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Solution-phase parallel synthesis of substituted chalcones and their antiparasitary activity against *Giardia lamblia*

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ABSTRACT

A library of 25-membered chalcones was prepared by parallel synthesis. Substituted acetophenones and benzaldehydes were condensed using the Claisen–Schmidt base-catalyzed aldol condensation. Several chalcones showed in vitro antiparasitic activity against *Giardia lamblia*. The highest activity observed for the IC_{50} values were 12.72, 15.05 and 15.31 μg/mL, respectively; these are potential leads for the development of antigiardial compounds.

1. Introduction

Chalcones (1,3-diphenyl-2-propen-1-one) bearing non natural substituents have been synthesized during the last decade in order to develop active drugs against cancer,[1,2] malaria,[3] leishmaniosis,[4] tuberculosis[5] and cardiovascular diseases.[6] These can also modulate diverse biochemical pathways such as the inflammatory process by scavenging nitric oxide[7,8] or processes in which the epidermal growth factor receptor (EGFR) is involved, chalcones inhibit the tyrosine kinase activity of EGFR.[9] Licochalcone A (Fig. 1) has been used extensively as a model compound in anti-parasitic research.[9–12] Chalcones are usually synthesized using the Claisen–Schmidt reaction in basic medium in polar solvents, and purified by separation, as the reaction results in a complex mixture of products. These reaction conditions have also been used for the creation of a combinatorial library of chalcones.[13]

Giardia lamblia is an intestinal protozoan parasite infecting humans and various other mammalian hosts. The most important clinical signs of giardiosis are diarrhea and malabsorption syndrome. The giardiosis is a worldwide infection; the prevalence is higher for 1–9 year old children and 30–39 year old adults.[14] Giardiosis is commonly treated with metronidazole, tinidazole, furazolidone, paramomycin or nitazoxanide.[15,16] Metronidazole affects electron transport and it is metabolized to produce a reactive reduced form which is cytotoxic for parasites.[17] However, many problems have been associated with the chemotherapeutic antigiardiasic agents currently used: treatment failures, unpleasant side effects, activity against normal intestinal flora, possible carcinogenicity and parasite resistance.[18,19]

In the development of new drugs, it is convenient to have a range of diverse chemical structures with the desired pharmacological activity. In respect to such diversity, a number of literature reports[20] suggest the value of synthetic chalcones as key intermediates for the combinatorial assembly of a number of different heterocyclic scaffolds.

As a part of our continuing efforts in the identification of new chemical entities (NCE) endowed with pharmacological activities, we synthesized a library of chalcones by a combinatorial approach and the activity against *G. lamblia* of the resulting compounds was evaluated.

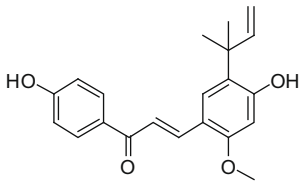

Figure 1. Licochalcone A.

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2. Results and discussion

Chalcone synthesis by condensation of acetophenones and aldehydes under the chosen conditions[21] (Scheme 1) is attractive since it specially generates (*E*)-isomer, normally in high yield. As observed from ^{1}H NMR spectra, all chalcones were geometrically pure and with trans-configuration (J Hα-Hβ = 15.5–16.0 Hz). Using this method, 25 substituted chalcones were obtained in satisfactory yields (Table 1).

Chalcones **4a–y** were obtained as white and yellow solids with sharp melting points. They were pure compounds as determined by HPLC and the spectroscopic and spectrometric data were consistent with the expected structure.

The antigiardial activity of the synthesized chalcones was from low to high as determined by the percentages of mortality and growth inhibition at 24 h of incubation; both values were lower than 50% showing that LD_{50} and IC_{50} values for most of these chalcones are relatively high. Remarkably, the chalcones **4n**, **4o** and **4w** showed the highest antigiardial activity (Table 2). By contrasting the LD_{50} values, the activity of the chalcone **4n** was comparable with that of metronidazole. Compound **4n** had a fluoro substituent at 4́-position and a methoxy group at the 4-position (Table 1). Compounds **4o** and **4w** were significantly less active (Table 2). It is interesting to note the effect of the non-bulky substitution in **4o** and **4w**, which maintained similar activities in spite of the pattern of substitution in the 4,4́ positions. Previous structure–activity studies on the activity of chalcones have emphasized the importance of ring A for activity. In particular, *para*-substitution of ring A with oxygenated and non-bulky substituents have been reported to be desirable.[2]

In the range of evaluated concentrations, chalcones **4b**, **4c**, **4d**, **4g**, **4j**, **4k** and **4v** showed slight antigiardiasic activities by considering mortality values but *Giardia lamblia* morphology was severely affected by treatment with **4v**. Culture media was unstable after mixing with the chalcones **4e**, **4f**, **4h**, **4m**, **4t** and **4y** and their antigiardial activities were not evaluated.

The chalcones studied here were not active against human bacterial pathogens (i.e., *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922), evaluated up to 100 μg/mL by the broth microdilution assay.

Chalcones **4n**, **4o** and **4w** are very promising structures to develop new antigiardial drugs and further studies must be carried out in order to obtain new structures with improved activity, to evaluate the toxicity as well as the in vivo antiparasitary effect.

Table 1
Yield of the Claisen–Schmidt reaction to produce the substituted chalcones **4a–y**

Acetophenones	Aldehydes	Chalcones[a] (yield %)
2a	**3a**	**4a** (80)
2a	**3b**	**4b** (68)[13]
2a	**3c**	**4c** (97)[23]
2a	**3e**	**4d** (90)[24]
2a	**3g**	**4e** (88)[24]
2a	**3k**	**4f** (94)[25]
2b	**3a**	**4g** (50)[13]
2b	**3b**	**4h** (61)[13]
2b	**3c**	**4i** (70)[23]
2b	**3d**	**4j** (80)[4]
2b	**3e**	**4k** (91)[4]
2b	**3f**	**4l** (50)[13]
2b	**3g**	**4m** (84)[24]
2b	**3h**	**4n** (94)[13]
2b	**3i**	**4o** (50)[24]
2b	**3j**	**4p** (78)[24]
2c	**3b**	**4q** (50)[26]
2c	**3c**	**4r** (85)[23]
2c	**3e**	**4s** (57)[26]
2d	**3b**	**4t** (53)[13]
2d	**3c**	**4u** (70)[23]
2d	**3e**	**4v** (99)[24]
2e	**3b**	**4w** (50)[24]
2e	**3c**	**4x** (83)[23]
2e	**3e**	**4y** (40)[24]

[a] Values are the yield of pure compounds recrystallized from anhydrous EtOH.

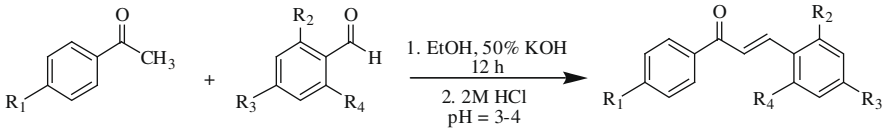
Table 2
Antigiardial activity of selected synthesized compounds[a]

Compounds	IC_{50}[b] (μg/mL)(CI)	LD_{50}[b] (μg/mL)(CI)
4n	12.72 (12.58–12.87)	1.99 (1.98–2.01)
4o	15.04 (14.99–15.09)	7.52 (7.50–7.54)
4w	15.31 (15.25–15.38)	9.67 (9.63–9.71)
Metronidazole	2.88 (2.83–2.93)	1.63 (1.61–1.64)

[a] Results are expressed as the mean of two independent experiments with two replicates.
[b] CI, 95% confidence intervals.

3. Conclusions

In conclusion, we have shown that the Claisen–Schmidt reaction can be used to perform solution-phase parallel synthesis of substituted chalcones. Three chalcones (**4n**, **4o** and **4w**) showed



(2a-e)
2a R_1 = H
2b R_1 = OMe
2c R_1 = OH
2d R_1 = Cl
2e R_1 = F

(3a-i)
3a $R_2 = R_3 = R_4$ = H
3b $R_2 = R_4$ = H, R_3 = Me
3c $R_2 = R_3 = R_4$ = Me
3d $R_2 = R_3$ = OMe, R_4 = H
3e $R_2 = R_4$ = H, R_3 = OMe
3f $R_2 = R_4$ = H, R_3 = OEt
3g $R_2 = R_4$ = H, $R_3 = NO_2$
3h $R_2 = R_3$ = H, R_4 = F
3i $R_2 = R_4$ = H, R_3 = Cl
3j Pyrrole-2-carboxaldehyde
3k Pyridine-4-carboxaldehyde

(4a-y)

Scheme 1. Reaction of substituted acetophenones with aldehydes.

in vitro antiparasitic activity against *Giardia lamblia*; the IC_{50} values were 12.72, 15.05 and 15.31 μg/mL, respectively. The LD_{50} values of the chalcone **4n** was comparable with that of metronidazole. These are potential leads for the development of antigiardial compounds.

4. Experimental part

4.1. General procedures

Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck). All compounds were detected using UV light. Melting points were obtained on an Electrothermal 88629 apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin Elmer FT-IR 1600 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance spectra at 200 Hz and 50.289 Hz, respectively, were recorded on a Varian Mercury 200 MHz Spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. Electrospray ionization mass spectra (ESI-MS) were obtained with an ion trap, and the intensities are reported as a percentage relative to the base peak after the corresponding *m*/*z* value. The purity was obtained on a High Pressure Liquid Chromatograph 1090 series II, column HPC-18.

4.2. Chemistry

The chalcones were synthesized by a base catalyzed Claisen–Schmidt condensation reaction[21] of substituted acetophenones and aldehydes (Scheme 1). The parallel synthesis was carried out in an Advanced ChemTech Instrument model Vantage. In most cases, the starting materials were commercially available. An EtOH solution of substituted acetophenones (**2a–e**) (1.0 equiv) and aldehydes (**3a–k**) (1.0 equiv) was added with 50% KOH (2.5 equiv). The reaction mixture was stirred overnight at room temperature; the pH was adjusted to 3–4 with aq 2 M HCl solution; the precipitate was collected by filtration and purified by recrystallization in EtOH. The purity of all chalcones was measured by HPLC. Structures of all products were confirmed by ^{1}H NMR spectra.

4.2.1. (*E*)-1,3-Diphenylprop-2-en-1-one (4a)

Pale yellow solid (0.833 g, 4.00 mmol, 80% yield). Mp 56–58 °C [lit.[22] 56–57 °C]. IR (KBr disk): 3058, 1659, 1601 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ *8.10–7.98* (m, 2H), 7.84 (d, *J* = 15.6 Hz, 1H), 7.70–7.36 (m, 9H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.5 (s), 144.8 (d), 138.2 (s), 135.0 (s), 132.8 (d), 130.5 (d), 128.9 (2 × d), 128.6 (2 × d), 128.5 (2 × d), 128.4 (2 × d), 122.1 (d). ESI-MS *m*/*z*: 209 $[M+H]^+$, 230 $[M+Na]^+$, 438 $[2M+Na]^+$.

4.2.2. (*E*)-3-(4-Methylphenyl)-1-phenylprop-2-en-1-one (4b)

Pale yellow solid (0.755 g, 6.79 mmol, 68% yield). Mp 95–97 °C [lit.[23] 92–96 °C]. IR (KBr disk): 3051, 2916, 1655, 1597 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 15.7 Hz, 1H), 7.60–7.44 (m, 6H), 7.22 (d, *J* = 8.1 Hz, 2H), 2.40 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.6 (s), 145.0 (d), 141.1 (s), 138.3 (s), 132. 7 (d), 132.1 (s), 129.7 (2 × d), 128.6 (2 × d), 128.5 (2 × d), 128.4 (2 × d), 121.1 (d), 21.5 (q). ESI-MS *m*/*z*: 223 $[M+H]^+$, 244 $[M+Na]^+$, 466 $[2M+Na]^+$.

4.2.3. (*E*)-1-Phenyl-3-(2,4,6-trimethylphenyl)prop-2-en-1-one (4c)

Pale yellow solid (1.21 g, 4.83 mmol, 97% yield). Mp 93–95 °C [lit.[24] 97–99 °C]. IR (KBr disk): 3055, 2912, 1657, 1597 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ *8.05–7.90* (m, 3H), 7.62–7.44 (m, 3H), 7.16 (d, *J* = 16.0 Hz, 1H), 6.93 (s, 2H), 2.40 (s, 6H), 2.30 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.5 (s), 143.3 (d), 138.6 (s), 138.2 (s), 137.2 (3 × s), 132.8 (d), 129.3 (2 × d), 128.6 (2 × d), 128.5 (2 × d), 127.3 (d), 21.3 (2 × q), 21.1 (q). ESI-MS *m*/*z*: 251 $[M+H]^+$, 272 $[M+Na]^+$, 522 $[2M+Na]^+$.

4.2.4. (*E*)-3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (4d)

Pale yellow solid (1.07 g, 4.49 mmol, 90% yield). Mp 70–73 °C [lit.[23] 73–74 °C]. IR (KBr disk): 3055, 2932, 1656, 1598, 1262 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00 (dd, J_1 = 8.3 Hz, J_2 = 1.8, 2H), 7.80 (d, *J* = 15.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.55–7.48 (m, 3H), 7.41 (d, *J* = 15.6 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.6 (s), 161. 7 (s), 144.7 (d), 138.5 (s), 132.6 (d), 130.2 (2 × d), 130.2 (2 × d), 128.6 (2 × d), 128.4 (2 × d), 127.6 (s), 119.8 (d), 55.4 (q). ESI-MS *m*/*z*: 239 $[M+H]^+$, 260 $[M+Na]^+$, 498 $[2M+Na]^+$.

4.2.5. (*E*)-3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (4e)

Pale brown solid (1.11 g, 4.40 mmol, 88% yield). Mp 155–157 °C [lit.[23] 158–160 °C]. IR (KBr disk): 3067, 2928, 1658, 1599, 1515 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.28 (d, *J* = 8.8 Hz, 2H), 8.04 (m, 2H), 7.83 (d, *J* = 15.8 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.71–7.48 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.6 (s), 141.5 (d, s), 141.0 (s), 137.5 (s), 133.4 (d), 128.9 (2 × d), 128.8 (2 × d), 128.6 (2 × d), 125.7 (d), 124.2 (2 × d). ESI-MS *m*/*z*: 254 $[M+H]^+$, 275 $[M+Na]^+$, 528 $[2M+Na]^+$.

4.2.6. (*E*)-1-Phenyl-3-(pyridin-4-yl)prop-2-en-1-one (4f)

White solid (0.88 g, 3.90 mmol, 78% yield). Mp 169–170 °C [lit.[25] 172–174 °C]. IR (KBr disk): 3057, 2940, 1670, 1595, 1183, 1092 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.81 (d, *J* = 5.1 Hz, 2H), 8.00–7.50 (m, 6H), 7.42 (d, *J* = 5.1 Hz, 2H), 7.27 (d, *J* = 16 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.0 (s), 149.2 (2 × d), 143.4 (d), 143.3 (s), 138.2 (s), 132.6 (d), 128.6 (4 × d), 122.2 (d), 120.5 (2 × d). ESI-MS *m*/*z*: 210 $[M+H]^+$, 231 $[M+Na]^+$, 440 $[2M+Na]^+$.

4.2.7. (*E*)-1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-one (4g)

White solid (0.59 g, 2.50 mmol, 50% yield). Mp 103–105 °C [lit.[26] 105–106 °C]. IR (KBr disk): 3053, 2932, 1653, 1602, 1256 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.9 Hz, 2H), 7.80 (d, *J* = 15.7 Hz, 1H), 7.66–7.61 (m, 2H), 7.54 (d, *J* = 15.6 Hz, 1H), 7.43–7.38 (m, 3H), 7.00 (d, *J* = 8.9 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.6 (s), 163.4 (s), 143.9 (d), 135.0 (s), 131.0 (s), 130.8 (2 × d), 130.3 (s), 128.9 (2 × d), 128.3 (2 × d), 121.8 (d), 113.8 (2 × d), 55.5 (q). ESI-MS *m*/*z*: 239 $[M+H]^+$, 260 $[M+Na]^+$, 498 $[2M+Na]^+$.

4.2.8. (*E*)-1-(4-Methoxyphenyl)-3-(4-methylphenyl)prop-2-en-1-one (4h)

Pale yellow solid (0.76 g, 3.01 mmol, 61% yield). Mp 124–126 °C [lit.[27] 126 °C]. IR (KBr disk): 3055, 2932, 1653, 1598, 1254 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.9 Hz, 2H), 7.80 (d, *J* = 15.5 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.50 (d, *J* = 15.5 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 3.90 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.7 (s), 163.3 (s), 144.1 (d), 140.8 (s), 138.4 (s), 132.3 (s), 130.8 (2 × d), 129.7 (2 × d), 128.4 (2 × d), 120.8 (d), 113.8 (2 × d), 55.5 (q), 21.5 (q). ESI-MS *m*/*z*: 253 $[M+H]^+$, 274 $[M+Na]^+$, 526 $[2M+Na]^+$.

4.2.9. (*E*)-1-(4-Methoxyphenyl)-3-(2,4,6-trimethylphenyl)prop-2-en-1-one (4i)

Pale yellow solid (0.98 g, 3.49 mmol, 70% yield). Mp 92–94 °C [lit.[24] 90–92 °C]. IR (KBr disk): 3067, 2916, 1654, 1607, 1257 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.9 Hz, 2H), 7.94 (d, *J* = 16.0 Hz, 1H), 7.15 (d, *J* = 16.0 Hz, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.92 (s, 2H), 3.87 (s, 3H), 2.38 (s, 6H), 2.30 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.7 (s), 163.4 (s), 142.4 (d), 138.3 (s), 137.1 (2 × s), 131.8 (s), 131.1 (s), 130.8 (2 × d), 129.2 (2 × d),

127.2 (d), 113.8 (2 × d), 55.5 (q), 21.2 (2 × q), 21.1 (q). ESI-MS *m/z*: 281 $[M+H]^+$, 302 $[M+Na]^+$, 582 $[2M+Na]^+$.

4.2.10. (*E*)-3-(2,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4j)

Pale yellow solid (1.47 g, 4.93 mmol, 99% yield). Mp 80–82 °C [lit.[23] 83–85 °C]. IR (KBr disk): 3063, 2932, 1644, 1601, 1280 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.05 (d, J = 15.6 Hz, 1H), 8.03 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 15.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 6.96 (d, J = 8.6 Hz, 2H), 6.53 (dd, J_1 = 8.4, J_2 = 2.3 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.4 (s), 163.0 (s), 162.8 (s), 160.3 (s), 139.7 (d), 131.7 (s), 130.8 (d), 130.7 (2 × d), 120.2 (d), 117.3 (s), 113.7 (2 × d), 105.3 (d), 98.4 (d), 55.5 (2 × q), 55.4 (q). ESI-MS *m/z*: 299 $[M+H]^+$.

4.2.11. (*E*)-1-(4-Methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4k)

Pale yellow solid (1.22 g, 4.54 mmol, 80% yield). Mp 98–100 °C [lit.[23] 97–99 °C]. IR (KBr disk): 3071, 2960, 1654, 1594, 1253 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.03 (d, J = 8.9 Hz, 2H), 7.80 (d, J = 15.5 Hz, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 15.5 Hz, 1H), 7.00 (dd, J_1 = 8.9, J_2 = 8.7 Hz, 4H), 3.90 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.7 (s), 163.2 (s), 161.5 (s), 143.8 (d), 131.3 (s), 130.7 (2 × d), 130.1 (2 × d), 127.7 (s), 119.5 (d), 114.3 (2 × d), 113.7 (2 × d), 55.5 (q), 55.4 (q). ESI-MS *m/z*: 269 $[M+H]^+$, 290 $[M+Na]^+$, 558 $[2M+Na]^+$.

4.2.12. (*E*)-3-(4-Ethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4l)

Pale yellow solid (0.70 g, 2.47 mmol, 50% yield). Mp 106–108 °C [lit.[13] 105–107 °C]. IR (KBr disk): 3063, 2936, 1654, 1596, 1254 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.03 (d, J = 8.9 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 15.6 Hz, 1H), 6.97 (d, J = 8.9 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 4.07 (c, J = 7.0 Hz, 2H), 3.88 (s, 3H), 1.43 (t, J = 7.0 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.7 (s), 163.2 (s), 160.9 (s), 143.9 (d), 131.4 (s), 130.7 (2 × d), 130.1 (2 × d), 127.6 (s), 119.4 (d), 114.8 (2 × d), 113.8 (2 × d), 63.6 (t), 55.5 (q), 14.7 (q). ESI-MS *m/z*: 283 $[M+H]^+$, 304 $[M+Na]^+$, 586 $[2M+Na]^+$.

4.2.13. (*E*)-1-(4-Methoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (4m)

Pale brown solid (1.18 g, 4.16 mmol, 84% yield). Mp 160–162 °C [lit.[28] 164 °C]. IR (KBr disk): 3079, 2932, 1657, 1596, 1513, 1264 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.72 (d, J = 8.9 Hz, 2H), 8.06 (d, J = 9.0 Hz, 2H), 7.82 (d, J = 15.8 Hz, 1H), 7.78 (d, J = 8.9 Hz, 2H), 7.65 (d, J = 15.8 Hz, 1H), 7.00 (d, J = 8.9 Hz, 2H), 3.90 (d, J = 9.00 Hz, 2H), 4.00 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 187.8 (s), 163.9 (s), 141.3 (s), 140.7 (d), 131.0 (2 × d), 130.4 (s), 128.8 (2 × d), 128. 6 (s), 125.6 (d), 124.2 (2 × d), 114.0 (2 × d), 55.6 (q). ESI-MS *m/z*: 284 $[M+H]^+$.

4.2.14. (*E*)-3-(6-Flourophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4n)

White solid (1.20 g, 4.68 mmol, 94% yield). Mp 101–103 °C [lit.[23] 100 °C]. IR (KBr disk): 3067, 2936, 1654, 1604, 1256, 1225 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.03 (d, J = 8.9 Hz, 2H), 7.89 (d, J = 15.8 Hz, 1H), 7.64 (d, J = 15.8 Hz, 1H), 7.65–7.58 (m, 1H), 7.43–7.05 (m, 3H), 6.97 (d, J = 8.9 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.6 (s), 164.2 (s), 163.5 (s), 159.1 (s), 136.6 (d), 131.7 (d), 131.5 (d), 130.9 (2 × d), 129.8 (s), 124.5 (d), 116.5 (d), 116.0 (d), 113.9 (2 × d), 55.5 (q). ESI-MS *m/z*: 257 $[M+H]^+$, 278 $[M+Na]^+$, 534 $[2M+Na]^+$.

4.2.15. (*E*)-3-(4-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4o)

White solid (0.68 g, 2.49 mmol, 50% yield). Mp 86–88 °C [lit.[28] 90–93 °C]. IR (KBr disk): 3063, 2931, 1652, 1600, 1257, 1100 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.16 (d, J = 15.7 Hz, 2H), 8.04 (d, J = 8.9 Hz, 1H), 7.74 (m, 1H), 7.50 (d, J = 15.7 Hz, 1H), 7.34 (m, 3H), 7.00 (d, J = 8.9 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.7 (s), 163.5 (s), 139.8 (d), 135.4 (s), 133.5 (s), 131.0 (2 × d), 130.3 (d), 127.8 (d), 127.0 (d), 124.7 (d), 113.9 (2 × d), 55.5 (q). ESI-MS *m/z*: 273 $[M+H]^+$, 294 $[M+Na]^+$, 566 $[2M+Na]^+$.

4.2.16. (*E*)-1-(4-Methoxyphenyl)-3-(1*H*-pyrrol-2-yl)prop-2-en-1-one (4p)

Green solid (0.88 g, 3.87 mmol, 78% yield). Mp 164–166 °C [lit.[13] 164 °C]. IR (KBr disk): 3233, 3087, 2964, 1650, 1604, 1542, 1241 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.88 (br s, 1H), 8.00 (d, J = 9.0 Hz, 2H), 7.73 (d, J = 15.5 Hz, 1H), 7.16 (d, J = 15.5 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 6.95 (m, 1H), 6.70 (m, 1H), 6.32 (m, 1H), 3.88 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 187.8 (s), 162.8 (s), 133.7 (d), 131.4 (s), 130.3 (2 × d), 129.5 (s), 123.2 (d), 115.7 (d), 114.9 (d), 113.6 (2 × d), 110.4 (d), 55.3 (q). ESI-MS *m/z*: 228 $[M+H]^+$, 249 $[M+Na]^+$.

4.2.17. (*E*)-1-(4-Hydroxyphenyl)-3-(4-methylphenyl)prop-2-en-1-one (4q)

Pale yellow solid (0.59 g, 2.47 mmol, 50% yield). Mp 178–180 °C [lit.[29] 183–185 °C]. IR (KBr disk): 3112, 2944, 1646, 1589, 1226 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 10.3 (s, 1H), 8.07 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 15.6 Hz, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 15.6 Hz, 1H), 7.26 (d, J = 8.1 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 2.35 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 187.0 (s), 162.0 (s), 142.7 (d), 140.2 (s), 132.1 (s), 131.0 (2 × d), 129.4 (2 × d), 129.1 (s), 128.6 (2 × d), 121.0 (s), 115.3 (2 × d), 21.0 (q). ESI-MS *m/z*: 239 $[M+H]^+$, 260 $[M+Na]^+$, 498 $[2M+Na]^+$.

4.2.18. (*E*)-1-(4-Hydroxyphenyl)-3-(2,4,6-trimethylphenyl)prop-2-en-1-one (4r)

Pale yellow solid (1.13 g, 4.25 mmol, 85%). Mp 144–146 °C [lit.[29] 145–147 °C]. IR (KBr disk): 3116, 3067, 2976, 1643, 1604, 1211 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 10.4 (s, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 16.0 Hz, 1H), 7.33 (d, J = 16.0 Hz, 1H), 6.94 (s, 2H), 6.90 (d, J = 8.8 Hz, 2H), 2.34 (s, 6H), 2.25 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 187.1 (s), 162.1 (s), 140.6 (d), 137.6 (s), 136.6 (2 × d), 131.2 (s), 131.0 (2 × d), 128.9 (3 × s), 126.9 (d), 115.4 (2 × d), 21.0 (2 × q), 20.6 (q). ESI-MS *m/z*: 267 $[M+H]^+$, 288 $[M+Na]^+$, 554 $[2M+Na]^+$.

4.2.19. (*E*)-1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4s)

Pale yellow solid (0.72 g, 2.85 mmol, 57%). Mp 168–170 °C [lit.[29] 166 °C]. IR (KBr disk): 3148, 3047, 2980, 1645, 1600, 1219 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 10.4 (s, 1H), 8.08 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 16.0 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 3.82 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 187.0 (s), 162.0 (s), 161.1 (s), 142.6 (d), 131.0 (2 × d), 130.5 (2 × d), 129.3 (s), 127.5 (s), 119.6 (d), 115.3 (2 × d), 114.3 (2 × d), 55.3 (q). ESI-MS *m/z*: 276 $[M+Na]^+$, 530 $[2M+Na]^+$.

4.2.20. (*E*)-1-(4-Chlorophenyl)-3-(4-methylphenyl)prop-2-en-1-one (4t)

Pale yellow solid (0.68 g, 2.64 mmol, 53% yield). Mp 155–157 °C [lit.[23] 158–160 °C]. IR (KBr disk): 3055, 2916, 1656, 1597, 1086 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 7.95 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 15.6 Hz, 1H), 7.53 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.8 Hz,

2H), 7.43 (d, J = 15.6 Hz, 1H), 7.22 (d, J = 8.3 Hz, 2H), 2.38 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.2 (s), 145.4 (d), 141.3 (s), 139.1 (s), 136.6 (s), 131.9 (s), 129.9 (2 × d), 129.7 (2 × d), 128.9 (2 × d), 128.5 (2 × d), 120.4 (d), 21.6 (q). ESI-MS m/z: 257 $[M+H]^+$, 278 $[M+Na]^+$, 534 $[2M+Na]^+$.

4.2.21. (*E*)-1-(4-Chlorophenyl)-3-(2,4,6-trimethylphenyl)prop-2-en-1-one (4u)

Pale yellow solid (0.99 g, 3.47 mmol, 70% yield). Mp 93–95 °C [lit.[24] 195 °C]. IR (KBr disk): 3059, 2964, 1659, 1599, 1088 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.97 (d, J = 16.0 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 16.0 Hz, 1H) 6.92 (s, 2H), 2.38 (s, 6H), 2.30 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.1 (s), 143.8 (d), 139.2 (s), 138.8 (s) 137.2 (2 × s), 136.5 (s), 131.3 (s), 129.9 (2 × d), 129.4 (2 × d), 128.9 (2 × d), 126.7 (d), 21.3 (2 × q), 21.1 (q). ESI-MS m/z: 285 $[M+H]^+$, 306 $[M+Na]^+$, 590 $[M+Na]^+$.

4.2.22. (*E*)-1-(4-Chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4v)

Pale yellow solid (1.35 g, 4.94 mmol, 99% yield). Mp 116–118 °C [lit.[23] 117–120 °C]. IR (KBr disk): 3063, 2972, 1656, 1592, 1258, 1087 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.94 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 15.6 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 15.6 Hz, 1H), 6.93 (d, J = 8.7 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 189.1 (s), 161.8 (s), 145.2 (d), 139.0 (s), 136.8 (s), 130.3 (2 × d), 129.8 (2 × d), 128.8 (2 × d), 127.4 (s), 119.1 (d), 114.4 (2 × d), 55.4 (q). ESI-MS m/z: 273 $[M+H]^+$, 294 $[M+Na]^+$, 566 $[2M+Na]^+$.

4.2.23. (*E*)-1-(4-Fluorophenyl)-3-(4-methylphenyl)prop-2-en-1-one (4w)

White solid (0.60 g, 2.49 mmol, 50% yield). Mp 135–137 °C [lit.[23] 138–141 °C]. IR (KBr disk): 3055, 2916, 1659, 1601, 1222 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05 (dd, $J_1 = J_2$ = 8.9 Hz, 2H), 7.80 (d, J = 15.6 Hz, H), 7.54 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 15.6 Hz, H), 7.22 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8.9 Hz, 2H), 2.40 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.9 (s), 145.4 (d), 141.3 (s), 139.1 (s), 136.6 (s), 131.9 (s), 129.9 (2 × d), 129.7 (2 × d), 128.9 (2 × d), 128.5 (2 × d), 120.4 (d), 21.6 (q). ESI-MS m/z: 241 $[M+H]^+$, 262 $[M+Na]^+$, 502 $[2M+Na]^+$.

4.2.24. (*E*)-1-(4-Fluorophenyl)-3-(2,4,6-trimethylphenyl)prop-2-en-1-one (4x)

White solid (1.11 g, 4.13 mmol, 83% yield). Mp 81–83 °C [lit.[24] 85–87 °C]. IR (KBr disk): 3063, 2912, 1659, 1599, 1217 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 8.00 (d, J = 8.6 Hz, 2H), 7.97 (d, J = 15.6 Hz, 1H), 7.13 (d, J = 15.6 Hz, 1H), 6.92 (s, 2H), 2.39 (s, 6H), 2.30 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.8 (s), 168.1 (s), 163.0 (s), 143.5 (d), 138.7 (s), 137.2 (s), 134.5 (s), 131.4 (s), 131.2 (d), 131.0 (d), 129.3 (2 × d), 126.8 (d), 116.0 (d), 115.5 (d), 21.3 (2 × q), 21.1 (q). ESI-MS m/z: 269 $[M+H]^+$, 558 $[2M+Na]^+$.

4.2.25. (*E*)-1-(4-Fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4y)

White solid (0.51 g, 1.99 mmol, 40% yield). Mp 103–105 °C [lit.[23] 108–110 °C]. IR (KBr disk): 3063, 2948, 1659, 1599, 1267, 1225 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05 (dd, $J_1 = J_2$ = 8.9 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.38 (d, J = 15.6 Hz, 1H), 7.19 (dd, $J_1 = J_2$ = 8.6 Hz, 2H), 6.93 (d, J = 8.9 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 188.8 (s), 168.0 (s), 162.9 (s), 161.7 (s), 144.9 (d), 131.0 (d), 130.9 (d), 130.3 (2 × d), 127.5 (s), 119.2 (d), 115.9 (d), 115.4 (d), 114.4 (2 × d), 55.4 (q). ESI-MS m/z: 257 $[M+H]^+$, 278 $[M+Na]^+$, 534 $[2M+Na]^+$.

4.3. Biology

Giardia lamblia trophozoites strain WB (ATCC 30957) were routinely cultivated at 37 °C in the TYI-S-33 medium, supplemented with 10% inactivated bovine fetal bovine serum and 0.2% antibiotic–antimycotic mixture. Trophozoites in the mid-logarithmic phase were resuspended in the media after cooling on ice for 20 min and counted in a hemocytometer. In counting, mortality was determined by cell staining (trypan blue) and absence of mobility. These cells were used as the inoculum for the antigiardial evaluation of the synthetic compounds.

In the test procedure, 24-well cell culture plates were used. The synthesized compounds were dissolved in DMSO and a concentrated stock solution was prepared in culture medium. In each well, trophozoites (300 μL inocula, 1.5 × 10^5 cells) were incubated (24 h/ 37 °C) with the evaluated compounds dissolved in 1.2 mL to get a final volume of 1.5 mL. In the first stage, the 25 synthetic chalcones were evaluated at 100 μg/mL; this was followed by selection of those chalcones with the highest activity (high mortality induction) and they were evaluated at 1, 5, 10, 15, 20, 25, 50, 75 and 100 μg/mL. The DMSO concentration per well was not higher than 5% (v/v). Control experiments were performed under similar experimental conditions without the synthesized compounds: positive with metronidazole (1, 2, 3, 4 and 5 μg/mL) and negative with solvent (DMSO) in which the synthetic compounds were incorporated. After 24 h incubation, the plates were cooled on ice (20 min) and the trophozoites were suspended by pipette mixing. The number of cells and viability was evaluated as described above. Probit analysis was used to calculate the 50% inhibitory concentration (IC_{50})[30] and the 50% lethal concentration (LD_{50}), only for the chalcones with the highest antigiardial activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.052.

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