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Original article

Novel antileishmanial chalconoids: Synthesis and biological activity of 1- or 3-(6-chloro-2*H*-chromen-3-yl)propen-1-ones

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ABSTRACT

A series of novel chalconoids containing a 6-chloro-2*H*-chromen-3-yl group were prepared through a convenient and efficient synthetic method by using 5-chloro-2-hydroxybenzaldehyde as starting material. The target compounds were evaluated against the promastigote form of *Leishmania major* using MTT assay. All of the evaluated compounds have shown high in vitro antileishmanial activity at concentrations less than 3.0 μM. The results of cytotoxicity assessment against mouse peritoneal macrophage cells showed that these compounds display antileishmanial activity at non-cytotoxic concentrations.

1. Introduction

Leishmaniasis is a parasitic infection caused by different species belonging to the genus *Leishmania*, a protozoan which is transmitted to humans by the bite of female phlebotomine sandfly. Leishmaniasis infection causes a wide spectrum of disease in humans, with many different clinical manifestations, such as cutaneous, mucocutaneous and visceral leishmaniasis, which can be fatal when untreated. Leishmaniasis has a major and increasing impact on global public health and is endemic in many tropical and subtropical regions of the world, particularly in Africa, Asia, and Latin America. It found in 88 countries and affects around 12 million people of the world and 350 million people are at risk of infection with *Leishmania* spp [1,2].

The management of leishmaniasis relies entirely on chemotherapy and so far no vaccine approved for human use is available [3]. The drugs available for the treatment of *Leishmania* infections is limited and includes pentavalent antimonials such as sodium stibogluconate (Pentostam®) and meglumine antimonate (Glucantime®), pentamidine, amphotericin B and miltefosine [4]. Despite the recent advances in new antileishmanial agents, the first-line therapy to all forms of leishmaniasis still requires multiple, potentially toxic and painful injections with pentavalent antimonials [5]. Furthermore, clinical resistance to pentavalent antimonials has been reported recently. This resistance occurred in 5–70% of patients in some areas of endemicity [6]. The chemotherapy with the second-line drug pentamidine is also far from satisfactory due to several side effects including renal and hepatic toxicity, pancreatitis, hypotension, dysglycemia, and cardiac abnormalities [7]. Although amphotericin B and its lipid complex, are quite effective for visceral leishmaniasis, they are expensive and do not appear to be suitable for treatment of non-visceral diseases [7].

Newly introduced oral miltefosine, a phosphocholine analogue, has shown promise in the treatment of visceral leishmaniasis [8], but presenting severe gastrointestinal problems and teratogenic effects [9,10]. It is also known that current drugs contribute to increased Leishmania–HIV co-infections. No treatment has proven to be effective in achieving radical cure of visceral leishmaniasis when it is associated with HIV infection. Therefore, there is an increasingly urgent need for the development of new, orally active, inexpensive, effective and safe drugs for the treatment of leishmaniasis.

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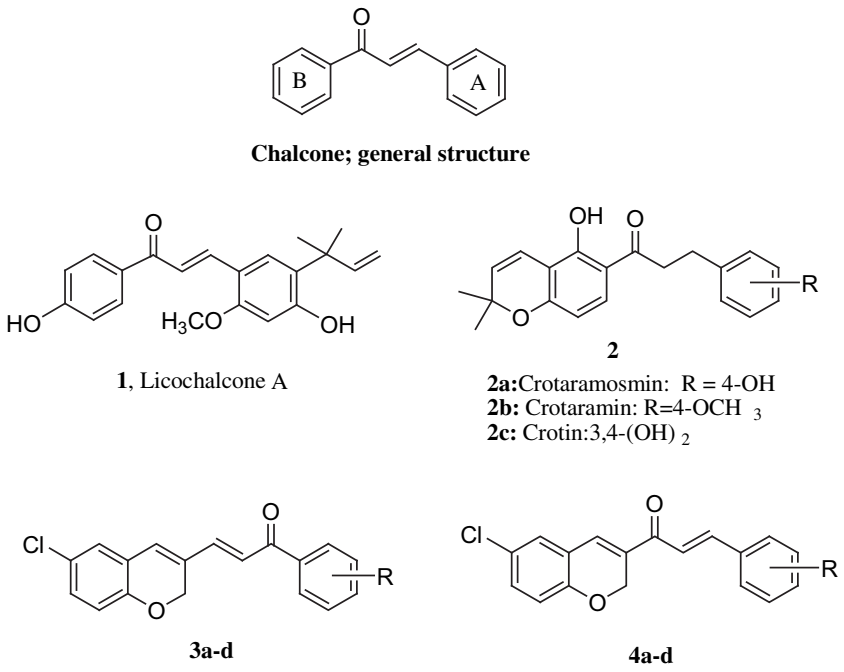


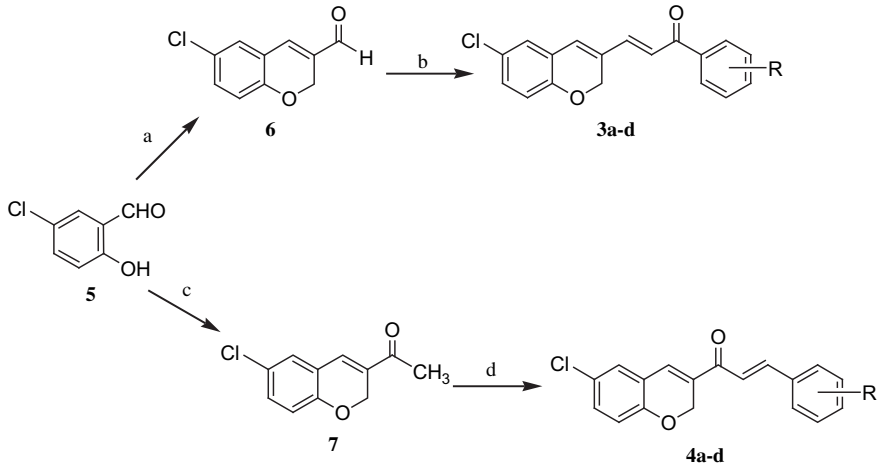
Fig. 1. Structure of chalcone, chalcone-like compounds **1** and **2** with antileishmanial activity, and designed chalconoids **3** and **4** as potential antileishmanial agents.

In the search for finding new antileishmanial drugs, both synthetic and natural origin compounds comprising a diverse group of chemical structures have been reported, including chalcones present in *Glycirrhyza glabra* and *Piper aduncum* [11,12]. Also, the antileishmanial activity of several chalcones have been reported in the literature [13–16]. The most promising member of this group is licochalcone A (**1**, Fig. 1), an oxygenated chalcone isolated from the roots of Chinese liquorice [15]. A number of chalcone-like compounds having 5-hydroxy-2*H*-chromen-6-yl moieties (compounds **2**) are reported to be potential agents against *Leishmania* in biological assays [17,18]. Based on these observations and our program for designing new antileishmanial agents [19–23] we hypothesized to synthesize 1- or 3-(6-chloro-2*H*-chromen-3-yl)propen-1-ones (**3** and **4**) in which ring A or ring B of typical chalcones was replaced by 6-chloro-2*H*-chromen-3-yl moiety (Fig. 1). Thus, we herein report the synthesis and in vitro antileishmanial activity of 1- or 3-(6-chloro-2*H*-chromen-3-yl)propen-1-ones.

2. Results and discussion

2.1. Chemistry

The general synthetic pathway used to synthesize the designated compounds **3** and **4** is outlined in Scheme 1. The treatment of 5-chloro-2-hydroxybenzaldehyde **5** with acrolein in refluxing dioxane in the presence of potassium carbonate afforded desired chromene-3-carbaldehyde **6**. Claisen–Schmidt condensation of compound **6** with various acetophenones in ethanolic solution of NaOH afforded 3-(6-chloro-2*H*-chromen-3-yl)propen-1-ones **3a–d**. The second type of target compounds as typified by general structure **4a–d** (Fig. 1), were prepared by a distinct rout (Scheme 1). First, 5-chloro-2-hydroxybenzaldehyde **5** was reacted with methyl vinyl ketone in refluxing dioxane in the presence of potassium carbonate to give 1-(6-chloro-2*H*-chromen-3-yl)ethanone **7**. Then, Claisen–Schmidt condensation of compound **7** with different aldehydes in ethanolic solution of NaOH yielded corresponding



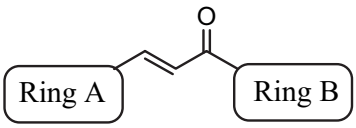
Scheme 1. Synthesis of chalconoids **3a–d** and **4a–d**. *Reagents and conditions*: (a) acrolein, 1,4-dioxane, K_2CO_3, reflux (b) appropriate acetophenone, NaOH, EtOH (c) methyl vinyl ketone, 1,4-dioxane, K_2CO_3, reflux (d) appropriate aldehyde, NaOH, EtOH.

1-(6-chloro-2*H*-chromen-3-yl)propen-1-ones **4a–d**. Yields for final step ranged from 13% to quantitative but were not optimized. The chalcones **3** and **4** were always obtained as (*E*)-isomer as judged by ^{1}H NMR spectroscopy. Examination of the ^{1}H NMR coupling constants of α and β-vinylic protons in compounds **3** and **4** clearly showed a large $J_{\alpha,\ \beta}$ values (15.5–16.0 Hz) and suggests a *trans*-configuration. The structures and physicochemical data of final compounds are illustrated in Table 1.

2.2. Biology

The parasitic protozoan *Leishmania* is digenetic and has two distinct stages in its life cycle. The motile flagellated promastigote stage lives in the alimentary canal of the sandfly vector, which, by inoculation, transmits the promastigotes into the mammalian host, where they enter macrophages differentiating and multiplying into non-motile amastigotes [24]. In our study, the target compounds

Table 1
Structures and physicochemical data of chalconoids **3a–d** and **4a–d**.



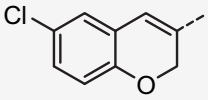
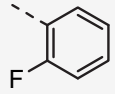
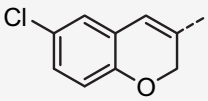
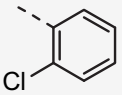
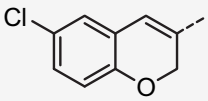
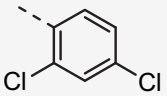
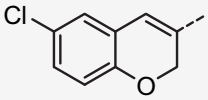
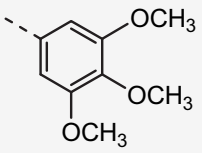
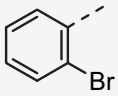
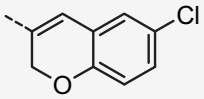
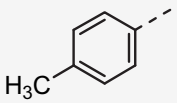
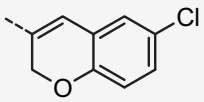
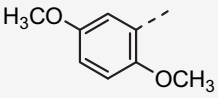
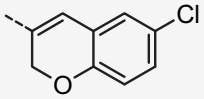
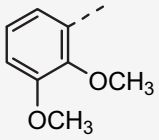
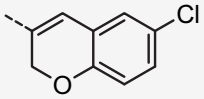
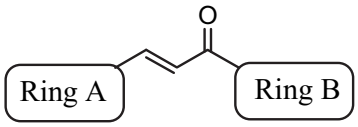
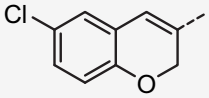
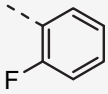
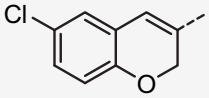
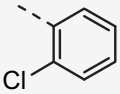
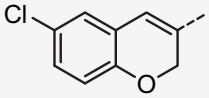
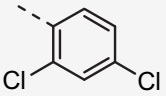
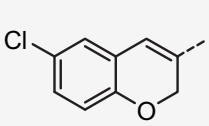
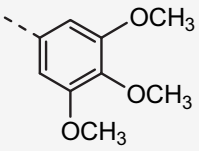
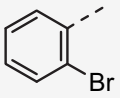
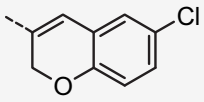
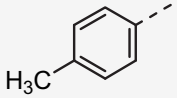
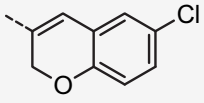
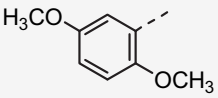
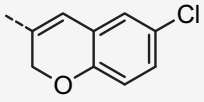
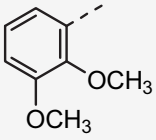
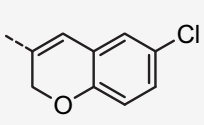
Compound	Ring A	Ring B	m.p. (°C)	M.W	Yield (%)	Formula
3a			184–185	314.74	89	$C_{18}H_{12}ClFO_2$
3b			191–192	331.19	45	$C_{18}H_{12}Cl_2O_2$
3c			226	365.64	88	$C_{18}H_{11}Cl_3O_2$
3d			207	386.83	13	$C_{21}H_{19}ClO_5$
4a			167	375.64	98	$C_{18}H_{12}BrClO_2$
4b			147	310.77	49	$C_{19}H_{15}ClO_2$
4c			146	356.80	46	$C_{20}H_{17}ClO_4$
4d			167	356.80	18	$C_{20}H_{17}ClO_4$

Table 2
In vitro activities of compounds **3a–d** and **4a–d** against promastigote form of *L. major*.



Compound	Ring A	Ring B	IC_{50} (μM)[a]
3a			2.22 ± 0.60
3b			1.22 ± 0.31
3c			1.91 ± 0.35
3d			2.06 ± 0.22
4a			1.33 ± 0.52
4b			2.75 ± 0.37
4c			3.00 ± 0.60
4d			2.80 ± 0.27
Glucantime®			30 ± 0.189[b]

[a] The values represent mean ± SD.
[b] The IC_{50} of Glucantime was in mg/mL.

were subjected to in vitro antileishmanial activity profile against the promastigote form of the *Leishmania major* using MTT assay, side by side the reference drug Glucantime®. The inhibitory concentrations for 50% of inhibition (IC_{50}) of *Leishmania* growth, at third day of incubation, were calculated based on a linear regression. All data were reported as the mean ± SD in Table 2.

Generally, the IC_{50} values of the test compounds **3a–d** and **4a–d** indicate that all compounds exhibit high activity against *L. major* ($IC_{50} \leq 3.0$ μM). Structurally, compounds **3a–d** have a group 6-chloro-2*H*-chromen-3-yl at the 3-position of propenone linkage (ring A), whereas compound **4a–d** have this pendant group at the position 1 of propenone (ring B). Obtained results based on the IC_{50} values of compounds **3a–d** and **4a–d** demonstrated that these restricted series of compounds showed small differences in their overall activity profile. It seems that in (6-chloro-2*H*-chromen-3-yl)propenones, the biological activity against *L. major* was slightly influenced by the type of secondary aryl ring attached to the 1- or 3-position of propenone linker. Thus, the structure–activity relationship study was not crucial. However, it is notable that compounds **3b** and **4a** (containing 2-chlorophenyl and 2-bromophenyl, respectively) with IC_{50} values of 1.22 ± 0.31 and 1.33 ± 0.52 μM proved to be statistically the most potent compounds. Comparison of the IC_{50} values reveals that certain substituents such as methyl, dimethoxy and trimethoxy on phenyl are permitted and well tolerated but cannot improve the inhibitory activity respect to halogen substituents against *Leishmania* promastigotes.

The in vitro toxicity of compounds **3a–d** and **4a–d** against mouse peritoneal macrophages was also assessed using MTT colorimetric assay. Macrophage cells were treated with synthesized compounds at the concentrations equal to IC_{50} values (against *L. major*) for 24 h, side by side with the reference drug Glucantime®. Whereas, the reference drug decreased viability of macrophages up to 40%, the test compound showed no effect on viability of macrophage cells at those levels of concentrations.

In conclusion, a series of novel chalconoids containing a 6-chloro-2*H*-chromen-3-yl group were prepared through an easy, convenient, and efficient synthetic method by using 5-chloro-2-hydroxybenzaldehyde as starting material. This new class of chalconoids has been identified as potential antileishmanial agents. All of the evaluated compounds have shown high in vitro antileishmanial activity at concentrations less than 3.0 μM. The results of cytotoxicity assessment showed that these compounds display antileishmanial activity at non-cytotoxic concentrations. Thus, (6-chloro-2*H*-chromen-3-yl)propenone can be considered as a promising lead for the development of an effective agent for chemotherapy of leishmaniasis and other protozoan infections.

3. Experimental

All chemicals and solvents used in this study were purchased from Merck chemical. Melting points were determined on a kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H NMR spectra was recorded using a Bruker 500 spectrometer and chemical shifts are reported in parts per million (ppm) relative to TMS as internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on CHN–O rapid elemental analyzer (GmbH–Germany) for C, H and N, and the results are within ±0.4% of the theoretical values. Merck silica gel F254 plates were used for analytical TLC.

3.1. Synthesis of 6-chloro-2H-chromene-3-carbaldehyde (*6*)

A mixture of 5-chloro-2-hydroxybenzaldehyde (7 mmol) and potassium carbonate (7 mmol) in 1,4-dioxane (12.5 mL) was treated with acrolein (0.5 mL). The mixture was heated at 100 °C for 8 h and allowed to cool. The mixture was diluted with water and extracted several times with ether. The combined ether extracts were dried

(Na_2SO_4) and evaporated to give compound **6** as a yellow solid which was crystallized from ethyl acetate–hexane. Yield 78%; m.p. 91–93 °C [25].

3.2. Synthesis of 1-(6-chloro-2H-chromen-3-yl)ethanone (**7**)

A mixture of 5-chloro-2-hydroxybenzaldehyde (5 mmol) and potassium carbonate (5 mmol) in 1,4-dioxane (5 mL) was treated with methyl vinyl ketone (5 mmol). The mixture was heated at 100 °C for 4 h and allowed to cool. The mixture was diluted with water and extracted several times with ether. The combined ether extracts were dried (Na_2SO_4) and evaporated to give compound **7** as a yellow solid, which was crystallized from ethyl acetate–hexane Yield 82%; m.p. 50–51 °C [25].

3.3. General procedure for the synthesis of 3-(6-chloro-2H-chromen-3-yl)-1-phenyl propen-1-one derivatives **3a–d**

To a well stirred solution of compound **6** (1 mmol) and substituted acetophenone (1 mmol) in absolute ethanol (5 mL), was added a 3.5 M NaOH solution (2 mL) and stirred overnight in an ice-bath. The reaction mixture was diluted with water and the precipitate was filtered and crystallized from ethanol to give corresponding chalconoids **3a–d**.

3.3.1. (E)-3-(6-Chloro-2H-chromen-3-yl)-1-(2-fluorophenyl)-prop-2-en-1-one (**3a**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.81 (dd, 1H, J = 7.5 and 1.5 Hz, H_6 phenyl), 7.57–7.52 (m, 1H, H_4 phenyl), 7.50–7.45 (m, 1H, H_5 phenyl), 7.40 (dt, 1H, J = 7.5 and 1.5 Hz, H_3 phenyl), 7.20–7.17 (m, 1H, H_3 propenone), 7.15 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.13 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.79 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.78–6.73 (m, 2H, H_2 propenone and H_4 chromene), 5.06 (s, 2H, OCH_2). ^{13}C NMR (125 MHz, $CDCl_3$): 188.8, 162.2, 160.2, 153.1, 146.6, 140.8, 134.1, 131.0, 130.5, 130.0, 127.3, 125.0, 124.6, 123.1, 119.4, 117.2, 116.6, 65.3. IR (KBr, cm^{-1}) ν_{max}: 1751 (C=O), 1214 (C–O). MS (*m*/*z*, %): 315 (M+1, 33), 314 (M^+, 92), 233 (12), 220 (8), 191 (91), 165 (17), 133 (12), 123 (100), 109 (11), 101 (18), 95 (48), 75 (36), 51 (15). Anal. Calcd for $C_{18}H_{12}ClFO_2$: C, 68.69; H, 3.84. Found: C, 68.60; H, 3.96.

3.3.2. (E)-3-(6-Chloro-2H-chromen-3-yl)-1-(2-chlorophenyl)-prop-2-en-1-one (**3b**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48–7.45 (m, 2H, H_4 and H_6 phenyl), 7.45–7.11 (m, 1H, H_3 phenyl), 7.39–7.35 (m, 1H, H_5 phenyl), 7.17 (dd, 1H, J = 16 and 0.5 Hz, H_3 propenone), 7.14 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.04 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.78 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.71 (brs, 1H, H_4 chromene), 6.49 (d, 1H, J = 16 Hz, H_2 propenone), 5.03 (s, 2H, OCH_2). IR (KBr, cm^{-1}) ν_{max}: 1741 (C=O). MS (*m*/*z*, %): 334 (M+4, 16), 332 (M+2, 33), 330 (M^+, 57), 314 (10), 260 (18), 222 (21), 192 (73), 167 (51), 141 (100), 130 (28), 114 (33), 58 (11). Anal. Calcd for $C_{18}H_{12}Cl_2O_2$: C, 65.28; H, 3.65. Found: C, 65.05; H, 3.66.

3.3.3. (E)-3-(6-Chloro-2H-chromen-3-yl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (**3c**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (d, 1H, J = 2 Hz, H_3 phenyl), 7.41 (d, 1H, J = 8.5 Hz, H_6 phenyl), 7.36 (dd, 1H, J = 8.5 and 2 Hz, H_5 phenyl), 7.18 (d, 1H, J = 16 Hz, H_3 propenone), 7.15 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.05 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.79 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.73 (s, 1H, H_4 chromene), 6.47 (d, 1H, J = 16 Hz, H_2 propenone), 5.02 (s, 2H, OCH_2). ^{13}C NMR (125 MHz, $CDCl_3$): 193.1, 153.1, 141.9, 138.8, 131.6, 131.2, 130.7, 130.3, 129.8, 129.4, 127.3, 126.9, 126.8, 126.5, 125.6, 122.9, 117.2, 65.2. IR (KBr, cm^{-1}) ν_{max}: 1654 (C=O), 1219 (C–O). MS (*m*/*z*, %): 369 (M+4, 13), 367 (M+2, 12), 365 (M^+, 17), 329 (50), 265 (13), 220 (12), 203 (12), 192 (64), 175 (50), 145 (29), 127 (50), 110 (37), 100 (34), 82 (41), 70 (100), 44 (35). Anal. Calcd for $C_{18}H_{11}Cl_3O_2$: C, 59.13; H, 3.03. Found: C, 58.88; H, 2.97.

3.3.4. (E)-3-(6-Chloro-2H-chromen-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3d**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.51 (d, 1H, J = 16 Hz, H_3 propenone), 7.21 (s, 2H, H_2 and H_6 phenyl), 7.15 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.09 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.82 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.81–6.78 (m, 2H, H_4 chromene and H_2 propenone), 5.10 (s, 2H, OCH_2), 3.95 (s, 9H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 188.6, 153.2, 152.9, 142.7, 140.9, 133.2, 130.7, 130.4, 129.9, 127.3, 126.6, 123.2, 121.1, 117.1, 106.1, 65.4, 61.0, 56.4. IR (KBr, cm^{-1}) ν_{max}: 1741 (C=O). MS (*m*/*z*, %): 388 (M+2, 40), 386 (M^+, 100), 371 (18), 312 (10), 220 (21), 191 (43), 167 (24), 154 (32), 127 (11). Anal. Calcd for $C_{21}H_{19}ClO_5$: C, 65.20; H, 4.95. Found: C, 65.20; H, 5.07.

3.4. General procedure for the synthesis of 1-(6-chloro-2H-chromen-3-yl)-3-phenyl propen-1-one derivatives **4a–d**

To a well stirred solution of 1-(6-chloro-2*H*-chromen-3-yl)-ethanone (**7**, 1 mmol) and appropriate aldehyde (1 mmol) in absolute ethanol (5 mL), was added a 3.5 M NaOH solution (2 mL) and stirred overnight in an ice-bath. The reaction mixture was diluted with water and the precipitate was filtered and crystallized from ethanol to give corresponding chalconoids **4a–d**.

3.4.1. (E)-3-(2-Bromophenyl)-1-(6-chloro-2H-chromen-3-yl)-prop-2-en-1-one (**4a**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.79 (t, 1H, J = 1 Hz, H_4 chromene), 7.65 (d, 1H, J = 15.5 Hz, H_3 propenone), 7.57–7.53 (m, 2H, H_3 and H_6 phenyl), 7.39 (m, 1H, H_5 phenyl), 7.34 (d, 1H, J = 15.5 Hz, H_2 propenone), 7.32–7.29 (m, 1H, H_4 phenyl), 7.23 (d, 1H, J = 2.5 Hz, H_5 chromene), 7.22–7.20 (m, 1H, H_7 chromene), 6.83 (d, 1H, J = 8 Hz, H_8 chromene), 5.12 (d, 2H, J = 1 Hz, OCH_2). ^{13}C NMR (125 MHz, $CDCl_3$): 186.5, 154.1, 142.2, 136.8, 133.3, 132.5, 132.1, 130.7, 130.5, 128.5, 127.3, 126.6, 123.1, 121.9, 120.9, 117.7, 64.8. IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O), 1178 (C–O). MS (*m*/*z*, %): 378 (M+2, 25), 376 (M^+, 85), 359 (25), 295 (11), 267 (7), 260 (26), 231 (27), 219 (7), 202 (10), 165 (49), 155 (6), 147 (19), 130 (19), 115 (12), 102 (100), 89 (5), 75 (30), 63 (10), 51 (19). Anal. Calcd for $C_{18}H_{12}BrClO_2$: C, 57.55; H, 3.22. Found: C, 57.41; H, 3.21.

3.4.2. (E)-1-(6-Chloro-2H-chromen-3-yl)-3-(4-methylphenyl)-prop-2-en-1-one (**4b**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.74 (d, 1H, J = 15.5 Hz, H_3 propenone), 7.54 (d, 2H, J = 8 Hz, H_2 and H_6 phenyl), 7.37 (brs, 1H, H_4 chromene), 7.33 (d, 1H, J = 15.5 Hz, H_2 propenone), 7.25 (d, 2H, J = 8 Hz, H_3 and H_5 phenyl), 7.22 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.19 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.83 (d, 1H, J = 8.5 Hz, H_8 chromene), 5.13 (d, 2H, J = 1 Hz, OCH_2), 2.41 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 187.0, 154.1, 144.1, 141.2, 132.8, 131.9, 131.8, 131.3, 129.7, 128.7, 128.5, 128.4, 126.5, 122.2, 118.7, 117.6, 65.0, 21.5. IR (KBr, cm^{-1}) ν_{max}: 1629 (C=O). MS (*m*/*z*, %): 312(M+2, 33), 310 (M^+, 100), 293 (27), 267 (12), 232 (15), 218 (18), 202 (7), 165 (31), 155 (9), 145 (54), 130 (12), 115 (86), 102 (33), 91 (56), 75 (23), 65 (30), 51 (23). Anal. Calcd for $C_{19}H_{15}ClO_2$: C, 73.43; H, 4.86. Found: C, 73.03; H, 4.78.

3.4.3. (E)-1-(6-Chloro-2H-chromen-3-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (**4c**)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.00 (d, 1H, J = 16 Hz, H_3 propenone), 7.42 (d, 1H, J = 16 Hz, H_2 propenone), 7.35 (brs, 1H, H_4 chromene), 7.22 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.19 (d, 1H, J = 2.5 Hz, H_5 chromene), 7.15 (d, 1H, J = 3 Hz, H_6 phenyl), 6.96

(dd, 1H, $J = 9$ and 3 Hz, H_4 phenyl), 6.90 (d, 1H, $J = 9$ Hz, H_3 phenyl), 6.83 (d, 1H, $J = 8.5$ Hz, H_8 chromene), 5.14 (s, 2H, OCH_2), 3.89 and 3.84 (2s, 6H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 187.5, 154.1, 153.5, 153.4, 139.4, 132.9, 131.7, 131.3, 128.3, 126.5, 124.3, 122.3, 120.9, 117.6, 117.2, 113.9, 112.5, 65.1, 56.1, 55.9. IR (KBr, cm^{-1}) ν_{max}: 1644 (C═O). MS (*m*/*z*, %): 358 (M+2, 38), 356 (M^+, 100), 324 (12), 306 (15), 297 (22), 281 (8), 262 (11), 191 (25), 176 (67), 164 (75), 148 (38), 133 (45), 102 (42), 89 (20), 77 (50), 63 (20), 51 (37). Anal. Calcd for $C_{20}H_{17}ClO_4$: C, 67.32; H, 4.80. Found: C, 67.11; H, 5.17.

3.4.4. (E)-1-(6-Chloro-2H-chromen-3-yl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (**4d**)

^1H NMR (500 MHz, $CDCl_3$) δ: 8.03 (d, 1H, $J = 16$ Hz, H_3 propenone), 7.43 (d, 1H, $J = 16$ Hz, H_2 propenone), 7.36 (brs, 1H, H_4 chromene), 7.26 (dd, 1H, $J = 8.5$ and 1.5 Hz, H_7 chromene), 7.21 (dd, 1H, $J = 8.5$ and 1.5 Hz, H_6 phenyl), 7.17 (d, 1H, $J = 1.5$ Hz, H_5 chromene), 7.12 (t, 1H, $J = 8.5$ Hz, H_5 phenyl), 6.99 (dd, 1H, $J = 8.5$ and 1.5 Hz, H_4 phenyl), 6.83 (d, 1H, $J = 8.5$ Hz, H_8 chromene), 5.14 (d, 2H, $J = 1.5$ Hz, OCH_2), 5.91 and 3.89 (2s, 6H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 187.3, 154.1, 153.2, 148.9, 138.9, 132.8, 131.8, 131.4, 128.9, 128.4, 126.5, 124.2, 122.2, 121.3, 119.5, 117.6, 114.2, 64.9, 61.3, 55.9. IR (KBr, cm^{-1}) ν_{max}: 1639 (C═O). MS (*m*/*z*, %): 358 (M+2, 41), 356 (M^+, 100), 325 (67), 306 (7), 297 (18), 281 (10), 262 (9), 191 (28), 176 (44), 164 (65), 155 (9), 148 (25), 133 (25), 102 (40), 91 (24), 77 (46), 63 (17), 51 (35). Anal. Calcd for $C_{20}H_{17}ClO_4$: C, 67.32; H, 4.80. Found: C, 67.21; H, 4.69.

3.5. Biological activity

3.5.1. Parasite and culture

The strain of *L. major* used in this study was the vaccine strain (MRHO/IR/75/ER), obtained from Pasteur Institute, Tehran (Iran). The infectivity of the parasites was maintained by regular passage in susceptible BALB/c mice. The promastigote form of parasite was grown in blood agar cultures at 25 °C. The stationary parasite inoculation was 2×10^6 cells/mL. For the experiments described here, the stationary phase of promastigotes were washed with phosphate buffered saline and recultured in RPMI 1640 medium (Sigma) at 2×10^6 cells/mL density, supplemented with 10% of heat-inactivated fetal bovine serum, 2 mM glutamine (Sigma), pH ~7.2, 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma).

3.5.2. In vitro antileishmanial activity

The antileishmanial evaluation of compounds **3a–d** and **4a–d** was performed using direct counting and MTT assay [26]. The growth curve of the *L. major* strain was determined daily under light microscope and counting in a Neubauer's chamber. Then, parasites (2×10^6/mL) in the logarithmic phase were incubated with a serial range of drug concentrations for 24 h at 25 °C. To determining 50% inhibitory concentrations (IC_{50}), the tetrazolium bromide salt (MTT) assay was used. Briefly, promastigotes from early log phase of growth were seeded in 96-well plastic cell culture trays, containing serial dilution of drug and phenol red free RPMI 1640 medium, supplemented with 10% of FCS, 2 mM glutamine, pH ~7.2 and antibiotics, in a volume of 200 μL. After 24 h of incubation at 25 °C, the media was renewed with 100 μg/well of MTT (0.5 mg/mL) and plates were further incubated for 4 h at 37 °C. The plates were centrifuged (2000 rpm × 5 min), the pellets were dissolved in 200 μL of DMSO. The samples were read using an ELISA plate reader at a wavelength of 492 nm. Two or more independent experiments in triplicate were performed for determination of sensitivity to each drug, the IC_{50} were calculated by linear regression analysis, expressed in mean ± SD. Control cells were incubated with culture medium plus DMSO.

3.5.3. Cytotoxicity against macrophages

In vitro toxicity against mouse peritoneal macrophages was assessed with cells plated in 96-well plates at 2×10^5 cells/well. After cell adherence, the medium was removed and replaced by the media containing IC_{50} concentration of each compounds. The plates were incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. Control cells were incubated with culture medium plus DMSO. Cell viability was determined by MTT colorimetric assay.

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