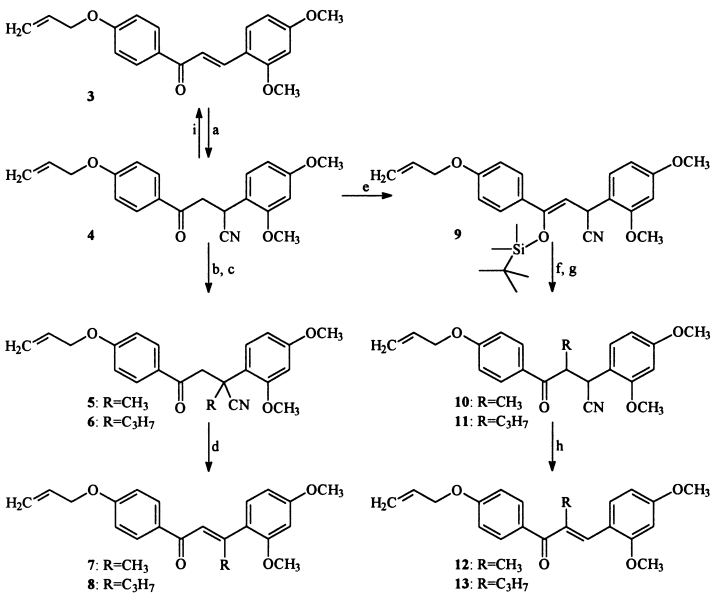
these have a limited scope. Published methods for preparing β-methylchalcones include cycloaddition of diazomethane to chalcones followed by pyrolysis,[28] self condensation of acetophenones catalysed by various catalyst to give β-methylchalcones with identical substitution pattern at the two aromatic rings,[29–32] palladium assisted condensation of substituted α-1,2-propadienylbenzenemethanols to halogenated benzenes,[33] and conjugate addition of lithium dimethylcuprate to give a dihydrochalcone, which is selenylated with phenylselenyl bromide followed by oxidation and elimination of phenylselenoxide.[34] In principle the last mentioned method can be used for introduction of alkyl groups larger than methyl. In our hands, however, this method failed because of limited solubility of the starting material. α-Alkylated chalcones are generally obtained by condensation of an arylketones with a benzaldehyde. In general, however, the yields are poor especially when larger arylketones are used as starting material. A number of catalysts including alkali hydroxides,[35] piperidinium acetate[36] and hydrochloric acid,[37] have not increased the yields. A further drawback is the difficult availability of the starting arylketones.

The present paper describes an efficient method for introducing alkyl groups in the α- and β-positions of chalcones. The activities of double bond modified chalcones, including the dihydrochalcones and the acetylenic analogues, are compared with the activities of the parent compounds.

Chemistry

Reduction of the chalcone double bond in **1** was performed by ionic hydrogenation using trifluoroacetic acid as a proton donor and triethylsilane as a hydride donor (Scheme 1).[38] The poor polarisation of the side chain double bond makes it inert towards this reagent. The use of equimolar amounts of silane and chalcone prevents reduction of the carbonyl group. The dihydrochalcone **2** is obtained in high yield and is easily isolated from the reaction mixture.

The previous described methods for α- or β-substitution of chalcones were found to be of limited value for modifying **1** and **3**. Instead **4**, which is obtained in high yield by conjugated addition of hydrogen cyanide to **3**, is found to be an excellent precursor for the α- as well as β-chalcone anion (Scheme 2). Compound **4** has two sets of activated protons, the protons α to the carbonyl group and the protons α to the cyano group. Since the α-protons of β-cyanoketones in general are more activated than the β-protons addition of two equivalents of LDA followed by an alkyl halide affords β-alkylation.[39] In the case of **4**, however, addition of one equivalent of



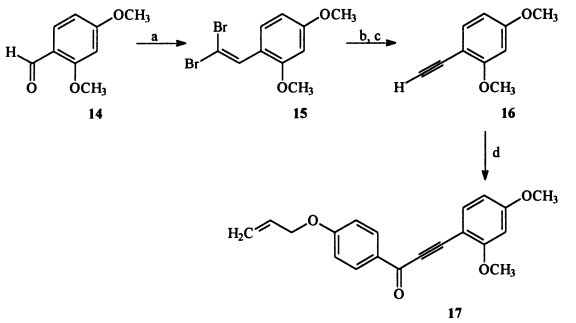
Scheme 2. (a) NaCN, NH_4Cl, DMF, 100 °C; (b) LDA, THF, −78 °C; (c) R-I; (d) NaH, toluene, reflux; (e) TBDMS-Cl, NaH, THF, rt; (f) R-I, DMF, rt; (g) CsF, rt; (h) NaH, toluene, reflux; (i) NaH, toluene, reflux.

LDA followed by addition of alkyl halide selectively afforded β-alkylation, indicating that the β-proton is more activated. Attempts to α-alkylate the diainion of **4** by adding surplus of LDA followed by an alkyl halide failed. Sodium hydride in boiling toluene cleanly eliminated hydrogen cyanide from **5** or **6** to give the β-alkylated chalcone **7** or **8**.

Attempts to synthesise α-alkylated chalcones from **4** via the dianion obtained by treatment with excess of sodium hydride failed since elimination of hydrogen cyanide affording the starting chalcone **3** was the dominating reaction. This elimination, however, proved that the α-protons were affected by sodium hydride. The α-alkylated chalcones were prepared in excellent yield *via* the silylenol ether **9** formed by treatment of the mono anion of β-cyanoketone **4** with *t*-butyldimethylsilyl chloride.[40] In contrast to the trimethylsilylenol ether the *t*-butyldimethylsilylenol ether can be isolated by column chromatography. Under absolutely anhydrous conditions the silylenol ether reacted with fluoride ions to create a partial negative charge on the α-carbon which was alkylated with iodomethane or iodopropane to give the α-alkylated-β-cyano ketone **10** and **11**, respectively. Even though poor solubility of cesium fluoride in the reaction medium only afforded a low fluoride concentration and consequently a poor reaction rate, this reagent was preferred for tetrabutylammonium fluoride, since the latter reagent could not be obtained in a sufficient dry state.[41,42] The α-alkylated chalcones **12** and **13** are formed by sodium hydride provoked elimination of hydrogen cyanide from **10** and **11**, respectively.

As evidenced by NOESY experiments the α-alkylchalcones **12** and **13** and the β-methylchalcone **7** was only obtained as the *E*-isomers, whereas the β-propylchalcone **8** was obtained as the *E*- as well as the *Z*-isomer. α,β-Unsubstituted chalcones are crystallising as the *E*-isomers but under the influence of daylight a solution of the *E*-isomer is converted into a mixture of the two geometric isomers, except when a hydroxy group is present in the 2- or 4-position.[43] The ease of light induced isomerization of chalcones is of importance, because it makes it difficult to distinguish between the biological activities of the two geometric isomers. The stabilities of the two β-propylchalcones, however, gives a possibility for answering the question in this case.

Whereas it was possible to follow the concept that the chalcone modified at the double bond should be prepared from the parent chalcone when the α- or β-alkylated chalcones and the dihydrochalcones were synthesised we had to abandon from this principle when preparing the acetylenic chalcone analogue **17** (Scheme 3). This compound was prepared by acylating the appropriate alkynylbenzene with an appropriate



Scheme 3. (a) PPh_3, CBr_4, CH_2Cl_2, 5 °C; (b) 2 equiv. *n*-BuLi, THF, −78 °C; (c) H_2O; (d) 4-allyloxybenzoylchloride, $(PPh_3)_2PdCl_2$-CuI, Et_3N, toluene, rt.

benzoyl chloride[44] in the presence of a bistriphenylphosphinpalladium(II)chloride copper(I) iodide complex according to a previously described procedure for similar compounds.[45] The alkynylbenzene **16** is obtained by elimination of hydrogen bromide from the dibromoethenylbenzene **15**, followed by halogen-metal exchange with *n*-butyllithium. Compound **15** is obtained by reacting the ylide formed from tetrabromomethane and triphenylphosphine with **14**, as has been described for analogous compounds.[46]

Biological Results

The antiparasitic activities were determined in vitro by incubating *Leishmania major* promastigotes or erythrocytes infected with *Plasmodium falciparum* schizonts with increasing concentrations of the double bond modified chalcones. The IC_{50} values were estimated by fitting the data to eq (1):

$$\mathrm{Inh} = \mathrm{Max}/(1 + (\mathrm{IC}_{50})^{n}/\mathrm{Concn}^{n}) \quad (1)$$

in which Concn is the concentration of the analogues, Inh is the percent inhibition compared to control (Max) and *n* is the Hill slope.[47] The estimated IC_{50} values are given in Table 1.

Discussion

Comparison of the IC_{50} values for the double bond modified chalcones with the values of the parent chalcones reveals that modifications of the double bond do not significantly affect the antiparasitic activities. The only two compounds that show activities slightly different from those of the unmodified chalcone are the (*Z*)-**8** and the acetylenic analogue **17**. Comparison of the IC_{50} values of licA (**1**) with the dihydro derivative **2** reveals that saturation of the double bond causes some but no

Table 1. IC_{50} values against *Leishmania major* and *Plasmodium falciparum* for double bond modified and parent chalcones

Compd	$IC_{50} \pm SD$ (μM)	
	Leishmania	*Plasmodium*
1	13 ± 0	5.6 ± 0.6
2	23 ± 2	7.3 ± 0.2
3	56 ± 3	24 ± 2
7	72 ± 4	36 ± 4
(*E*)-**8**	84 ± 8	39 ± 4
(*Z*)-**8**	35 ± 3	10 ± 1
12	54 ± 5	26 ± 1
13	86 ± 9	37 ± 1
17	13 ± 1	12 ± 1

drastic reduction of activity. Especially the remaining activity of **2** and the high activity of **17** but also the activities of the α- and β-alkylated analogues of **3** justify the conclusion that the alkylating properties of the α,β-unsaturated ketone is of minor importance for the antiparasitic activities of the chalcones. This conclusion concerning antiparasitic effects is different from previous suggestions, that the antibacterial effect is caused by an inactivation of essential enzymes by a conjugated addition of the chalcones to thiolo groups or other nucleophilic centres.[5]

Based on modelling studies of the active site of *Plasmodium* proteases a number of inhibitors, including chalcones, for these enzymes have been designed.[24,48] According to this model, the pharmacophore of chalcones is the two aromatic rings and the α,β-unsaturated ketone only functions as a spacer. The optimum length of the spacer is calculated to be approximately the length of four connected atoms. *Leishmania* mutants lacking cysteine proteases were as sensitive to some of the present chalcones as wild type parasites indicating that cysteine proteases are not the target for these chalcones (data not shown). Even though, the data given in Table 1 do confirm that the pharmacophore is the two aromatic rings and that the propanone chain just functions as a spacer. Substituents on the spacer only have a minor influence of the antiparasitic activity. The described antiparasitic activities of neolignans[49] can also be explained by this model, if it is assumed that the lignans have the same target molecule as the chalcones.

The limited flexibility of the spacer in the *E*- and *Z*-chalcones and in the acetylene analogue **17** causes a different spacial orientation of the two benzene rings, whereas the flexibility of the propanone-spacer in **2** allows the two benzene rings to be located as in the *E*- as well as in the *Z*-isomer. Apparently these conformational differences only affect the biological activity to a minor extent, indicating that the major part of the differences in biological activity observed in series of chalcones is caused by the different substitution patterns at the two benzene rings. At the present we are performing a study on the biological activity of a series of chalcones in order to develop 2D- and 3D-QSAR model, which will reveal the substitution patterns affording increased antiparasitic activities.

Experimental

The NMR spectra were recorded on a Bruker AC-200F spectrometer. Splitting pattern are described as singlet (s), doublet (d), triplet (t), quartet (q) and broad (b). An asterisk indicates that signals with similar shifts might be interchanged. Mass spectra were recorded on a JEOL AX505W mass spectrometer. Melting points were determined on a Electrothermal melting point apparatus, and were not corrected. Elemental analyses are within 0.4% of the calculated values, unless otherwise stated. All moisture sensitive reactions were performed under nitrogen using oven dried glassware. Solvents were dried before use: tetrahydrofurane (THF) was freshly distilled from sodium/benzophenone, toluene was distilled and stored over sodium. HPLC grade dimethylformamide (DMF) was dried and stored over 4 A molecular sieves. 1-Iodopropane was distilled from CaH_2 before use. Cesium flouride was dried at 150 °C under oil pump vacuum for 2 h and stored in a desiccator. Samples of *E*-**8**, *Z*-**8** and **13** for biological testing were purified by HPLC using a Waters 6000 A pump, a prepacked Knauer column (16×250 mm, LiChrosorp RP18, eluent: acetonitrile–water) and a Shimadzu SPD 6A UV detector at 254 mn. Column chromatography was performed on silica gel (Merck, 0.040–0.063 mm) using mixtures of toluene and ethyl acetate as eluents.

1-(4-Hydroxyphenyl)-3-(5-(1,1-dimethylpropenyl)-4-hydroxy-2-methoxyphenyl)propanone (2). Trifluoroacetic acid (770 μL, 10.0 mmol) was slowly added to a stirred suspension of licochalcone A[50] (0.507 g, 1.5 mmol) in dichloromethane (5 mL) and triethylsilane (240 μL, 1.5 mmol). Water was added after stirring for 2 h. The aqueous phase was extracted with dichloromethane (2×10 mL) and the combined organic phases were concentrated in vacuo and the residue purified by column chromatography to give **2** (0.359 g, 70.4%) as colourless crystals, mp 153.5–154.4 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.91 (AA′ part of an AA′MM′ system, H2′, H6′), 7.00 (s, H6), 6.90 (MM′ part of an AA′MM′ system, H3′, H5′), 6.40 (s, H3), 6.24 (dd J = 10.5, 17.7 Hz, H2″), 5.33 (dd, J = 17.7, 1.0 Hz, H3″*trans*), 5.28 (dd, J = 10.5, 1.0 Hz, H3″*cis*), 3.76 (s, OCH_3), 3.18 (bt, J = 7.0 Hz, Hα), 2.96 (bt, J = 7.0 Hz, Hβ), 1.38 (s, CH_3). ^{13}C NMR ($CDCl_3$) δ 200.6 (C=O), 160.8* (C4′), 157.2*

(C2), 154.0* (C4), 148.3 (C2″), 130.9 (C2′, C6′), 129.8 (C1′), 127.7 (C6), 123.4 (C5), 121.0 (C1), 115.4 (C3′, C5′), 113.3 (C3″), 100.7 (C3), 55.3 (OCH_3), 39.7 (C1″), 39.2 (Cα), 27.0 (CH_3), 26.1 (Cβ). Anal. ($C_{21}H_{24}O_4$) C, H.

2-(2,4-Dimethoxyphenyl)-4-(4-(2-propenyloxy)phenyl)-4-oxobutanenitrile (4). A stirred solution of 2,4-dimethoxy-4′-allyloxychalcon, **3** (19.44 g, 60.0 mmol), sodium cyanide (29.4 g, 600 mmol) and ammonium chloride (3.24 g, 60 mmol) in DMF (240 mL) was heated to 100 °C for 20 min. The reaction was allowed to cool to rt, filtered, slowly added 4 M HCl (240 mL) and extracted with ethyl acetate (3×200 mL). The combined organic phase were concentrated in vacuo and the residue freeze-dried to give **4** (17.8 g, 84.4%) as colourless crystals, mp 112.2–112.7 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.90 (AA′ part of an AA′MM′ system, H2′, H6′), 7.37 (d, J=8.4 Hz, H6), 6.91 (MM′ part of an AA′MM′ system, H3′, H5′), 6.50 (dd, J=8.4, 2.0 Hz, H5), 6.47 (bs, H3), 6.03 (ddq, J=17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J=17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J=10.5, 1.3 Hz, H3″*cis*), 4.67 (dd, J=8.4, 5.0 Hz, Hβ), 4.58 (bd, J=5.2 Hz, H1″), 3.82 (s, OCH_3), 3.79 (s, OCH_3), 3.58 (dd, J=17.4, 8.4 Hz, Hα), 3.40 (dd, J=17.4, 5.0 Hz, Hα′); ^{13}C NMR ($CDCl_3$) δ 193.6 (C=O), 162.4* (C4′), 160.6* (C2), 156.8* (C4), 132.0 (C2″), 129.9 (C2′, C6′), 129.0 (C6), 128.6 (C1′), 120.6 (C≡N), 117.7 (C3″), 115.0 (C1), 114.1 (C3′, C5′), 104.2 (C5), 98.5 (C3), 68.4 (C1″), 55.2 (OCH_3), 55.0 (OCH_3), 41.4 (Cα), 26.5 (Cβ). Anal. ($C_{21}H_{21}NO_4$) C, H, N.

2-(2,4-Dimethoxyphenyl)-2-methyl-4-(4-(2-propenyloxy)phenyl)-4-oxobutanenitrile (5). To a cold (−78 °C) solution of **4** (3.52 g, 10 mmol) in anhydrous THF (40 mL) was slowly added LDA (11 mL of a 2 M soln in diethyl ether, 22 mmol). After 5 min, iodomethane (4.0 mL, 50 mmol) was added and the reaction was stirred for additional 10 min. The reaction was heated to rt (30 min) and water (50 mL) was added. The mixture was extracted with ethyl acetate (3×50 mL) and the combined organic phases were concentrated in vacuo and the residue purified by column chromatography, to give **5** (2.85 g, 78.0%) as yellow crystals, mp 77.8–78.5 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.88 (AA′ part of an AA′MM′ system, H2′, H6′), 7.47 (d, J=8.5 Hz, H6), 6.91 (MM′ part of an AA′MM′ system, H3′, H5′), 6.50 (dd, J=8.5, 2.3 Hz, H5), 6.46 (d, J=2.3 Hz, H3), 6.04 (ddq, J=17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J=17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J=10.5, 1.3 Hz, H3″*cis*), 4.60 (bd, J=5.2 Hz, H1″), 3.96 (d, J=17.1 Hz, Hα), 3.84 (s, OCH_3), 3.80 (s, OCH_3), 3.56 (d, J=17.1 Hz, H-α′), 1.95 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 194.2 (C=O), 162.6* (C4′), 160.6* (C4), 157.6* (C2), 132.4 (C2″), 130.3 (C2′, C6′), 129.8 (C1′), 128.6 (C6), 120.4 (C≡N), 119.3 (C1), 118.2 (C3″), 114.4 (C3′, C5′), 104.3 (C5), 99.8 (C3), 68.8 (C1″), 55.5 (OCH_3), 55.3 (OCH_3), 45.2 (Cα), 37.1 (Cβ), 25.2 (β-CH_3). Anal. ($C_{22}H_{23}NO_4$) C, H, N.

2-(2,4-Dimethoxyphenyl)-2-propyl-4-(4-(2-propenyloxy)phenyl)-4-oxobutanenitrile (6). To a cold (−78 °C) solution of **4** (3.52 g, 10 mmol) in anhydrous THF (40 mL) was slowly added LDA (11 mL of a 2 M soln in diethyl ether, 22 mmol). After 5 min 1-iodopropane (4.8 mL, 50 mmol) was added and the reaction was stirred for additional 10 min. The reaction was heated to rt (30 min) and water (50 mL) was added. The mixture was extracted with ethyl acetate (3×50 mL) and the combined organic phases were concentrated in vacuo and the residue purified by column chromatography, to give **6** (3.21 g, 81.6%) as colourless crystals, mp 86.1–87.1 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.86 (AA′ part of an AA′MM′ system, H2′, H6′), 7.61 (d, J=8.6 Hz, H6), 6.90 (MM′ part of an AA′MM′ system, H3′, H5′), 6.50 (dd, J=8.6, 1.5 Hz, H5), 6.40 (d, J=1.5 Hz, H3), 6.03 (ddq, J=17.3, 10.5, 5.2 Hz, H2″), 5.41 (dd, J=17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J=10.5, 1.3 Hz, H3″*cis*), 4.59 (bd, J=5.2 Hz, H1″), 4.10 (d, J=17.1 Hz, Hα), 3.78 (s, OCH_3), 3.76 (s, OCH_3), 3.56 (d, J=17.1 Hz, Hα′), 2.32 (td, J=12.6, 4.5 Hz, Hβ1), 2.09 (td, J=12.6, 4.5 Hz, Hβ1′), 1.53 (m, Hβ2), 1.21 (m, Hβ2′), 0.91 (t, J=7.3 Hz, Hβ3); ^{13}C NMR ($CDCl_3$) δ 194.4 (C=O), 162.4* (C4′), 160.1* (C4), 157.1* (C2), 132.3 (C2″), 130.6 (C6), 130.1 (C2′, C6′), 129.8 (C1′), 122.7 (C1), 118.1 (C3″), 117.1 (C≡N), 114.2 (C3′, C5′), 104.0 (C5), 99.6 (C3), 68.7 (C1″), 55.2 (OCH_3), 44.5 (Cα), 43.8 (Cβ), 39.4 (Cβ1), 18.7 (Cβ2),13.9 (Cβ3). Anal. ($C_{24}H_{27}NO_4$) C, H, N.

1-(4-(2-Propenyloxy)phenyl)-3-(2,4-dimethoxyphenyl)-but-2-enone-1 (7). A solution of **5** (1.38 g, 3.8 mmol) in anhydrous toluene (60 mL) was added sodium hydride (1.53 g 60% dispersion, 38.0 mmol) and refluxed for 20 min. Water was added and the mixture was extracted with ethyl acetate (2×90 mL). The combined organic phases were concentrated in vacuo and the residue purified by column chromatography, to give **7** (1.18 g, 91.4%) as yellow crystalls, mp 79.7–80.1 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.97 (AA′ part of an AA′MM′ system, H2′, H6′), 7.20 (d, J=9.0 Hz, H6), 6.96 (s, Hα), 6.91 (MM′ part of an AA′MM′ system, H3′, H5′), 6.50 (m, H3, H15), 6.06 (ddq, J=17.3, 10.5, 5.2 Hz, H2″), 5.41 (dd, J=17.3, 1.3 Hz, H3″*trans*), 5.31 (dd, J=10.5, 1.3 Hz, H3″*cis*), 4.59 (bd, J=5.2 Hz, H1″), 3.83 (s, OCH_3), 2.50 (s, β-CH_3). In a NOESY spectrum correlations between Hα and H2′, H6′ and H6 were found. ^{13}C NMR ($CDCl_3$) δ 190.4 (C=O), 161.8* (C4′), 160.9* (C4), 157.7* (C2), 154.3 (Cβ), 132.4 (C2″), 130.4 (C2′, C6′), 129.6 (C6), 126.1 (C1′), 123.3 (Cα), 118.6 (C1), 118.0 (C3″), 114.1 (C3′, C5′), 104.1 (C5), 98.8 (C3), 68.7 (C1″), 55.4 (OCH_3), 55.3 (OCH_3), 20.5 (β-CH_3). Anal. ($C_{21}H_{22}O_4$) C, H.

1-(4-(2-Propenyloxy)phenyl)-3-(2,4-dimethoxyphenyl)-hex-2-enone-1 (8). A solution of **6** (0.79 g, 2.0 mmol) in anhydrous toluene (40 mL) was added sodium hydride (0.15 g of 60% NaH in oil, 3.8 mmol) and refluxed for 90 min. Water was added and the mixture was extracted with ethyl acetate (2×50 mL). The combined organic phases were concentrated in vacuo and the residue purified by column chromatography, to give *E*-**8** (0.13 g, 18.1%) and *Z*-**8** (0.37 g, 51.0%) as yellow oils. *E*-**8**: ^{1}H NMR ($CDCl_3$) δ 7.97 (AA′ part of an AA′MM′ system, H2′, H6′), 7.14 (d, J = 9.0 Hz, H6), 6.93 (MM′ part of an AA′MM′ system, H3′, H5′), 6.83 (s, Hα), 6.50 (d, J = 2.2 Hz, H3), 6.47 (dd, J = 9.0, 2.2 Hz, H5), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.41 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.59 (bd, J = 5.2 Hz, H1″), 3.81 (s, OCH_3), 2.97 (t, J = 7.5 Hz, Hβ1), 1.40 (6.tet, J = 7.5 Hz, Hβ2), 0.88 (t, J = 7.5 Hz, Hβ3). In a NOESY spectrum correlations between Hα and H2′, H6′ and H6 were found. ^{13}C NMR ($CDCl_3$) δ 190.2 (C=O), 161.8* (C4′), 160.7* (C4), 158.3* (C2), 157.6 (Cβ), 132.4 (C2″), 132.3 (C1′), 130.4 (C2′, C6′), 130.0 (C6), 124.7 (C1), 124.0 (Cα), 117.8 (C3″), 114.1 (C3′, C5′), 104.0 (C5), 98.6 (C3), 68.6 (C1″), 55.3 (OCH_3), 55.1 (OCH_3), 34.4 (Cβ1), 21.8 (Cβ2), 14.0 (Cβ3). HRMS (FAB+): 367.1937 (calcd $C_{23}H_{27}O_4$: 367.1909).

Z-**8**: ^{1}H NMR ($CDCl_3$) δ 7.82 (AA′ part of an AA′MM′ system, H2′, H6′), 6.90 (d, J = 7.9 Hz, H6), 6.81 (MM′ part of an AA′MM′ system, H3′, H5′), 6.63 (s, Hα), 6.35 (dd, J = 9.0, 2.3 Hz, H5), 6.31 (d, J = 2.3 Hz, H3), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.41 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.59 (bd, J = 5.2 Hz, H1″), 3.70 (s, OCH_3), 3.61 (s, OCH_3), 2.52 (t, J = 7.1 Hz, Hβ1), 1.45 (6.tet, J = 7.1 Hz, Hβ2), 0.94 (t, J = 7.1 Hz, Hβ3). In a NOESY spectrum correlations between Hα and H2′, H6′ and the allylic protons in the propyl group were found. ^{13}C NMR ($CDCl_3$) δ 191.8 (C=O), 161.7* (C4′), 160.3* (C4), 156.9* (C2), 152.7 (Cβ), 132.6 (C2″), 131.4 (C1′), 130.7 (C2′, C6′), 129.7 (C6),124.5 (Cα), 121.8 (C1), 117.9 (C3″), 113.8 (C3′, C5′), 103.9 (C5), 98.5 (C3), 68.6 (C1″), 55.1 (OCH_3), 55.0 (OCH_3), 41.0 (Cβ1), 21.0 (Cβ2), 13.7 (Cβ3). HRMS (FAB+): 367.1938 (calcd $C_{23}H_{27}O_4$: 367.1909).

1-(2,4-Dimethoxyphenyl)-3-(4-(2-propenyloxy)phenyl)-3-*t*-butyldimethylsilyloxy-prop-2-enenitrile (9). A solution of **4** (3.5 g, 10 mmol) and *t*-butyldimethylsilyl chloride (2.0 g, 13 mmol) in anhydrous THF (50 mL) was added sodium hydride (1.6 g 60% dispersion, 40 mmol). After violent stirring for 60 min, water (30 mL) was slowly added. The mixture was extracted with ethyl acetate (2×50 mL) and the combined organic phases were concentrated in vacuo and the residue purified by column chromatography to give **9** (3.4 g, 74.0%) as a yellow oil. ^{1}H NMR (CD_3CN) δ 7.41 (AA′ part of an AA′MM′ system, H2′, H6′), 7.31 (d, J = 8.3 Hz, H6), 6.90 (MM′ part of an AA′MM′ system, H3′, H5′), 6.58 (d, J = 2.3 Hz, H3), 6.53 (dd, J = 2.3, 8.3 Hz, H5), 5.99 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.40 (dd, J = 17.3, 1.5 Hz, H3″*trans*), 5.36* (d, J = 9.7 Hz, Hα), 5.27 (dd, J = 10.5, 1.5 Hz, H3″*cis*), 5.20* (d, J = 9.7 Hz, Hβ), 4.54 (bd, J = 5.2 Hz, H1″), 3.87 (s, OCH_3), 3.79 (s, OCH_3), 1.00 (s, $C(CH_3)_3$), 0.04 (s, Si-CH_3), −0.13 (s, Si-CH_3); ^{13}C NMR (CD_3CN) δ 160.4* (C2), 158.5* (C4), 156.9* (C4′), 151.9 (C-OSi), 132.9 (C2″), 129.8 (C1′), 128.2 (C6), 127.3 (C2′, C6′), 120.1 (C1), 116.4 (C3″), 115.8 (C≡N), 113.6 (C3′, C5′), 104.4 (C5), 102.5 (Cα), 98.2 (C3), 67.9 (C1″), 54.9 (OCH_3), 54.5 (OCH_3), 26.1 (Cβ), 24.5 (($\mathbf{C}H_3)_3$-C-Si), 17.3 (**C**-Si), −5.3 (**C**H_3-Si), −5.4 (**C**H_3-C-Si).

2-(2,4-Dimethoxyphenyl)-3-methyl-4-oxo-4-(4-(propenyloxy)phenyl)-butanenitrile (10). To a solution of **9** (1.16 g, 2.5 mmol) and iodomethane (313 μL, 5.0 mmol) in anhydrous DMF (12.5 mL) was added cesium flouride (0.76 g, 5.0 mmol). The mixture was stirred for 6 h, water (10 mL) was added and the mixture was extracted with ethyl acetate (4×20 mL). The combined organic phases were concentrated in vacuo to give a mixture of the two epimeric racem pairs of **10** (2.2 mmol, 89.0%) as colourless crystals, mp 123.6–124.7 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.95/7.86 (AA′ part of an AA′MM′ system, H2′, H6′), 7.29/7.23 (d, J = 9.1/9.1 Hz, H6), 6.94/6.90 (MM′ part of an AA′MM′ system, H3′, H5′), 6.42 (m, H5, H3), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.58 (bd, J = 5.2 Hz, H1″), 4.44/4.50 (d, J = 8.9/7.1 Hz, Hβ), 4.03 (bp, $J \approx 8$ Hz, Hα), 3.82/3.85 (s, OCH_3), 3.80/3.76 (s, OCH_3), 1.10/1.39 (d, J = 7.1/7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 198.8/198.6 (C=O), 162.6* (C4′), 160.8* (C2), 157.6/157.2* (C4), 132.2 (C2″), 130.4 (C2′, C6′), 130.3 (C6), 128.4 (C1′) 119.6 (C≡N), 118.1 (C3″), 114.7 (C1), 114.4 (C3′, C5′), 104.6/104.2 (C5), 98.6/98.9 (C3), 68.7 (C1″), 55.4 (OCH_3), 55.2 (OCH_3), 41.7/41.9 (Cα), 33.4/34.9 (Cβ), 16.3/15.6 (CH_3). Anal. ($C_{22}H_{23}NO_4$) C, H, N.

2-(2,4-Dimethoxyphenyl)-3-propyl-4-oxo-4-(4-(propenyloxy)phenyl)butanenitrile (11). A solution of **9** (0.93 g, 2.0 mmol) and 1-iodopropane (390 μL, 4.0 mmol) in anhydrous DMF (10 mL) was added cesium flouride (0.61 g, 4.0 mmol). The mixture was stirred for 6 h, water (10 mL) was added and the mixture was extracted with ethyl acetate (4×20 mL). The combined organic phases were concentrated in vacuo the residue was purified by column chromatography to give a mixture of the two epimeric racemic pairs of **11** (1.2 mmol, 62.1%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.87/7.80 (AA′ part of an AA′MM′ system, H2′, H6′), 7.28/7.12 (d, J = 9.0/9.0 Hz, H6), 6.91/6.87 (MM′ part of an AA′MM′

system, H3′, H5′), 6.43/6.36 (m, H5, H3), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.58 (bd, J = 5.2 Hz, H1″), 4.44/4.34 (d, J = 7.9/8.6 Hz, Hβ), 4.10 (m, Hα), 3.84/3.85 (s, OCH_3), 3.76/3.71 (s, OCH_3), 1.8/1.9 (m, Hα1), 1.2 (m, Hα2), 0.79/0.85 (t, J = 7.2 Hz, Hα3); ^{13}C NMR ($CDCl_3$) δ 199.0/198.9 (C = O), 162.5* (C4′), 160.8* (C2), 157.4* (C4), 132.2 (C2″), 130.4/130.5 (C1′), 130.3/130.2 (C2′, C6′), 130.0/130.1 (C6), 120.0/119.7 (C≡N), 118.0 (C3″), 114.1/114.5 (C1), 114.3/114.2 (C3′, C5′), 104.4/104.3 (C5), 98.6/98.9 (C3), 68.7 (C1″), 55.4 (OCH_3), 55.1 (OCH_3), 47.0/46.3 (Cα), 35.2 (Cβ), 32.9/33.2 (Cα1), 19.8/20.1 (Cα2), 13.7/13.8 (Cα3).

3-(2,4-Dimethoxyphenyl)-2-methyl-1-(4-(propenyloxy)phenyl)prop-2-enone-1 (12). A solution of **10** (50 mg, 0.14 mmol) in anhydrous toluene (10 mL) was added sodium hydride (50 mg 60% dispersion, 12.5 mmol) and refluxed for 2 h. Water (5 mL) was added, the mixture was extracted with ethyl acetate (2×10 mL) and the combined organic phases were concentrated in vacuo. The residue was purified by column chromatography to give **12** (43.1 mg, 91.0%) as colourless crystals, mp 60.3–61.7 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.79 (AA′ part of an AA′MM′ system, H2′, H6′), 7.38 (d, J = 8.5 Hz, H6), 7.31 (bs, Hβ), 6.95 (MM′ part of an AA′MM′ system, H3′, H5′), 6.54 (dd, J = 8.5, 2.3 Hz, H5), 6.46 (d, J = 2.3 Hz, H3), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.58 (bd, J = 5.2 Hz, H1″), 3.84 (s, OCH_3), 3.77 (s, OCH_3), 2.18 (d, J = 1.4 Hz, α-CH_3). NOE: α-CH_3-H2′H6′, α-CH_3-H6; ^{13}C NMR ($CDCl_3$) δ 198.1 (C = O), 161.5* (C4′), 161.4* (C4), 158.9* (C2), 136.7 (C2″), 134.7 (Cα), 132.7 (C2′, C6′), 132.0 (Cβ), 131.4 (C1′), 130.9 (C6), 118.1 (C3″), 118.0 (C1), 114.0 (C3′, C5′), 104.1 (C5), 98.2 (C3), 68.8 (C1″), 55.4 (OCH_3), 14.8 (α-CH_3). Anal. ($C_{21}H_{22}O_4$) C, H.

3-(2,4-Dimethoxyphenyl)-2-propyl-1-(4-(propenyloxy)phenyl)prop-2-enone-l (13). A solution of **11** (197 mg, 0.5 mmol) in anhydrous toluene (20 mL) was added sodium hydride (200 mg 60% dispersion, 15 mmol) and refluxed for 6 h. Water (10 mL) was added, the mixture was extracted with ethyl acetate (2×20 mL) and the combined organic phases was concentrated in vacuo. The residue was purified by column chromatography to give **13** (135.8 mg, 74.1%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.88 (AA′ part of an AA′MM′ system, H2′, H6′), 7.33 (d, J = 8.4 Hz, H6), 7.16 (bs, Hβ), 6.94 (MM′ part of an AA′MM′ system, H3′, H5′), 6.56 (dd, J = 8.4. 2.4 Hz, H5), 6.47 (d, J = 2.4 Hz, H3), 6.03 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.42 (dd, J = 17.3, 1.3 Hz, H3″*trans*), 5.32 (dd, J = 10.5, 1.3 Hz, H3″*cis*), 4.58 (bd, J = 5.2 Hz, H1″), 3.85 (s, OCH_3), 3.72 (s, OCH_3), 2.65 (bt, J ≈ 8 Hz, Hα1), 1.55 (6.tet., J ≈ 8 Hz, Hα2), 0.94 (t, J ≈ 8 Hz, Hα3). In the NOESY spectrum correlations between the protons in the propyl group and H6 were found. ^{13}C NMR ($CDCl_3$) δ 198.1 (C = O), 161.5* (C4′), 161.1* (C4), 158.5* (C2), 140.1 (Cα), 135.0 (C2″), 132.5 (Cβ), 132.0 (C2′, C6′), 131.4 (C1′), 130.0 (C6), 117.9 (C3″), 117.6 (C1), 113.8 (C3′, C5′), 104.0 (C5), 98.1 (C3), 68.7 (C1″), 55.3 (OCH_3), 55.2 (OCH_3), 30.1 (Cα1), 21.9 (Cα2), 14,2 (Cα3). HRMS (FAB+): 367.1949 (calcd $C_{23}H_{27}O_4$: 367.1909).

1,3-Dimethoxy-4-(2,2-dibromoethenyl)benzene (15). To a solution of **14** (0.88 g, 5 mmol) and triphenyl phosphine (2.62 g, 10 mmol) in anhydrous dichloromethane (10 mL) was added a solution of tertabromomethane (1.91 g, 5.8 mmol) in dichloromethane (3 mL), keeping the temperature kept below 5 °C. The reaction mixture was stirred for additional 30 min, filtred and concentrated in vacuo and the residue purified by column chromatography to give **15** (1.27 g, 72.1%) as colourless crystals. mp 147.0–147.8 °C (ethanol–water). ^{1}H NMR ($CDCl_3$) δ 7.67 (d, J = 8.4 Hz, H5), 7.51 (s, H1′), 6.46 (dd, J = 8.4, 2.5 Hz, H6), 6.38 (d, J = 2.5 Hz, H2), 3.79 (s, OCH_3), 3.75 (s, OCH_3); ^{13}C NMR ($CDCl_3$) δ 161.2* (C1), 157.8* (C3), 132.2 (C1′), 129.6 (C5), 117.0 (C4), 104.0 (C6), 98.0 (C2), 87.5 (C2′), 55.4 (OCH_3), 55.3 (OCH_3).

1,3-Dimethoxy-4-ethynylbenzene (16). A solution of **15** (1.10 g, 3.0 mmol) in THF (20 mL) was cooled to −78 °C, and slowly added *n*-butyllithium (2.52 mL of a 2.5 M soln, 6.3 mmol). The mixture was stirred for 15 min, water added (10 mL) and extracted with ethyl acetate (20 mL). The organic phase was concentrated in vacuo and the residue purified by column chromatography to give **16** (0.296 g, 65.6%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ 7.37 (d, J = 9.0 Hz, H5), 6.44 (dd, J = 9.0, 2.3 Hz, H6), 6.42 (d, J = 2.3 Hz, H2), 3.86 (s, OCH_3), 3.80 (s, OCH_3), 3.25 (s, H2′); ^{13}C NMR (CDCN) δ 166.9* (C3), 166.7* (C1), 139.6 (C5), 110.1 (C6), 108.2 (C4), 103.2 (C2), 85.2 (C2′), 85.1 (C1′), 60.3 (OCH_3), 60.1 (OCH_3).

1-(4-(2-Propenyloxy)phenyl)-3-(2,4-dimethoxyphenyl)-2-propynone-1 (17). A solution of **16** (1.3 mL, 6.15 mmol), 4-allyloxybenzoyl chloride[7] (2.42 g, 12.3 mmol), copper(I)iodide (0.24 g, 1.2 mmol) and bis[triphenylphosphine]dichloropalladium(II) (0.24 g) in toluene (9 mL) and triethylamine (19 mL) was stirred for 16 h at rt The mixture was washed with water and the organic phase was concentrated in vacuo. The residue was purified by column chromatography to give **17** (0.56 g, 28.3%) as yellow crystals, mp 109.8–110.6 °C (hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 8.26 (AA′ part of an AA′MM′ system, H2′, H6′), 7.54 (d, J = 8.4 Hz, H6), 6.98 (MM′ part of an AA′MM′ system, H3′, H5′), 6.51 (dd, J = 8.4, 2.3 Hz, H5), 6.46 (d, J = 2.3 Hz, H3), 6.05 (ddq, J = 17.3, 10.5, 5.2 Hz, H2″), 5.44 (dd, J = 17.3, 1.5 Hz, H3″*trans*),

5.33 (dd, $J = 10.5$, 1.5 Hz, H3″*cis*), 4.62 (bd, $J = 5.2$ Hz, H1″), 3.94 (s, OCH_3), 3.85 (s, OCH_3); ^{13}C NMR ($CDCl_3$) δ 176.8 (C=O), 163.5* (C2), 163.3* (C4′), 163.1* (C4), 136.3 (C2″), 132.4 (C6), 132.0 (C2′, C6′), 130.8 (C1′), 118.2 (C3″), 114.4 (C3′, C5′), 105.5 (C5), 102.1 (C1), 98.3 (C3), 91.2* (Cβ), 91.1* (Cα), 68.9 (C1″), 55.9 (OCH_3), 55.6 (OCH_3). Anal. ($C_{20}H_{18}O_4$) C, H.

Biological methods

Effect on *Leishmania* promastigotes. The efffect of the chalcones on promastigotes was assessed a described elswhere.[19,51] Briefly, progmastigotes of *L. major* (a World Health Organization reference vaccine strain originally isolated from a patient in Iran, MHOM/IL/LRC-L 137) were incubated at 26 °C in RPMI 1640 containing 25 mM HEPES, 4 mM L-glutamine, 0.02 mg of gentiamicin per ml, and 10% heat inactivated fetal calf serum in the presence of different concentration of the chalcones or the medium alone in 96-well flat-bottomed microtiter plates (NUNC, Denmark). The chalcones were added to the incubation medium by adding 10 μL of an appropriate diluted dimethylsulfoxide solution. After 2 h, 1 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA, USA) was added to each well. Parasites were harvested 18 h later, and [^{3}H]thymidine incorporation was measured. All experiments were performed eight times in triplicates.

Effect on malaria parasites. The effect of the chalcones on a chloroquine-susceptible strain (3D7) of *P. falciparum* (kindly provided by D. Walliker, Edinburgh, UK) was assessed by a modification of the method originally described by Jensen et al.[52] Fifty microliters of parasitized erythrocytes (parasitemia of approximately 1%) at a concentration of 5×10^8 cell/mL and fifty microliters of the medium containing the chalcone were added into each well of 96-well flat-bottomed microtiter plates (NUNC, Denmark). The cultures were incubated for 48 h. Twenty four hours before termination of the cultures, 20 μL of [^{3}H]-hypoxanthine (40 μCi/mL, New England Nuclear, Boston, MA, USA) were added to each well. The cultures were harvested on filter paper by a cell harvester (Skatron, Liebyen, Norway) and counted in a scintillation counter (Minaxi TriCarb 400, United Technologies, Packard Instruments Co. Inc Rockville, MD, USA). All experiments were performed at least four times in triplicate.

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