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Synthesis of chromenochalcones and evaluation of their in vitro antileishmanial activity☆

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Abstract—A large number of novel chromenochalcones were synthesized by pyridine-catalysed chromenylation of mono-chelated meta-dihydric acetophenones with the monoterpene, citral dimethyl acetal and subsequent Claisen–Schmidt condensation of the resultant acylchromenes with appropriate aromatic aldehydes. These chromenochalcones **1**–**19** were screened against in vitro extracellular promastigotes and intracellular amastigotes of *Leishmania donovani*. The most potent compound in this series was compound **9** with a pyridine ring-A, which showed 99% inhibition of promastigotes at 10 μg/ml, 82% at 0.25 μg/ml and 96% at 10 μg/ml concentration against amastigotes.

1. Introduction

Various species of the protozoan parasite *Leishmania* cause a broad spectrum of diseases ranging from cutaneous healing skin lesions caused by *L. major* to a fatal visceral form called kala azar caused by *L. donovani*.[1,2] Leishmaniasis is widespread in many parts of the world with the highest prevalence in Africa, Asia and Latin America. It is estimated that 12 million people suffer from the disease, with 400,000 new cases and 100,000 deaths each year.[3] No fewer than 550 million individuals in 88 countries, including some southern European countries, are at risk of infection.[4] Therapy to treat patients with leishmaniasis still poses a serious problem. The drugs of first choice are pentavalent antimonial compounds, which were developed before 1960,[5] and in general, require long-term treatment and have severe side effects.[4] The reported large-scale clinical resistance to antimonial agents in India and Sudan[6] has created an urgent need for the development of new, efficient and safe drugs for the treatment of the disease.[7] The discovery of new drugs from traditional medicine is not a new phenomenon. Licochalcone A (Fig. 1), isolated from Chinese licorice roots, efficiently inhibited the proliferation of *L. donovani* and *L. major* promastigotes and amastigotes in vitro by inhibiting fumarate reductase,[8] a selective target present in the parasite mitochondria. Recently, we have described the isolation[9] and synthesis[10] of few naturally occurring 2′,2′-dimethyl chromenochalcones and dihydro chromenochalcones, that is, crotaramosmin, crotaramin and crotin (Fig. 1) and evaluation of their antileishmanial activity, which were found to be promising in our studies. These compounds contain a 2′2′-di-

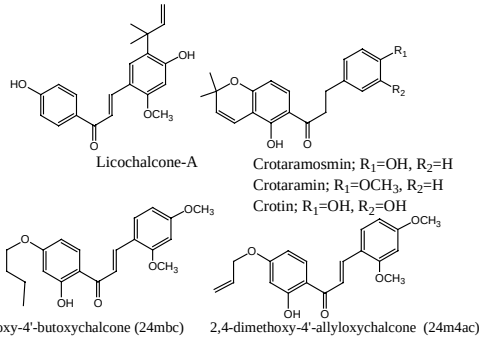

Figure 1. Few naturally occurring and synthetic antileishmanial chalcones.

Keywords: Chromenochalcones; Antileishmanial activity; Synthesis.
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methyl benzopyran system that is frequently encountered in many natural products. Some of them have been reported to exhibit significant biological activities[10] including anti-HIV, insecticidal, anti-inflammatory, etc., and appeared to be more potent antileishmanial compounds than normal chalcones.[10] Several synthetic chalcones have appeared in the literature, which act as fumarate reductase inhibitors (FRD)[11] and further studies on antileishmanial chalcones have revealed that large substitution on the 4′-hydroxyl group of ring-B, as in compounds 2,4-dimethoxy-4′-butoxychalcone (24mbc) and 2,4-dimethoxy-4′-allyloxy chalcone (24m4ac) (Fig. 1), has been shown to be more active.[11] We therefore synthesized a large number of such novel chromenochalcones and evaluated their antileishmanial activity.

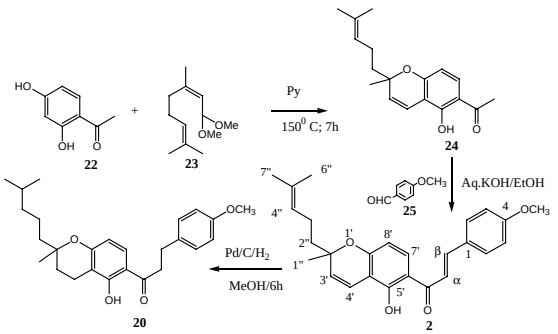
2. Synthesis

The general synthesis of chalcones involves the Claisen–Schmidt condensation of acetophenone with an appropriate aldehyde.[12] For the preparation of acyl chromenes, the pyridine-catalysed condensation between citral dimethyl acetal **23** and 2,4-dihydroxyacetophenone **22** has been exploited in which one of the two hydroxyl groups of acetophenone is engaged in chelation (Scheme 1).[13] The resultant acyl chromenes **24** were subjected to Claisen–Schmidt condensation with various substituted aromatic aldehydes to give chromenochalcones (Scheme 1).

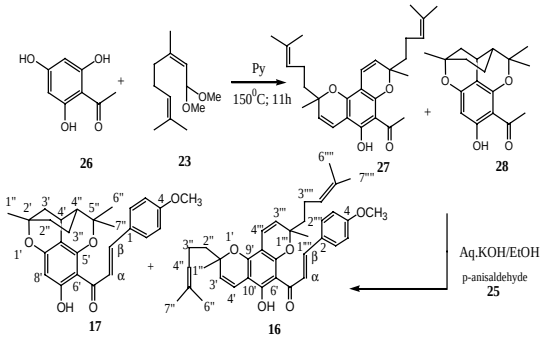
To evaluate the activity of dichromenylated chalcones, we carried out the same chromenylation reaction on 2,4,6-trihydroxy acetophenone **26** and ended up with a compound **28** with a menthylidene ring system and with the dichromene derivative **27** (Scheme 2).

3. In vitro antileishmanial activity

Chalcones, natural[14] as well as synthetic, have been reported as potential antileishmanial agents.[15] Based on our earlier findings,[10] we were prompted to synthesize various substituted chromenochalcones **1**–**19** (Table 1) to improve the antileishmanial activity. All the compounds were tested against extracellular promastigotes[16] and intracellular amastigotes[17] of *L. donavani* residing within



Scheme 1. Synthesis of chromenochalcones and chromanodihydrochalcones.



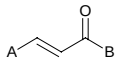
Scheme 2. Synthesis of dicromenochalcone and unusual chalcone.

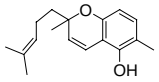
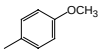
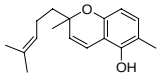
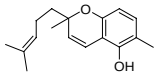
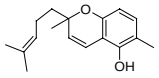
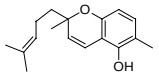
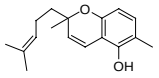
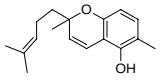
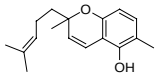
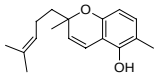
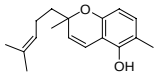
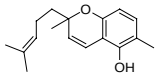
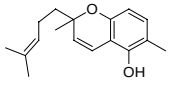
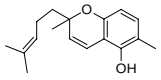
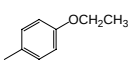
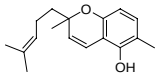
murine macrophages. These chromenochalcones have exhibited inhibition of parasites in a concentration ranging from 50 to 0.25 μg/ml. The promastigote inhibition was 12– 99% at 10 μg/ml, while the inhibition was 34–99% at 25 μg/ml against amastigotes (Table 1). The most potent compound in this series was compound **9** with a pyridine ring-A. It showed 99% inhibition at 10 μg/ml and 82% at 0.25 μg/ml concentration against promastigotes, whereas the inhibition was 96% at 10 μg/ml against amastigotes. Compound **1**, which has a hydroxyl group at the 4-position in ring-A, showed significant inhibition of parasites. The inhibition was 97% at 10 μg/ml and 66% at 5 μg/ml against promastigotes and 89% at 25 μg/ml against amastigotes. On the other hand, its dihydroderivative **19** exhibited diminished activity with 95% at 10 μg/ml and 31% inhibition at 5 μg/ml in promastigotes and 47% at 25 μg/ml in amastigotes. This revealed that unsaturation at α, β-carbons and in the chromene part played a significant role in enhancing the activity.

The O-alkylated derivatives of compound **1** viz., compounds **2** and **14** did not show any significant activity, implying the increased role of hydroxyl group in ring-A as compared to O-alkylated groups. The presence of nitrogenous substituents either at the 4-position or at the 3-position in ring-A imparted promising activity to these chromenochalcones. Compound **3** having a nitro group at the 4-position exhibited 99% at 10 μg/ml and 86% inhibition at 0.25 μg/ml against promastigotes. Compound **12** with nitro group at the 3-position also showed significant results at 10 μg/ml with 99% and 1 μg/ml with 95% inhibition against promastigotes and at 10 μg/ml with 84% inhibition against amastigotes. On the contrary, the presence of halogens in ring-A did not impart any significant activity. Compound **4** showed 86% at 10 μg/ml and no inhibition at all below this concentration in promastigotes. There was no inhibition against amastigotes, even at 50 μg/ml concentration. These findings reveal that substituent effect in ring-A is pivotal in determining the antileishmanial activity of chromenochalcones.

Ring-B in these chromenochalcones is also contributing to antileishmanial activity against the parasites. This was revealed by compound **18** in which the chromene group was absent, showing 60 and 15% inhibition at 50 μg/ml against promastigotes and amastigotes, respectively. Whereas its chromenylated derivative **2** showed 87 and 49% inhibition at the same concentration. Thus,

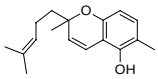
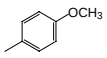
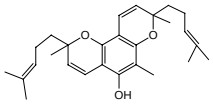
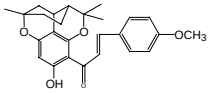
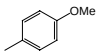
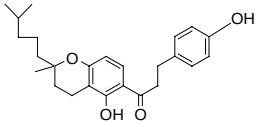
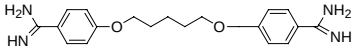
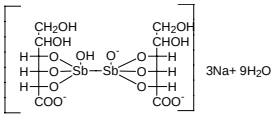
Table 1. In vitro antileishmanial activity of synthetic chromenochalcones



Compound	A	B	Concentration (μg/ml)	Promastigotes (%inhibition)	Concentration (μg/ml)	Amastigotes (%inhibition)
1			10	97	25	89
2			50	87	50	49
3			10	99	25	34
4			10	86	25	NI[a]
5			10	95	25	NI
6			10	80	25	NI
7			10	5	25	NI
8			10	20	25	NI
9			10	99	25	99
10			10	85	25	NI
11			10	91	25	NI
12			10	99	25	93
13			10	49	25	NI
14			10	16	25	NI

(*continued on next page*)

Table 1 (*continued*)

Compound	A	B	Concentration (μg/ml)	Promastigotes (%inhibition)	Concentration (μg/ml)	Amastigotes (%inhibition)
15			10	99	25	NI
16			10	12	25	NI
17			10	24	25	31
18			50	60	50	15
19			10	98	25	47
20	 Pentamidine		1	90	50	92
21	 Sodium stibogluconate		500	45	50	21

[a] NI, no inhibition.

introduction of a chromene ring with its 5-membered side chain at the 4′position in ring-B led to enhanced activity.

Dichromenochalcones viz., compounds **16** and **17** did not exhibit any significant activity, which suggests that monochromene ring is vital in determining the antileishmanial potential of these chromenochalcones.

4. Conclusion

We have synthesized few naturally occurring chalcones, chromenochalcones and synthetic chromenochalcones and investigated their antileishmanial activity to provide a scientific rationale for the antiprotozoal potency of plants used in ethnomedicine in the search of new antiprotozoal drugs. Our results disclose that chromenochalcones have potent antileishmanial activity and further work is in progress to improve the potency of these compounds.

5. Experimental

5.1. General

Melting points were recorded on a Buchi-530 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WM 200 MHz spectrometer in deuterated chloroform with TMS as internal reference. IR spectra of the compounds were recorded on a Perkin–Elmer AC-1 spectrometer using KBr pellets. FAB mass spectra of all compounds were measured with a Jeol SX 102/DA 6000 mass spectrometer using a beam of Argon (2–8 ev). Microanalysis were determined on a Carlo Erba EA-1108 element analyzer. Thin-layer chromatography was performed on Merck pre-coated silica gel plates. For column chromatography, silica gel of 60–120 mesh was used.

5.2. General procedure for the synthesis of 1-(5-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2H-chromen-6-yl) ethanone (24)

To a stirred solution of 2,4-dihydroxyacetophenone **22** (10.64 g, 0.07 mol) in pyridine (9.48 g, 0.12 mol) was added citral dimethyl acetal **23** (13.66 g, 0.035 mol) slowly. The mixture was refluxed for 4 h at 150 °C. More citral dimethyl acetal (13.66 g, 0.035 mol) was added and refluxed for further 3 h. The reaction mixture was concentrated under reduced pressure and the resultant concentrate was subjected to silica gel chromatography using hexane/ethyl acetate (94:6) system as eluent.

5.3. General procedure for the synthesis of dichromene of 2,4,6-trihydroxyacetophenone (27)

To a stirred solution of 2,4,6-trihydroxyacetophenone **26** (3.36 g, 0.02 mol) in pyridine (4.74 g, 0.06 mol) was added citral dimethyl acetal **23** (3.96 g, 0.02 mol) slowly. The mixture was refluxed for 6 h at 150 °C. More citral dimethyl acetal (1.98 g, 0.01 mol) was added and refluxed for further 5 h. The reaction mixture was concentrated under reduced pressure and the resultant concentrate was subjected to silica gel chromatography using hexane/ethyl acetate (96:4) system as eluent.

5.4. General procedure for the synthesis of chromenochalcones (2)

To a solution of chromeno/dichromeno acetophenone 24:28 (0.500 g, 0.02 mol) in aqueous potassium hydroxide (2-pellets) in ethanol (5 ml) was added the aldehyde 25 (0.04 mol). The mixture was kept at room temperature for 48 h. The resultant mixture was quenched in ice-cold water, acidified with 1 N HCl. The crude product was filtered under suction and extracted with ethyl acetate and organic layer concentrated under reduced pressure. The resultant concentrate was subjected to silica gel column chromatography using different polarity systems of hexane/ethyl acetate.

5.5. General procedure for the synthesis of nitrochromenochalcones (3, 12)

To a stirred solution of chromene of 2,4-dihydroxyacetophenone **25** (0.572 g, 0.002 mol) in THF (5 ml) was added sodium hydride (0.115 g, 0.005 mol) slowly and stirred at rt for 20 min. Then the nitro substituted aldehyde (0.302 g, 0.002 mol) in THF was added and the mixture was stirred at rt under nitrogen for 8 h. The mixture was poured into ice-cold water, acidified with 1 N HCl. Filtration, extraction with ethyl acetate and concentration under reduced pressure and silica gel column chromatography led to isolation of the desired compound. (eluent: hexane/ethyl acetate).

5.6. 1-(5-Hydroxy–2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-hydroxy-phenyl)-propenone (1)

Yield: 44%; semisolid; FAB MS 391 (M+1); IR (KBr) 3436 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR(200 MHz, $CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.08 (q, J = 8 Hz, 2H, H-3″), 5.09 (t, J = 8 Hz, 1H, H-4″), 5.53 (d, J = 10.1 Hz, 1H, H-3′), 6.37 (d, J = 8.8 Hz, 1H, H-7′), 6.79 (d, J = 10.1 Hz, 1H, H-4′), 6.88 (d, J = 8.4 Hz, 2H, H-3,5), 7.42 (d, J = 15.3 Hz, 1H, H-α), 7.53 (d, J = 8.4 Hz, 2H, H-2,6), 7.70 (d, J = 8.8 Hz, 1H, H-8′), 7.82 (d, J = 15.3 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{26}O_4$: C, 76.90; H, 6.71; O, 16.39. Found: C, 76.80; H, 6.69.

5.7. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-methoxy-phenyl)-propenone (2)

Yield: 50%; semisolid; FAB MS 405 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.67 (s, 3H, H-7″), 1.72 (t, J = 6 Hz, 2H, H-2″), 2.08 (q, J = 6 Hz, 2H, H-3″), 3.83 (s, 3H, OMe), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.52 (d, J = 10.2 Hz, 1H, H-3′), 6.35 (d, J = 8.8 Hz, 1H, H-7′), 6.78 (d, J = 10.1 Hz, 1H, H-4′), 6.90 (d, J = 8.4 Hz, 2H, H-3,5), 7.41 (d, J = 15.4 Hz, 1H, H-α), 7.58 (d, J = 8.4 Hz, 2H, H-2,6), 7.69 (d, J = 8.8 Hz, 1H, H-8′), 7.83 (d, J = 15.4 Hz, 1H, H-β). Anal. Calcd for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98; O, 15.82. Found: C, 77.40; H, 7.10.

5.8. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)- 3-(4-nitro-phenyl)-propenone (3)

Yield: 30%; mp 140 °C; FAB MS 420 (M+1); IR (KBr) 3425 cm^{-1} (OH), 1638 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 3H, H-1″), 1.56, (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.08 (q, J = 8 Hz, 2H, H-3″), 5.09 (t, J = 6 Hz, 1H, H-4″), 5.54 (d, J = 10.1 Hz, 1H, H-3′), 6.36 (d, J = 8.8 Hz, 1H, H-7′), 6.77 (d, J = 10.1 Hz, 1H, H-4′), 7.63 (d, J = 15.5 Hz, 1H, H-α), 7.69 (d, J = 8.7 Hz, 2H, H-2,6), 7.76 (d, J = 8.8 Hz, 1H, H-8′), 7.85 (d, J = 15.5 Hz, 1H, H-β), 8.26 (d, J = 8.7 Hz, 2H, H-3,5). Anal. Calcd for $C_{25}H_{25}NO_5$: C, 77.58; H, 6.01; N, 3.34; O, 19.07. Found: C, 77.62; H, 6.07; N, 3.39.

5.9. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-chloro-phenyl)-propenone (4)

Yield: 38%; mp 130 °C ; FAB MS 409 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.35 (s, 3H, H-1″), 1.49 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.72 (t, J = 6 Hz, 2H, H-2″), 2.04 (q, J = 6 Hz, 2H, H-3″), 5.01 (t, J = 6 Hz, 1H, H-4″), 5.45 (d, J = 10.1 Hz, 1H, H-3′), 6.29 (d, J = 8.7 Hz, 1H, H-7′), 6.70 (d, J = 10.1 Hz, 1H, H-4′), 7.30 (d, J = 8.2 Hz, 2H, H-3,5), 7.46 (d, J = 15.4 Hz, 1H, H-α), 7.48 (d, J = 8.2 Hz, 2H, H-2,6), 7.60 (d, J = 8.8 Hz, 1H, H-8′), 7.72 (d, J = 15.4 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{25}O_3Cl$: C, 73.43; H, 6.16; O, 11.74; Cl, 8.67. Found: C, 73.31; H, 6.18.

5.10. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-fluoro-phenyl)-propenone (5)

Yield: 37%; mp 110 °C; FAB MS 392 (M+1); IR (KBr) 3424 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.10 (q, J = 8 Hz, 2H, H-3″), 5.09 (t, J = 6 Hz, 1H, H-4″), 5.54 (d, J = 10.1 Hz, 1H, H-3′), 6.37 (d, J = 8.8 Hz, 1H, H-7′), 6.79 (d, J = 10.1 Hz, 1H, H-4′), 7.12 (t, J = 8.4 Hz, 2H, H-3,5), 7.46 (d, J = 15.4 Hz, 1H, H-α), 7.61 (d, J = 8.4 Hz, 2H, H-2,6), 7.63 (d, J = 8.8 Hz, 1H, H-8′), 7.83 (d, J = 15.4 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{25}O_3F$.

5.11. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-bromo-phenyl)-propenone (6)

Yield: 46%; mp 96 °C; FAB MS 454 (M+1); IR (KBr) 3428 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR (200 MHz,

$CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4Hz, 2H, H-2″), 2.08 (q, J = 8Hz, 2H, H-3″), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.54 (d, J = 10.1 Hz, 1H, H-3′), 6.36 (d, J = 8.8 Hz, 1H, H-7′), 6.77 (d, J = 10.1 Hz, 1H, H-4′), 7.48 (d, J = 8.8 Hz, 2H, H-2,6), 7.50 (d, J = 8.8 Hz, 2H, H-3,5), 7.52 (d, J = 15.4 Hz, 1H, H-α), 7.68 (d, J = 8.8 Hz, 1H, H-8′), 7.78 (d, J = 15.4 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{25}O_3Br$: C, 66.23; H, 5.56; Br, 17.62; O, 10.59. Found: C, 65.98; H, 5.23.

5.12. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4,4,*N*,*N*-dimethylamino-phenyl)-propenone (7)

Yield: 48%; semisolid; FAB MS 418 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.35 (s, 3H, H-1″), 1.49 (s, 3H, H-6″), 1.51 (s, 3H, H-7″), 1.50 (t, J = 6 Hz, 2H, H-2″), 2.04 (q, J = 6 Hz, 2H, H-3″), 2.96 (s, 6H, NMe_2), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.44 (d, J = 10.1 Hz, 1H, H-3′), 6.27 (d, J = 8.8 Hz, 1H, H-7′), 6.60 (d, J = 8.4 Hz, 2H, H-3,5), 6.72 (d, J = 10.1 Hz, 1H, H-4′), 7.27 (d, J = 15.6 Hz, 1H, H-α), 7.47 (d, J = 8.4 Hz, 2H, H-2,6), 7.63 (d, J = 8.8 Hz, 1H, H-8′), 7.78 (d, J = 15.6 Hz, 1H, H-β). Anal. Calcd for $C_{27}H_{31}NO_3$: C, 77.67; H, 7.48; N, 3.35; O, 11.50. Found: C, 77.50; H, 7.27; N, 3.15.

5.13. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(2,4-dichloro-phenyl)-propenone (8)

Yield: 26%; mp 76 °C; FAB MS 443 (M+1); IR (KBr) 3433 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.68 (s, 3H, H-7″), 1.73 (t, J = 6Hz, 2H, H-2″), 2.08 (q, J = 6 Hz, 2H, H-3″), 5.07 (t, J = 6 Hz, 1H, H-4″), 5.54 (d, J = 10.1 Hz, 1H, H-3′), 6.36 (d, J = 8.9 Hz, 1H, H-7′), 6.79 (d, J = 10.1 Hz, 1H, H-4′), 7.29 (dd, J = 8.2, 1.8 Hz, 1H, H-5), 7.46 (d, J = 1.8 Hz, 1H, H-3), 7.50 (d, J = 15.4 Hz, 1H, H-α), 7.63 (d, J = 8.9 Hz, 1H, H-8′), 7.68 (d, J = 8.2 Hz, 1H, H-6), 8.16 (d, J = 15.4 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{24}O_3Cl_2$: C, 67.73; H, 5.46; Cl, 15.99; O, 10.83. Found: C, 67.40; H, 5.18.

5.14. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(3-pridyl)-propenone (9)

Yield: 46%; semisolid; FAB MS *m*/*z* 376 (M+1); IR (KBr) 3425 cm^{-1} (OH), 1638 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 3H, H-1″), 1.15 (s, 3H, H-6″), 1.36 (s, 3H, H-7″), 1.49 (t, J = 5 Hz, 2H, H-2″), 2.05 (q, J = 8 Hz, 2H, H-3″), 5.01 (t, J = 8 Hz, 1H, H-4″), 5.46 (d, J = 10.1 Hz, 1H, H-3′), 6.31 (d, J = 8.8 Hz, 1H, H-7′), 6.71 (d, J = 10.1 Hz, 1H, H-4′), 7.29 (dd, J = 7.7 Hz, 1H, H-5), 7.54 (d, J = 15.6 Hz, 1H, H-α), 7.61 (d, J = 8.8 Hz, 1H, H-8′), 7.77 (d, J = 15.5 Hz, 1H, H-β), 7.87 (d, J = 7.4 Hz, 1H, H-6), 8.54 (d, J = 3.4 Hz, 1H, H-4), 8.79 (s, 1H, H-2). Anal. Calcd for $C_{24}H_{25}NO_3$: C, 76.77; H, 6.71; N, 3.73; O, 12.78. Found: C, 76.82; H, 6.76; N, 3.79.

5.15. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(3,5-dichloro-phenyl)-propenone (10)

Yield: 26%; mp 92–95 °C; FAB MS 443 (M+1); IR (KBr) 3429 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.08 (q, J = 8 Hz, 2H, H-3″), 5.07 (t, J = 6 Hz, 1H, H-4″), 5.53 (d, J = 10.1 Hz, 1H, H-3′), 6.37 (d, J = 8.8 Hz, 1H, H-7′), 6.77 (d, J = 10.1 Hz, 1H, H-4′), 7.41 (d, J = 1.6 Hz, 1H, H-6), 7.44 (d, J = 1.6 Hz, H-2), 7.50 (d, J = 15.0 Hz, 1H, H-α), 7.68 (d, J = 8.3 Hz, 1H, H-8′), 7.74 (d, J = 15.0 Hz, 1H, H-β). Anal. Calcd for $C_{25}H_{24}O_3Cl_2$: C, 67.73; H, 5.46; Cl, 15.99; O, 10.83. Found: C, 67.64; H, 5.20.

5.16. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(3,4,5-trimethoxy-phenyl)-propenone (11)

Yield: 30%; semisolid; FAB MS 465 (M+1); IR (KBr) 3424 cm^{-1} (OH), 1638 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.57 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.12 (q, J = 6 Hz, 2H, H-3″), 3.91 (s, 9H, OMe), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.54 (d, J = 10.1 Hz, 1H, H-3′), 6.38 (d, J = 8.8 Hz, 1H, H-7′), 6.80 (d, J = 10.1 Hz, 1H, H-4′), 6.86 (s, 1H, H-2), 7.26 (s, 1H, H-6), 7.41 (d, J = 15.3 Hz, 1H, H-α), 7.72 (d, J = 8.8 Hz, 1H, H-8′), 7.79 (d, J = 15.3 Hz, 1H, H-β). Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.40; H, 6.98.

5.17. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(3-nitro-phenyl)-propenone (12)

Yield: 30%; mp: 115–118 °C MS 420 (M+1); IR (KBr) 3430 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 3H, H-1″), 1.49 (s, 3H, H-6″), 1.58 (s, 3H, H-7″), 1.68 (t, J = 4 Hz, 2H, H-2″), 2.03 (q, J = 8 Hz, 2H, H-3″), 5.01 (t, J = 8 Hz, 1H, H-4″), 5.47 (d, J = 10.1 Hz, 1H, H-3′), 6.33 (d, J = 8.9 Hz, 1H, H-7′), 6.70 (d, J = 10.1 Hz, 1H, H-4′), 7.58 (d, J = 15.4 Hz, 1H, H-α), 7.60 (d, J = 8.9 Hz, 1H, H-8′), 7.64 (dd, J = 5.9 Hz, 1H, H-5), 7.80 (d, J = 15.4 Hz, 1H, H-β), 8.18 (dd, J = 9.9, 1.9 Hz, 1H, H-6) (d, J = 8.1 Hz, 1H, H-4), 8.43 (s, 1H, H-2). Anal. Calcd for $C_{25}H_{25}NO_5$: C, 77.58; H, 6.01; N, 3.34; O, 19.07. Found: C, 77.64; H, 6.05; N, 3.39.

5.18. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(2-methoxy-phenyl)-propenone (13)

Yield: 41%; semisolid; FAB MS 405 (M+1); IR (KBr) 3428 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) 1.43 (s, 3H, H-1″), 1.57 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.12 (q, J = 8 Hz, 2H, H-3″), 3.92 (s, 3H, OMe), 5.08 (t, J = 8 Hz, 1H, H-4″), 5.53 (d, J = 10.1 Hz, 1H, H-3′), 6.36 (d, J = 8.8 Hz, 1H, H-7′), 6.80 (d, J = 10.1 Hz, 1H, H-4′), 6.97 (dd, J = 8.6 Hz, 2H, H-2,6), 7.37 (d, J = 15.5 Hz, 1H, H-α), 7.64 (dd, J = 6, 10 Hz, 2H, H-3,5), 7.70 (d, J = 8.6 Hz, 1H, H-8′), 8.18

(d, J = 15.5 Hz, 1H, H-β). Anal. Calcd for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98; O, 15.82. Found: C, 77.30; H, 7.05.

5.19. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-ethoxy-phenyl)-propenone (14)

Yield: 29%; semisolid; FAB MS 419 (M+1); IR (KBr) 3434 cm^{-1} (OH), 1636 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.67 (s, 3H, H-7″), 1.71 (t, J = 8 Hz, 3H, OCH_2CH_3), 1.77 (t, J = 4 Hz, 2H, H-2″), 2.08 (q, J = 8 Hz, 2H, H-3″), 4.05 (q, J = 8 Hz, 2H, OCH_2CH_3), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.52 (d, J = 10.1 Hz, 1H, H-3′), 6.35 (d, J = 8.8 Hz, 1H, H-7′), 6.78 (d, J = 10.1 Hz, 1H, H-4′), 6.89 (d, J = 8.6 Hz, 2H, H-3,5), 7.40 (d, J = 14.9 Hz, 1H, H-α), 7.56 (d, J = 8.8 Hz, 2H, H-2,6), 7.69 (d, J = 8.6 Hz, 1H, H-8′), 7.83 (d, J = 14.9 Hz, 1H, H-β). Anal. Calcd for $C_{27}H_{30}O_4$: C, 77.48; H, 7.22; O, 15.29. Found: C, 77.2; H, 7.02.

5.20. 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)2H-chromen-6-yl)-3-(4-cyano-phenyl)-propenone (15)

Yield: 29%; mp 130 °C; FAB MS 400 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 3H, H-1″), 1.56 (s, 3H, H-6″), 1.65 (s, 3H, H-7″), 1.73 (t, J = 4 Hz, 2H, H-2″), 2.08 (q, J = 8 Hz, 2H, H-3″), 5.08 (t, J = 6 Hz, 1H, H-4″), 5.55 (d, J = 10.1 Hz, 1H, H-3′), 6.38 (d, J = 8.8 Hz, 1H, H-7′), 6.77 (d, J = 10.1 Hz, 1H, H-4′), 7.61 (d, J = 15.4 Hz, 1H, H-α), 7.65 (d, J = 7.6 Hz, 2H, H-3, 5), 7.67 (d, J = 7.6 Hz, 2H, H-2,6), 7.82 (d, J = 15.4, 1H, H-β) Anal. Calcd for $C_{26}H_{25}NO_3$: C, 78.17; H, 6.31; N, 3.51; O, 12.02. Found: C, 78.12; H, 6.17; N, 3.39.

5.21. 1-(5-Hydroxy-2,8-dimethyl-2,8-bis-(4-methylpent-3-enyl)-2H,8H-pyrano(2,3-f)chromen-6-yl)-3-(4-methoxy-phenyl)-propenone (16)

Yield: 51%; semisolid; FAB MS; 555 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1636 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 3H, H-1″), 1.49 (s, 6H, H-6″6‴), 1.57 (s, 3H, H-1‴), 1.64 (s, 6H, H-7″,7‴), 1.83 (t, J = 4 Hz, 4H, H-2″,2‴), 2.08 (q, J = 8 Hz, 4H, 3″,3‴), 3.85 (s, 3H, OMe), 5.09 (t, J = 6 Hz, 2H, H-4″,4‴), 5.40 (d, J = 10.1 Hz, 1H, H-3′), 5.44 (d, J = 10.1 Hz, 1H, H-3‴), 6.65 (d, J = 10.1Hz, 1H, H-4′), 6.73 (d, J = 10.1 Hz, 1H, H-4‴), 6.91 (d, J = 8.8 Hz, 2H, H-3,5), 7.54 (d, J = 8.8 Hz, 2H, H-2,6), 7.75 (d, J = 15.6 Hz, 1H, H-α), 7.98 (d, J = 15.6 Hz, 1H, H-β). Anal. Calcd for $C_{36}H_{42}O_5$: C, 77.95; H, 7.63; O, 14.42. Found: C, 77.90; H, 7.58.

5.22. Methylidene ring derivative (17)

Yield: 53%; mp 190–192 °C; FAB MS m/z 421 (M+1); IR (KBr) 3435 cm^{-1} (OH), 1635 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 1.06 (s, 3H, H-6″), 1.25–1.32 (m, 2H, H-3″), 1.39 (s, 3H, H-7″), 1.44 (m, 1H, H-5″), 1.65 (s, 3H, H-1″), 1.89–1.90 (m, 2H, H-2″), 2.12–2.17 (m, 2H, H-3′), 2.77 (t, 1H, H-5′), 3.84 (s, 3H, OMe), 6.09 (s, 1H, ArH), 6.93 (d, J = 8.8 Hz, 2H, H-3,5), 7.57 (d, J = 8.8 Hz, 2H, H-2,6), 7.75 (d, J = 15.6 Hz, 1H, H-α), 8.14 (d, J = 15.6 Hz, 1H, H-β).

5.23. 1-(2,4-dihydroxy-phenyl)-3-(4-methoxy-phenyl)-propenone (18)

Yield: 14%; FAB MS 271 (M+1); IR (KBr) 3436 cm^{-1} (OH), 1640 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 3.87 (s, OMe, 3H), 6.27 (d, J = 2Hz, 1H, H-3′), 6.39 (dd, J = 8.6, 2 Hz, 1H, H-5′), 6.83 (d, J = 8.4 Hz, 2H, H-3,5), 7.41 (d, J = 15.4 Hz, 1H, H-α), 7.62 (d, J = 8.4 Hz, 2H, H-2,6), 7.73 (d, J = 15.4 Hz, 1H, H-β), 8.14 (d, J = 8.6 Hz, 1H, H-6′). Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22; O, 23.68.

5.24. 1-(5-Hydroxy-2-methyl-2-(4-methylpentanyl)2H-chroman-6-yl)-3-(4-hydroxy-phenyl)-propenone (19)

Yield: 73%; semisolid; FAB MS 397 (M+1); IR (KBr) 3436 cm^{-1} (OH), 1650 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) δ 0.77 (s, 3H, H-6″), 0.88 (s, 3H, H-7″), 1.17 (m, 2H, H-3″), 1.19 (m, 2H, H-4″), 1.21 (s, 3H, H-1″), 1.43–152 (m, 3H, H-2″,5″), 1.72 (t, J = 6.02 Hz, 2H, H-3′), 2.58 (t, J = 6.3 Hz, 2H, H-4′), 2.90 (t, J = 6.8 Hz, 2H, H-β), 3.07 (t, J = 8 Hz, 2H, H-α), 6.23 (d, J = 10 Hz, 1H, H-8′), 6.68 (d, J = 8.2 Hz, 2H, H-3,5), 7.00 (d, J = 8.1 Hz, 2H, H-2,6), 7.41 (d, J = 8.5 Hz, 1H, H-7′). Anal. Calcd for $C_{25}H_{32}O_4$: C, 75.73; H, 8.13; O, 16.14. Found: C, 75.80; H, 8.20.

5.25. Procedure for in vitro antileishmanial screening

5.25.1. Antipromastigote activity. Luciferase transfected *L. donovani* promastigotes (MHOM/IN/80/Dd-8, obtained from Imperial College, London), which are more stable under the influence of G 418,[16] were maintained at 25 ± 1 °C in medium 199 (Sigma Chemical, USA) supplemented with 10% foetal calf serum (Gibco). The in vitro effect of compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 μl medium 199/well in 96-well flat-bottomed microtitre (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (0.25–10 μg/ml) in DMSO.[16] Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μl) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo$^{(R)}$ reagent (Promega) and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU).

$$\text{Percentage inhibition} = N\text{-}n/\text{NX}100,$$

where N is average relative luminescence unit (RLU) of control wells; n is average RLU of treated wells.

5.25.2. Antiamastigotes activity. For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing luciferase firefly reporter gene

was used.[17] Cells were seeded in a 96-well plate (5×10^4 cells/100 μl/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (2.5×10^5/100 μl/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test material in appropriate concentrations (50 and 10 μg/ml) in complete medium was added after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 24 h or more. After incubation, the drug-containing medium was decanted and 50 μl PBS was added in each well and mixed with an equal volume of steady Glo reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer.

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