Chem Biol Drug Des 2010; **75:** 590–596

Research Article

doi: 10.1111/j.1747-0285.2010.00959.x

Chromene-Based Synthetic Chalcones as Potent Antileishmanial Agents: Synthesis and Biological Activity

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Two types of regioisomeric chromene-based chalcones namely, 1-(6-methoxy-2*H*-chromen-3-yl)-3-phenylpropen-1-ones and 3-(6-methoxy-2*H*-chromen-3-yl)-1-phenylpropen-1-ones were prepared and investigated for their antileishmanial activity against promastigotes form of *Leishmania major*. The obtained results from *in vitro* biological assays indicated that chloro-substituted 1-(6-methoxy-2*H*-chromen-3-yl)-3-phenylpropen-1-ones exhibited excellent activity against *Leishmania major* at non-cytotoxic concentrations.

Key words: antileishmanial activity, chalcones, chromene, *Leishmania major*, promastigotes

Received 13 August 2009, revised 4 February 2010, accepted for publication 6 February 2010

Leishmaniasis comprises a group of parasitic diseases typically transmitted by the bite of female phlebotomine sandfly and manifest with visceral, cutaneous and mucutaneous forms (1). Human leishmaniasis is mainly distributed in the tropic and subtropic area with a prevalence of 12 million cases and approximate incidence of 0.5 million cases of visceral leishmaniasis and 1.5 million cases of cutaneous leishmaniasis. Overall, 350 million individuals are at risk of leishmaniasis infection in the 88 countries (2). Whereas no vaccine exists, the current chemotherapy for leishmaniasis possesses a set of problems because of the emergence of drug resistant strains, limited efficacy, long-term treatment, cost expensive and severe side effects (3–5). Thus, the necessity for the development of new, efficient, affordable and safe drug is felt. Also, because of the high toxicity associated with the currently used antileishmanial drugs, efforts are being made to identify new structures from natural and synthetic compounds.

Chalcones (1,3-diaryl-2-propen-1-ones) are natural or synthetic compounds belonging to the flavonoid family with widespread distribution in vegetables, fruits, spices and tea and are present in a variety of plant species (6). Chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. Chalcones have been reported to possess many pharmacological activities (7), including anti-inflammatory (8), immunomodulatory (9), antifungal (10), anticancer (11,12), antioxidant (13), antibacterial (14), antimalarial (15,16) and antileishmanial (17,18) properties.

In the past decade, synthetic or naturally occurring chalcones emerged as a new class of antileishmanial compounds. The most studied antileishmanial chalcones are licochalcone A (**1**) isolated from the roots of Chinese liquorice, licochalcone C (**2**) and oxygenated chalcones **3** (19). Recently, Narender *et al.* (20,21) have described the promising antileishmanial activity of few naturally occurring chromenochalcones **4**, including crotaramosmin, crotaramin and crotin (Figure 1). These compounds contain a benzopyran system, which is frequently found in many natural products.

In the search for new synthetic chromene-based chalcones (22), we prepared two types of novel chromenochalcones **5a–e** and **6a–e**, to investigate their antileishmanial activity against promastigotes form of *Leishmania major* (Figure 1). The structure **5a–e** possessing the carbonyl group close to chromene ring is called Type A chalcones. The structure **6a–e** possessing the carbonyl group away from chromene ring is called Type B chalcones (Figure 1). The synthesis and *in vitro* antileishmanial activity of both regioisomeric chromene-based chalcones are reported in this article.

Experimental Section

All chemical and solvent used in this study were purchased from Merck AG and Aldrich. 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

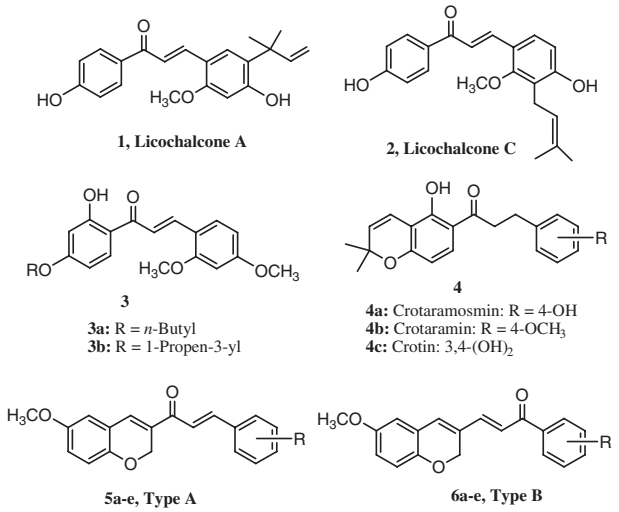


Figure 1: Structures of naturally occurring and synthetic antileishmanial chalcones **1–4** and newly designed compounds (**5** and **6**) as chromene-based antileishmanial chalcones.

disks). ^{1}H NMR spectra was recorded using a Bruker 500 MHz spectrometer and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, San Jose, CA, USA) at 70 eV. Elemental analyses were carried out on CHN-O rapid elemental analyzer (Heraeus GmbH, Hanau, 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.

Synthesis of 1-(6-methoxy-2H-chromen-3-yl) ethanone (9)

A mixture of 5-methoxy-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. It was then diluted with water and extracted several times with ether. The combined ether extracts were dried (Na_2SO_4) and evaporated to give **9** as a yellow solid, which was crystallized from ethyl acetate-hexane (23).

Synthesis of 6-methoxy-2H-chromene-3-carbaldehyde (10)

A mixture of 5-methoxy-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. It was then diluted with water and extracted several times with ether. The combined ether extracts were dried (Na_2SO_4) and evaporated to give **10** as a yellow solid, which was crystallized from ethyl acetate-hexane (23).

General procedure for the synthesis of (E)-1-(6-methoxy-2H-chromen-3-yl)-3-phenyl prop-2-en-1-one derivatives 5a–e

To a solution of compound **9** (1 mmol) and appropriate aldehyde (1 mmol) in absolute ethanol (5 mL), NaOH solution (3.5 M, 2 mL) was added 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 the corresponding chalcones **5a–e**.

(E)-1-(6-Methoxy-2H-chromen-3-yl)-3-phenylprop-2-en-1-one (5a)

IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O), 1224 (C-O). ^{1}H NMR ($CDCl_3$) δ: 7.74 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.63 (dd, 2H, J = 7.5 and 2 Hz, H2, H_6 phenyl), 7.44 (s, 1H, H_4 chromene), 7.42 (m, 3H, H_3, H_4 and H_5 phenyl), 7.38 (d, 1H, J = 15.6 Hz, H_2 propenone), 6.84 (m, 2H, H_7 and H_8 chromene), 6.75 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.08 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3). MS (m/z, %): 293 (M + 1, 38), 291 (60), 273 (38), 270 (16), 159 (38), 130 (50), 100 (98), 74 (100). Anal. Calcd for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52. Found: C, 77.87; H, 5.69.

(E)-3-(2-Chlorophenyl)-1-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (5b)

IR (KBr, cm^{-1}) ν_{max}: 1639 (C=O), 1219 (C-O). ^{1}H NMR ($CDCl_3$) δ: 8.1 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.73 (dd, 1H, J = 7 and 2 Hz, H_6 phenyl), 7.44 (m,1H, H_4 phenyl), 7.43 (s, 1H, H_4 chromene), 7.33 (d, 1H, J = 15.6 Hz, H_2 propenone),7.32 (m, 2H, H_3 and H_5 phenyl), 6.84 (m, 2H, H_7 and H_8 chromene), 6.74 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.07 (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 15), 326 (M^+, 45), 309 (40), 199 (11), 185 (100), 143 (33), 137 (94), 109 (80), 73 (46). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 70.03; H, 4.77.

(E)-3-(3-Chlorophenyl)-1-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (5c)

IR (KBr, cm^{-1}) ν_{max}: 1644 (C=O), 1229 (C-O). ^{1}H NMR ($CDCl_3$) δ: 7.81 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.73 (m, 1H, H_6 phenyl), 7.58 (m, 2H, H_2 and H_4 phenyl), 7.43 (s,1H, H_4 chromene), 7.32 (d, 1H, J = 15.6 Hz, H_2 propenone), 7.18 (m, 1H, H_5 phenyl), 6.84 (m, 2H, H_7 and H_8 chromene), 6.73 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.08 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 33), 326 (M^+, 90), 309 (50), 201 (8), 189 (14), 159 (60), 146 (43), 101 (100), 88 (30). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 69.71; H, 4.80.

(E)-3-(4-Chlorophenyl)-1-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (5d)

IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O), 1224 (C-O). ^{1}H NMR ($CDCl_3$) δ: 7.68 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.56 (d, 2H, J = 8.2 Hz, H_2 and H_6 phenyl), 7.43 (s, 1H, H_4 chromene), 7.39 (d, 2H, J = 8.2 Hz, H_3 and H_5 phenyl), 7.35 (d, 1H, J = 15.6 Hz, H_2 propenone), 6.84 (m, 2H, H_7 and H_8 chromene), 6.75 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.07 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 30), 326 (M^+, 90), 307 (42), 275 (17), 227 (8), 165 (50), 159 (63), 135 (67), 101 (100), 89 (58), 45 (50). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: 69.52; H, 4.49.

(E)-3-(2,4-Dichlorophenyl)-1-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (5e)

IR (KBr, cm^{-1}) ν_{max}: 1647 (C=O), 1222 (C-O). ^1H NMR ($CDCl_3$) δ: 8.02 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.66 (d, 2H, J = 8.4 Hz H_6 phenyl), 7.47 (d, 1H, J = 2 Hz, H_3 phenyl), 7.42 (s, 1H, H_4 chromene), 7.32 (d, 1H, J = 15.6 Hz, H_2 propenone), 7.28 (dd, 2H, J = 8.4 and 2 Hz, H_5 phenyl), 6.86 (dd, 1H, J = 8.8 and 2.5 Hz, H_7 chromene), 6.83 (d, 1H, J = 8.8 Hz, H_8 chromene), 6.74 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.07 (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3). MS (m/z, %): 364 (M + 4, 10), 362 (M + 2, 65), 361 (M^+, 21), 360 (100), 325 (58), 309 (17), 260 (8), 202 (10), 198 (35), 161 (50), 118 (62), 89 (45), 63 (20). Anal. Calcd for $C_{19}H_{14}Cl_2O_3$: C, 63.18; H, 3.91. Found: C, 62.96; H, 4.05.

General procedure for the synthesis of 3-(6-methoxy-2H-chromen-3-yl)-1-phenyl prop-2-en-1-one derivatives 6a–e

To a solution of compound **10** (1 mmol) and appropriate acetophenone (1 mmol) in absolute ethanol (5 mL), NaOH solution (3.5 M, 2 mL) was added 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 the corresponding chalcones **6a–e**.

(E)-3-(6-Methoxy-2H-chromen-3-yl)-1-phenylprop-2-en-1-one (6a)

IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O), 1229 (C-O). ^1H NMR ($CDCl_3$) δ: 7.96 (d, 2H, J = 7.5 Hz, H_2 and H_6 phenyl), 7.58 (m, 1H, H_4 phenyl), 7.52 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.50 (m, 2H, H_3 and H_5 phenyl), 6.86 (d, 1H, J = 15.6 Hz, H_2 propenone), 6.82 (s, 1H, H_4 chromene), 6.80 (d, 1H, J = 8.7 Hz, H_8 chromene), 6.76 (dd, 1H, J = 8.7 and 2.5 Hz, H_7 chromene), 6.65 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.03 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3). MS (m/z, %): 292 (M^+, 60), 277 (30), 187 (39), 142 (23), 113 (24), 105 (75), 75 (88), 45 (100). Anal. Calcd for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52. Found: 78.32; H, 5.51.

(E)-1-(2-Chlorophenyl)-3-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (6b)

IR (KBr, cm^{-1}) ν_{max}: 1659 (C=O), 1219 (C-O). ^1H NMR ($CDCl_3$) δ: 7.45 (m, 2H, H_4, H_6 phenyl), 7.41 (m, 1H, H_3 phenyl), 7.36 (m, 1H, H_5 phenyl), 7.18 (d, 1H, J = 16 Hz, H_3 propenone), 6.77 (m, 2H, H_7, H_8 chromene), 6.75 (s, 1H, H_4 chromene), 6.61 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.48 (d, 1H, J = 16 Hz, H_2 propenone), 4.97 (s, 2H, OCH_2), 3.76 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 22), 326 (M^+, 66), 311 (48), 236 (11), 201 (11), 185 (100), 141 (40), 139 (80), 109 (80), 67 (38), 55 (55). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 69.83; H, 4.70.

(E)-1-(3-Chlorophenyl)-3-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (6c)

IR (KBr, cm^{-1}) ν_{max}: 1654 (C=O), 1219 (C-O). ^1H NMR ($CDCl_3$) δ: 7.93 (s, 1H, H_2 phenyl), 7.83 (d, 1H, J = 7.75 Hz, H_4 phenyl), 7.56 (m, 1H, H_6 phenyl), 7.53 (d, 1H, J = 15.5 Hz, H_3 propenone), 7.44 (t, 1H, J = 7.75 Hz, H_5 phenyl), 6.84 (s, 1H, H_4 chromene), 6.80 (d, 1H, J = 15.5 Hz, H_2 propenone), 6.78 (m, 2H, H_7, H_8 chromene), 6.65 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.02 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 28), 326 (M^+, 84), 309 (76), 189 (11), 159 (60), 146 (45), 101 (100), 87 (28). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 69.99; H, 4.48.

(E)-1-(4-Chlorophenyl)-3-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (6d)

IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O), 1229 (C-O). ^1H NMR ($CDCl_3$) δ: 7.90 (d, 2H, J = 8.5 Hz, H_2 and H_6 phenyl), 7.52 (d, 1H, J = 15.6 Hz, H_3 propenone), 7.47 (d, 2H, J = 8.5 Hz, H_3 and H_5 phenyl), 6.83 (s,1H, H_4 chromene), 6.80 (m, 2H, H_7 and H_8 chromene), 6.79 (d, 1H, J = 15.6 Hz, H_2 propenone), 6.65 (d, 1H, J = 2.5 Hz, H_5 chromene), 5.02 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3). MS (m/z, %): 328 (M + 2, 20), 326 (M^+, 93), 323 (68), 311 (46), 308 (25), 245 (11), 202 (11), 187 (95), 184 (32), 139 (100), 111 (80), 109 (46), 75 (28). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 70.21; H, 4.49.

(E)-1-(2,4-Dichlorophenyl)-3-(6-methoxy-2H-chromen-3-yl)prop-2-en-1-one (6e)

IR (KBr, cm^{-1}) ν_{max}: 1662 (C=O), 1224 (C-O). ^1H NMR ($CDCl_3$) δ: 7.47 (d, 1H, J = 2 Hz, H_3 phenyl), 7.40 (d, 1H, J = 8 Hz, H_6 phenyl), 7.35 (dd, 1H, J = 8 and 2 Hz, H_5 phenyl), 7.19 (d, 1H, J = 16 Hz, H_3 propenone), 6.77 (m, 2H, H_7 and H_8 chromene), 6.61 (d, 1H, J = 2 Hz, H_5 chromene), 6.46 (d, 1H, J = 16 Hz, H_2 propenone), 4.96 (s, 2H, OCH_2), 3.77 (s, 3H, OCH_3). MS (m/z, %): 365 (M + 4, 7), 363 (M + 2, 42), 361 (M^+, 63), 325 (46), 279 (60), 261 (18), 206 (8), 189 (23), 187 (100), 149 (100), 113 (48), 71 (64), 57 (70). Anal. Calcd for $C_{19}H_{14}Cl_2O_3$: C, 63.18; H, 3.91. Found: C, 63.20; H, 4.04.

Biological activity

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 was washed with phosphate-buffered saline and recultured in RPMI 1640 medium (Sigma, St. Louis, MO, USA) at 2×10^6 cells/mL density, supplemented with 10% of heat-inactivated fetal bovine serum, 2-mM glutamine (Sigma), pH approximately 7.2, 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma).

In vitro *antileishmanial activity*

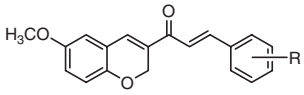
The antileishmanial evaluation of compounds **5a–e** and **6a–e** was performed using direct counting and MTT assay (24,25). The growth curve of the *L. major* strain was determined daily under light microscope and counting in a Neubauer's chamber. Then, parasites (2×10^6/mL) in the logarithmic phase were incubated with a serial range of drug concentrations for 24 h at 25 °C. To determine 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 approximately 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 (700 g × 5 min) and 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} was calculated by linear regression analysis, expressed in mean ± SD. Control cells were incubated with culture medium plus DMSO.

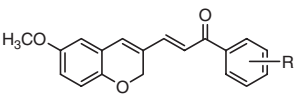
Cytotoxicity against macrophages

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 compound. 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 using MTT colorimetric assay.

Table 1: Structures and physicochemical data of chromene-based chalcones **5a–e** and **6a–e**



5a-e (Type A)



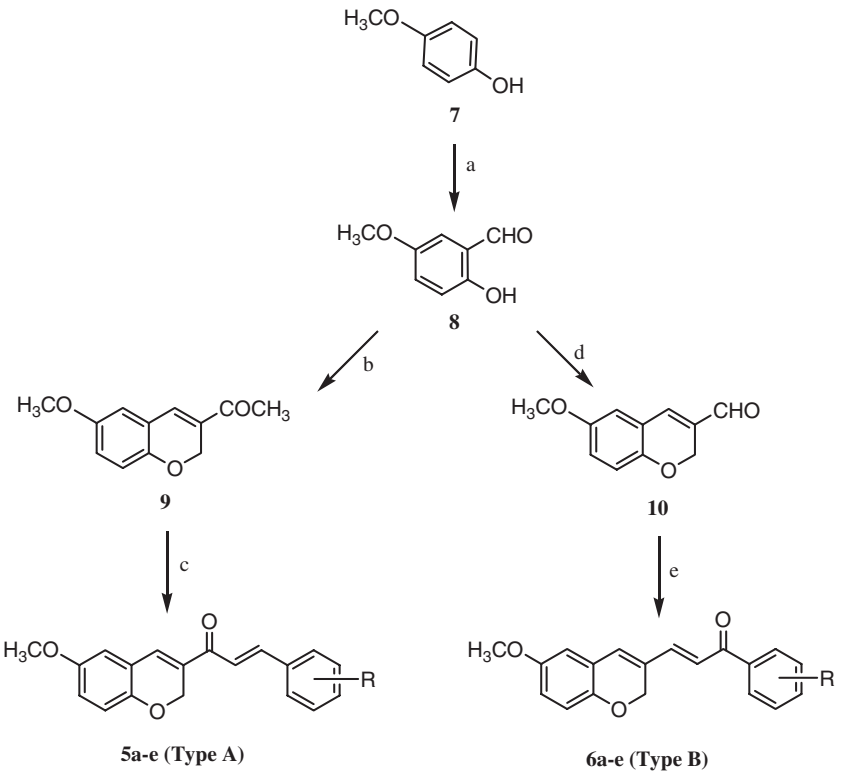
6a-e (Type B)

Compound	R	m.p. (°C)	M.W.	Yield	Formula
5a	H	73–75	292.33	76	$C_{19}H_{16}O_3$
5b	2-Cl	87–89	326.77	62	$C_{19}H_{15}ClO_3$
5c	3-Cl	54–56	326.77	59	$C_{19}H_{15}ClO_3$
5d	4-Cl	111–113	326.77	34	$C_{19}H_{15}ClO_3$
5e	2,4-Cl_2	124–125	361.22	89	$C_{19}H_{14}Cl_2O_3$
6a	H	156–158	292.33	55	$C_{19}H_{16}O_3$
6b	2-Cl	91–93	326.77	61	$C_{19}H_{15}ClO_3$
6c	3-Cl	146–148	326.77	64	$C_{19}H_{15}ClO_3$
6d	4-Cl	187–189	326.77	78	$C_{19}H_{15}ClO_3$
6e	2,4-Cl_2	137–138	361.22	97	$C_{19}H_{14}Cl_2O_3$

Results and discussion

Chemistry

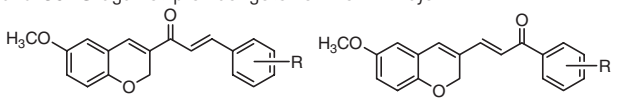
As illustrated in Scheme 1, the routes to synthesis of both target compounds **5a–e** and **6a–e** was started from phenolic



R= H; 2-Cl; 3-Cl; 4-Cl; 2,4-Cl_2

Scheme 1: Synthesis of chromene-based chalcones **5a–e** and **6a–e**. *Reagents and conditions*: (a) NaOH, $CHCl_3$, H_2O, reflux (b) methyl vinyl ketone, 1,4-dioxane, K_2CO_3, reflux (c) appropriate aldehyde, NaOH, EtOH, (d) acrolein, 1,4-dioxane, K_2CO_3, reflux (e) appropriate acetophenone, NaOH, EtOH.

Table 2: *In vitro* activities of chromene-based chalcones **5a–e** and **6a–e** against promastigote form of *L. major*



Compound	R	% Inhibition at the concentration of 10 μM[a] Day 1	Day 2	Day 3	IC_{50} (μM)[b]
5a	H	0.0	18.8	93	30 ± 0.06
5b	2-Cl	100	100	100	5 ± 0.14
5c	3-Cl	100	100	100	0.8 ± 0.26
5d	4-Cl	100	100	100	0.7 ± 0.3
5e	2,4-Cl_2	98.1	100	100	0.75 ± 0.23
6a	H	0.0	12.8	62.8	45 ± 0.15
6b	2-Cl	0.0	0.0	1.0	>50
6c	3-Cl	0.0	0.0	75.2	45 ± 0.22
6d	4-Cl	0.0	0.0	5.4	>50
6e	2,4-Cl_2	4.3	4.3	8.4	>50
Glucantime®					81.97 ± 3.85[c]

[a]The growth inhibitory effect of compounds on *Leishmania* parasite in three consecutive days.
[b]The values represent mean ± SD.
[c]IC_{50} in mM.

compound **7**. Compound **7** was converted to salicylaldehyde derivative **8** according to the general literature method (26,27). Then, compound **8** was reacted with methyl vinyl ketone, as a Michael acceptor, in refluxing dioxane in the presence of K_2CO_3 to give 3-acetylchromene **9**. Claisen-Schmidt condensation of 3-acetylchromene **9** with different aldehydes in ethanolic solution of NaOH yielded corresponding type A chalcones **5a–e**. For obtaining type B chalcones **6a–e**, a slightly different strategy was used. Treatment of salicylaldehyde **8** with acrolein in refluxing dioxane in the presence of K_2CO_3 afforded chromene-3-carbaldehyde **10**. Subsequently, condensation of aldehyde **10** with appropriate acetophenone in ethanolic solution of NaOH afforded type B chalcones **6a–e**. The structures of desired products were established with IR, NMR, mass spectrometry and elemental analysis. ^{1}H NMR spectra showed that only (*E*)-isomer of chalcones were obtained. The structures and physicochemical data of target compounds are listed in Table 1.

Antileishmanial activity

The life cycle of *Leishmania* parasites consists of two evolutionary stages: promastigotes, flagellated extracellular parasites and amastigotes, non-flagellated, non-motile stages that is more sensitive and live in macrophages. In this study, the chromene-based chalcones **5a–e** and **6a–e** were evaluated for their *in vitro* activity against the promastigote form of the *Leishmania major* using MTT assay. In primary screening assay, the *Leishmania* parasite was affected by 10 μM concentration of the synthesized compounds **5a–e** and **6a–e** for three consecutive days and the growth inhibitory effect of these compounds was monitored during Day 1, Day 2 and Day 3 and the results are reported in Table 2. The obtained results indicate that compounds **5b–e** (Type A chalcones) exhibited excellent activity against *Leishmania* (100% inhibition) at the concentration of 10 μM. Compound **5a** that showed no inhibition at the first day of exposure exhibited potent inhibitory activity after 3 days (% inhibition = 93%). In addition, remaining compounds (Type B chalcones) showed weak to moderate inhibitory activity at this level of concentration.

The IC_{50} values of type A and type B chalcones against *L. major* in comparison with meglumine antimonate (Glucantime®, Aventis, Paris, France) are presented in Table 2. The IC_{50} values of the test compounds against *L. major* indicate that most compounds possessed good leishmanicidal activity ($IC_{50} \leq 50$ μM) with respect to reference drugs. The most potent compounds against the promastigote form of *L. major* were found to be chloro-substituted Type A chalcones **5c–e** with IC_{50} values less than 1.0 μM. The activity profile of these compounds (**5c–e**) against promastigotes demonstrated that there are no significant differences in their IC_{50} values.

The effect of positional substitution was investigated by preparing all three possible chloro-substitutions (2-Cl, 3-Cl or 4-Cl) and 2,4-dichloro-substitutions on phenyl ring attached to propenone scaffold. Although chlorine substitution on phenyl ring increases the activity in Type A chalcones, (compounds **5b–e** in comparison with **5a**) this alteration in Type B compounds cannot improve antileishmanial activity (compounds **6b–e** in comparison with **6a**). The better results were achieved with 3-chloro- or 4-chloro-containing analogues in the Type A series.

The cytotoxicity of target compounds was also assessed using MTT colorimetric assay on macrophage cells. Macrophage cells were treated with synthesized compounds at the concentration equal to IC_{50} values for 24h, side by side with the reference drug Glucantime® (25). The results showed that these compounds display antileishmanial activity at non-cytotoxic concentrations.

Chemically, chalcones consist of open-chain flavonoids in which the two aromatic ring A and ring B are joined by a three-carbon α,β-unsaturated carbonyl linker. Ring A is attached to the β-position respect to the carbonyl group and ring B is aryl moiety connected to the carbonyl group. Based on previous studies on antileishmanial chalcones, ring A and its substitution pattern are generally considered less important for antileishmanial activity compared to ring B (28,29). Our results with chromene-based chalcones revealed that very good antileishmanial activity was observed when ring B is 6-methoxy-2*H*-chromen-3-yl and ring A is 3- or 4-chlorophenyl moiety (compounds **5c–e**). In this work, the mechanisms by which the chromene-based chalcones showed antileishmanial activity were not addressed but based on the literature, it can be predicted that chalcones could potentially interfere with the function of parasite mitochondria and inhibit the activity of fumarate reductase, succinate dehydrogenase, NADH dehydrogenase, or succinate- and NADH-cytochrome *c* reductases (30–32).

In conclusion, we prepared two types of regioisomeric chromene-based chalcones namely, 1-(6-methoxy-2*H*-chromen-3-yl)-3-phenylpropen-1-ones and 3-(6-methoxy-2*H*-chromen-3-yl)-1-phenylpropen-1-ones and investigated their antileishmanial activity against promastigotes

form of *Leishmania major*. The obtained results from *in vitro* biological assays indicated that chloro-substituted 1-(6-methoxy-2*H*-chromen-3-yl)-3-phenylpropen-1-ones exhibited excellent activity against *Leishmania major* at non-cytotoxic concentrations. The marked activity and simple synthesis of these chalcones suggest that they are potential leads for the development of antileishmanial compounds and further work is in progress to improve the potency of these compounds.

Acknowledgment

This work was supported by grants from the research council of Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

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