Natural Chromenes and Chromene Derivatives as Potential Anti-trypanosomal Agents

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The aim of the study was to investigate the anti-trypanocidal activities of natural chromene and chromene derivatives. Five chromenes were isolated from *Piper gaudichaudianum* and *P. aduncum*, and a further seven derivatives were prepared using standard reduction, methylation and acetylation procedures. These compounds were assayed *in vitro* against epimastigote forms of *Trypanosoma cruzi*, the causative agent of Chagas disease. The results showed that the most of the compounds, especially those possessing electron-donating groups as substituents on the aromatic ring, showed potent trypanocidal activity. The most active compound, [(2*S*)-methyl-2-methyl-8-(3″-methylbut-2″-enyl)-2-(4′-methylpent-3′-enyl)-2*H*-chromene-6-carboxylate], was almost four times more potent than benznidazole (the positive control) and showed an IC_{50} of 2.82 μM. The results reveal that chromenes exhibit significant anti-trypanocidal activities and indicate that this class of natural product should be considered further in the development of new and more potent drugs for use in the treatment of Chagas disease.

Key words chromene; *Trypanosoma cruzi*; *Piper aduncum*; *Piper gaudichaudianum*; Piperaceae

The flagellate protozoan *Trypanosoma cruzi*, the etiological agent of Chagas disease, affects more than 18 million people in Latin America and is responsible for approximately 400000 deaths per year.[1] Only two drugs are commercially available for the treatment of this disease, namely, nifurtimox (a 2-nitrofuran derivative) and benznidazole (a 2-nitroimidazole acetamide). These drugs are, however, not consistently effective and, moreover, exhibit serious side effects including cardiac and/or renal toxicity.[2] There is thus particular interest in the discovery of natural products that might be developed to generate safer and more efficient chemotherapeutic agents against *T. cruzi*.[3]

A number of biologically-active chromenes have been isolated from species of the genus *Piper*, including the prenylated chromene **1** from *Piper gaudichaudianum*[4] and chromenes **2**—**5** (Fig. 1) from *P. aduncum*.[5] Whilst these chromenes have been shown to exhibit anti-fungal[4] and anti-tumour properties,[5] no evaluation has yet been made with respect to their activity against *T. cruzi*. The object of the present study was to examine a range of natural chromenes and chromene derivatives in order to determine their potential for further development to treat Chagas disease.

METHODS AND RESULTS

General All reagents were of analytical grade. For methylation reactions, an ethereal solution of diazomethane (30 ml) was prepared by dissolving 2.14 g of *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (Diazald; Aldrich, Steinheim, Germany) in 10.0 ml of ethanol containing 4.0% potassium hydroxide.[6] Hydrogenation reactions were carried out under an atmosphere of hydrogen in the presence of palladium (3.0 or 10.0%) on activated charcoal (Acros Organics, New Jersey, U.S.A.) as catalyst.[7] The acetylated chromene was prepared by treatment with acetic anhydride (20.0 ml) and pyridine (20.0 ml) overnight.[8] Benznidazole, employed as positive control in the assays of trypanocidal activity, was obtained from Roche (Rio de Janeiro, Brazil).

Plant Material Specimens of *P. gaudichaudianum* and *P. aduncum* were cultivated from seed under greenhouse conditions at the Institute of Chemistry, UNESP, Araraquara-SP, Brazil. Plant material was authenticated by Dr. Guillermo E. D. Paredes (Universidad Pedro Ruiz Gallo, Lambayeque, Peru) and voucher specimens (with codes Kato 0093 and 0057, respectively) were deposited at the Herbarium of the

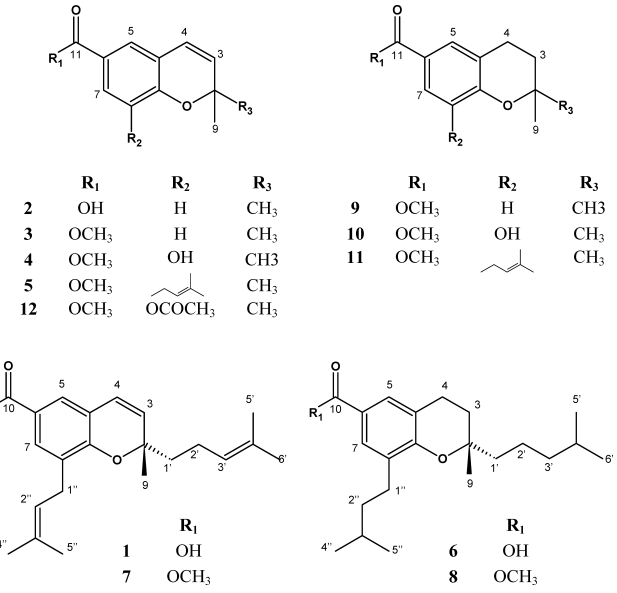

Fig. 1. Structures of Chromenes Isolated from *Piper gaudichaudianum* (**1**), *P. aduncum* (**2**—**5**) and Chromene Derivatives (**6**—**12**)

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Isolation of Chromenes and Preparation of Derivatives Compounds **1** and **2**—**5** (Fig. 1) were isolated from stems of *Piper gaudichaudianum* and leaves of *P. aduncum*, respectively, according to previously published methods.[4,5] Various reduced, methylated and acetylated derivatives of chromenes **1** and **3**—**5** (Fig. 1) were prepared using standard methods, under room temperature.[6—8] Compound **6** was obtained from **1** (CH_2Cl_2, Pd/C–10%, H_2, 12—16 h; yielding 90%), **7** was prepared from **1** (CH_2Cl_2, CH_2N_2, 5 min; 98%), **8** was prepared from **7** (CH_2Cl_2, Pd/C–10%, H_2, 12—16 h; 90%), **9** was obtained from **3** (CH_2Cl_2, Pd/C–10%, H_2, 12—16 h; 90%), **10** was obtained from **4** (CH_2Cl_2, Pd/C–10%, H_2, 12—16 h; 90%), **12** was prepared from **4** (Py, Ac_2O, overnight; 85%) and **11** was prepared from **5** (CH_2Cl_2, Pd/C–3%, H_2, 2 h; 95%).

Characterization of Chromenes and Derivatives The structures of all isolates and their derivatives were confirmed from the NMR data as shown below. Assignments were based on 2D-NMR experiments including *g*HMQC and *g*HMBC. The spectral data of the natural products (2*S*)-2-methyl-8-(3″-methylbut-2″-enyl)-2-(4′-methylpent-3′-enyl)-2*H*-chromene-6-carboxylic acid (**1**),[4] 2,2-dimethyl-2*H*-chromene-6-carboxylic acid (**2**),[5] methyl-2,2-dimethyl-2*H*-chromene-6-carboxylate (**3**),[9] methyl-8-hydroxy-2,2-dimethyl-2*H*-chromene-6-carboxylate (**4**),[10] methyl-2,2-dimethyl-8-(3′-methylbut-2′-enyl)-2*H*-chromene-6-carboxylate (**5**),[11] were compared with literature values and were consistent with the structure described. The spectral data of the derivatives obtained, as described in Experimental, were described below.

(2*S*)-8-Isopentyl-2-methyl-2-(4′-methylpentyl)chroman-6-carboxylic acid (**6**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.79 (1H, m, H-3), 1.85 (1H, m, H-3), 2.80 (2H, dd, J=14.0, 8.0 Hz, H-4), 7.71 (2H, s, H-5, H-7), 1.26 (3H, s, H-9), 2.59 (2H, m, H-1′), 1.46 (2H, m, H-2′), 1.22 (2H, m, H-3′), 1.46 (1H, m, H-4′), 0.90 (6H, d, J=6.5 Hz, H-5′, H-6′), 2.37 (2H, t, J=7.0 Hz, H-1″), 1.46 (2H, m, H-2″), 1.58 (1H, m, H-3″), 0.95 (6H, d, J=6.5 Hz, H-4″, H-5″). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 77.3 (C-2), 34.0 (C-3), 22.2 (C-4), 119.5 (C-4a), 129.6 (C-5, C-7), 131.4 (C-6), 129.9 (C-8), 156.9 (C-8a), 23.9 (C-9), 40.5 (C-1′), 39.0 (C-2′), 39.3 (C-3′), 27.9 (C-4′), 22.6 (C-5′, C-6′), 34.0 (C-1″), 21.2 (C-2″), 40.5 (C-3″), 22.5 (C-4″, C-5″), 172.0 (C-10).

(2*S*)-Methyl-2-methyl-8-(3″-methylbut-2″-enyl)-2-(4′-methylpent-3′-enyl)-2*H*-chromene-6-carboxylate (**7**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.50 (1H, d, J=10.0 Hz, H-3), 6.30 (1H, d, J=10.0 Hz, H-4), 7.59 (1H, d, J=2.0 Hz, H-7), 7.44 (1H, d, J=2.1 Hz, H-5), 1.32 (3H, s, H-9), 1.70 (2H, m, H-1′), 2.03 (2H, m, H-2′), 5.03 (1H, m, H-3′), 1.48 (3H, s, H-5′), 1.58 (3H, s, H-6′), 3.21 (2H, d, J=7.0 Hz, H-1″), 5.21 (1H, m, H-2″), 1.65 (6H, s, H-4″, H-5″), 3.78 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 79.7 (C-2), 129.4 (C-3), 122.0 (C-4), 120.1 (C-4a), 126.0 (C-5), 121.7 (C-6), 131.1 (C-7), 128.8 (C-8), 155.0 (C-8a), 27.0 (C-9), 167.1 (C-10), 41.8 (C-1′), 22.7 (C-2′), 123.9 (C-3′) 131.7 (C-4′) 17.5 (C-5′), 25.7 (C-6′), 28.2 (C-1″), 122.6 (C-2″), 132.4 (C-3″), 25.7 (C-4″), 17.8 (C-5″), 51.7 (OCH_3).

(2*S*)-Methyl-8-isopentyl-2-methyl-2-(4′-methylpentyl)chroman-6-carboxylate (**8**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.68 (1H, m, H-3), 1.75 (1H, m, H-3), 2.71 (2H, dd, J=14.0, 8.5 Hz, H-4), 7.56 (1H, d, J=2.0 Hz, H-7), 7.55 (1H, d, J=2.0 Hz, H-5), 1.18 (3H, s, H-9), 2.49 (2H, m, H-1′), 1.37 (2H, m, H-2′), 1.12 (2H, m, H-3′), 1.51 (1H, m, H-4′), 0.81 (6H, d, J=6.5 Hz, H-5′, H-6′), 2.49 (2H, t, J=2.0 Hz, H-1″), 1.36 (2H, m, H-2″), 1.51 (1H, m, H-3″), 0.87 (6H, d, J=6.5 Hz, H-4″, H-5″), 3.78 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 77.1 (C-2), 30.8 (C-3), 22.2 (C-4), 120.5 (C-4a), 129.1 (C-5, C-7), 131.2 (C-6), 131.4 (C-8), 156.1 (C-8a), 23.9 (C-9), 40.5 (C-1′), 39.0 (C-2′), 39.4 (C-3′), 27.9 (C-4′), 22.6 (C-5′, C-6′), 27.9 (C-1″), 21.2 (C-2″), 40.5 (C-3″), 22.5 (C-4″, C-5″), 167.4 (C-10), 51.6 (OCH_3).

Methyl-2,2-dimethylchroman-6-carboxylate (**9**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.76 (2H, t, J=6.5 Hz, H-4), 1.77 (2H, t, J=6.5 Hz, H-3), 7.79 (1H, d, J=2.0 Hz, H-5), 7.76 (1H, dd, J=8.5, 2.0 Hz, H-7), 6.75 (1H, d, J=8.5 Hz, H-8), 1.37 (6H, s, H-9, H-10), 3.79 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 77.3 (C-2), 32.7 (C-3), 21.9 (C-4), 120.6 (C-4a), 128.7 (C-5), 122.5 (C-6), 131.0 (C-7), 116.1 (C-8), 157.1 (C-8a), 26.3 (C-9, C-10), 166.8 (C-11), 51.7 (OCH_3).

Methyl-8-hydroxy-2,2-dimethylchroman-6-carboxylate (**10**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.82 (2H, t, J=6.5 Hz, H-4), 1.87 (2H, t, J=6.5 Hz, H-3), 7.43 (1H, d, J=2.0 Hz, H-5), 7.42 (1H, d, J=2.0 Hz, H-7), 1.40 (6H, s, H-9, H-10), 3.88 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 76.6 (C-2), 21.9 (C-3), 32.7 (C-4), 120.6 (C-4a), 121.6 (C-5), 122.7 (C-6), 113.1 (C-7), 145.2 (C-8), 144.8 (C-8a), 26.8 (C-9, C-10), 167.1 (C-11), 51.8 (OCH_3).

Methyl-2,2-dimethyl-8-(3′-methylbut-2′-enyl)chroman-6-carboxylate (**11**): Pale yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.72 (1H, t, J=6.5 Hz, H-4), 1.74 (1H, t, J=6.5 Hz, H-3), 7.56 (2H, s, H-5, H-7), 3.20 (2H, d, J=7.5 Hz, H-1′), 5.20 (1H, m, H-2′), 1.65 (6H, s, H-4′, H-5′), 1.27 (6H, s, H-9, H-10), 3.78 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 75.1 (C-2), 22.5 (C-3), 32.5 (C-4), 120.7 (C-4a), 128.9 (C-5), 132.2 (C-6), 129.1 (C-7), 131.2 (C-8), 156.1 (C-8a), 26.9 (C-9, C-10), 28.5 (C-1′), 122.3 (C-2′), 27.0 (C-3′), 25.7 (C-4′), 17.8 (C-5′), 167.4 (C-11), 51.6 (OCH_3).

Methyl-8-(ethanoyloxy)-2,2-dimethyl-2*H*-chromene-6-carboxylate (**12**): Amorphous solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.61 (1H, d, J=10 Hz, H-3), 6.29 (1H, d, J=10 Hz, H-4), 7.49 (1H, d, J=2.0 Hz, H-7), 7.51 (1H, d, J=2.0 Hz, H-5), 1.36 (6H, s, H-9, H-10), 2.23 (3H, s, H-13), 3.79 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 78.3 (C-2), 131.4 (C-3), 121.4 (C-4), 122.1 (C-4a), 124.1 (C-5), 138.5 (C-6), 125.4 (C-7), 148.8 (C-8), 148,9 (C-8a), 28.3 (C-9, C-10), 166.1 (C-11), 51.9 (OCH_3), 33.5 (CH_3), 168.5 (C=O).

Evaluation of Trypanocidal Activity Assays of the antiparasitic activities of the isolates and their derivatives were conducted by screening their effects on the proliferation of epimastigotes of *T. cruzi* Y-strain grown in axenic culture in LIT medium. Stock solutions (containing 1.0 mg/ml) of the natural chromenes and their derivatives were prepared in dimethylsulphoxide and serially diluted (1 : 3) in liver infusion tryptose medium to yield sample solutions with concentrations of 0.41, 1.23, 3.70, 11.1, 33.3, 100.0 and 300.0 μg/ml. The number of remaining viable protozoa was established by counting in a Neubauer chamber following incubation at 28 °C for 72 h with the test sample. All assays were conducted in triplicate. The 50% inhibitory concentration (IC_{50}) values were determined by linear regression analysis. The IC_{50} values for compounds **1**—**12** and for benznidazole (em-

ployed as positive control) are shown in Table 1. For the statistical analysis, method of probits was employed.

DISCUSSION

As part of an on-going screening program for new anti-chagasic drugs, the prenylated chromene [(2*S*)-2-methyl-8-(3″-methylbut-2″-enyl)-2-(4′-methylpent-3′-enyl)-2*H*-chromene-6-carboxylic acid] (**1**), the major metabolite of the ethanolic extract of *Piper gaudichaudianum*,[4] was evaluated with respect to its toxicity against epimastigotes of *T. cruzi* and exhibited a dose-dependent effect with an IC_{50} value of 33.8 μM. On the basis of this finding, the natural benzopyrans **2**—**5** (isolated from *P. aduncum*) were assayed for trypanocidal activity together with some novel chromene derivatives obtained from **1**, **3**, **4** and **5** by simple chemical transformations. Specifically, hydrogenation[7] of the isoprene units and of the double bond at C-3 of the pyran ring of **1** produced the derivative **6**. The benzopyran ester **7** was obtained from **1** by methylation with diazomethane,[6] and this ester was subsequently reduced to **8** by catalytic hydrogenation. Similarly, derivatives **9** and **10** were obtained from chromenes **3** and **4**, respectively, whilst derivative **11** was obtained by selective hydrogenation of **5**. Finally the chromene **4** was acetylated to **12**.

Most of the isolates together with their derivatives showed significant trypanocidal activities in comparison with the positive control benznidazole (IC_{50} of 10.3 μM). Compound **7** proved to be the most active with an IC_{50} value of 2.82 μM, *i.e.* almost four times more potent than benznidazole.

All compounds that showed activity possessed electron-donating substituents. Within this group the prenylated compounds **1**, **5**, **7** and **11** were the most active, whilst the ester derivative **7** (containing two prenyl units) was an extremely powerful trypanocidal agent with a potency *ca.* 10-fold greater than that of the corresponding acid. This finding suggests that both prenyl and ester substituents are necessary for the strongest anti-parasitic activity. The lower activity exhibited by chromene **2** compared with **3** corroborates the importance of an ester group, and indicates a direct correlation between the electronic nature of the substituents and trypanocidal activity. Moreover, the observed reduction in activity of **12** occasioned by the introduction of an electron-withdrawing acetyl group at C-8 following acetylation[8] of **4**, substantiates the influence of the electronic nature of the substituents (Table 1).

Reduction of the double bonds of the prenyl substituents (as in derivatives **6** and **8**) gave rise to a significant decrease in toxicity indicating their essential role in trypanocidal activity. Furthermore, reduction of the double bond at C-3 of the pyran ring (as in derivatives **9** and **10**) resulted in the total loss of toxicity suggesting that a double bond conjugated with the aromatic ring is essential for trypanocidal activity. These results suggest that substituents on the aromatic ring that contribute to an increase in lipophilicity are also required for activity (Table 1).

Table 1. Inhibition of Epimastigote Forms of *T. cruzi* by Chromene **1** from *Piper gaudichaudianum*, Chromenes **2**—**5** from *P. aduncum*, and Chromene Derivatives **6**—**12**

Compounds	IC_{50} (μM)
1	33.8
2	558.3
3	190.1
4	44.8
5	33.2
6	NA[a]
7	2.82
8	NA
9	NA
10	230.7
11	22.5
12	128.5
Benznidazole[b]	10.3

a) NA=not active; *b*) positive control.

In conclusion, several natural chromenes from *P. gaudichaudianum* and *P. aduncum*, along with a series of semi-synthetic derivatives, have been shown to exhibit potential trypanocidal activities against epimastigotes of *T. cruzi*. The results suggest that chromenes should be considered further in the development of new and more potent drugs for use in the treatment of Chagas disease.

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