Experimental Parasitology 130 (2012) 141–145

Contents lists available at SciVerse ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

In vitro antileishmanial and antitrypanosomal activities of flavanones from *Baccharis retusa* DC. (Asteraceae)

Simone dos S. Grecco [a], Juliana Q. Reimão [b], André G. Tempone [b], Patricia Sartorelli [a], Rodrigo L.O.R. Cunha [c], Paulete Romoff [d], Marcelo J.P. Ferreira [d], Oriana A. Fávero [d], João Henrique G. Lago [a,*]

[a] *Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Diadema, SP, Brazil*
[b] *Departamento de Parasitologia, Instituto Adolfo Lutz, São Paulo, SP, Brazil*
[c] *Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, SP, Brazil*
[d] *Centro de Ciências e Humanidades, Universidade Presbiteriana Mackenzie, São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 13 October 2011
Accepted 15 November 2011
Available online 28 November 2011

Keywords:
Baccharis retusa DC
Asteraceae
Leishmania
Trypanosoma cruzi
Flavanones

ABSTRACT

Leishmaniasis and Chagas' are parasitic protozoan diseases that affect the poorest population in the world, causing a high mortality and morbidity. As a result of highly toxic and long-term treatments, novel, safe and more efficacious drugs are essential. In this work, the CH_2Cl_2 phase from MeOH extract from the leaves of *Baccharis retusa* DC. (Asteraceae) was fractioned to afford two flavonoids: naringenin (**1**) and sakuranetin (**2**). These compounds were *in vitro* tested against *Leishmania* spp. promastigotes and amastigotes and *Trypanosoma cruzi* trypomastigotes and amastigotes. Compound **2** presented activity against *Leishmania* (*L.*) *amazonensis*, *Leishmania* (*V.*) *braziliensis*, *Leishmania* (*L.*) *major*, and *Leishmania* (*L.*) *chagasi* with IC_{50} values in the range between 43 and 52 μg/mL and against *T. cruzi* trypomastigotes (IC_{50} = 20.17 μg/mL). Despite of the chemical similarity, compound **1** did not show antiparasitic activity. Additionally, compound **2** was subjected to a methylation procedure to give sakuranetin-4′-methyl ether (**3**), which resulted in an inactive compound against both *Leishmania* spp. and *T. cruzi*. The obtained results indicated that the presence of one hydroxyl group at C-4′ associated to one methoxyl group at C-7 is important to the antiparasitic activity. Further drug design studies aiming derivatives could be a promising tool for the development of new therapeutic agents for Leishmaniasis and Chagas' disease.

1. Introduction

The genus *Baccharis*, one of the most important genera of Asteraceae family, is widespread throughout Central and South America (Nesom, 2000). Some of these species have been used in the folk medicine to the treatment of stomach and liver diseases, to reduce inflammatory processes and to cure ulcers and skin wounds (Melo et al., 2001; Korbes, 1995; Corrêa, 1984; Franco, 1995; Bandoni et al., 1978; Cortadi et al., 1999; Verdi et al., 2005). Previous chemical studies on *Baccharis* species have been carried out and several compounds such as terpenoids, tricotecenes, chromenes, and flavonoids were found (Bohlmann et al., 1979; Labbe et al., 1986; Zdero et al., 1989; Jarvis et al., 1991; Verdi et al., 2005; Grecco et al., 2010a). Additionally, several works demonstrated that some of these species have been sources of antileishmanial compounds, mainly phenolics and triterpenoids (Fournet et al., 1994; da Silva Filho et al., 2009; Zalewski et al., 2011).

Previous works have described that the *Baccharis uncinella* EtOH extract as well as its main derivatives (oleanolic/ursolic acids, pectolinaringenin, caffeic acid, and ferulic acid) showed activity against promastigotes and amastigotes forms of both *Leishmania* (*L.*) *amazonensis* and *Leishmania* (*V.*) *braziliensis* (Passero et al., 2011). It was also reported that 5,6,7-trihydroxy-4′-methoxyflavanone, an isolated derivative from MeOH extract of *Baccharis retusa*, showed activity against cutaneous species of *Leishmania* (Grecco et al., 2010b). In continuation with *B. retusa* investigation, the present study was undertaken to determine the effects of main compounds isolated from MeOH extract from the leaves of *B. retusa* against *Leishmania* spp. and *Trypanosoma cruzi*: naringenin (**1**) and sakuranetin (**2**). Additionally, sakuranetin-4′-methyl ether (**3**) was prepared after methylation of **2** and tested against the same parasites.

2. Materials and methods

2.1. General experimental procedures

Silica gel (Merck, 230–400 mesh) was used for column chromatographic separation, while silica gel 60 PF_{254} (Merck) was used

* Corresponding author.
E-mail address: joao.lago@unifesp.br (J.H.G. Lago).

0014-4894/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2011.11.002

for analytical TLC (0.25 mm). ^{1}H NMR and ^{13}C spectra were recorded, respectively, at 300 and 75 MHz in a Bruker DPX-300 spectrometer. $CDCl_3$ (Aldrich) was used as solvent and TMS (Aldrich) as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (J) in Hz. LREIMS were measured in a HP 5990/5988A mass spectrometer.

2.2. Plant material

Baccharis retusa DC. leaves were collected in Campos do Jordão, SP, Brazil, in October 2008 and were identified by Dr. Oriana A. Fávero. Voucher specimen has been deposited at Herbarium of Instituto de Botânica-SEMA, São Paulo, SP, Brazil.

2.3. Extraction and isolation

Dried and powdered leaves of *B. retusa* (460 g) were exhaustively defatted with hexane (at room temperature). Sequentially, the plant material was extracted with MeOH (six times at room temperature) affording 32 g of a syrupy green extract, after solvent remotion under reduced pressure. This extract was partitioned between MeOH:H_2O (1:2) and CH_2Cl_2. After evaporation under reduced pressure, the CH_2Cl_2 phase (13 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2 containing increasing amounts of EtOAc (up to 100%) and with EtOAc containing increasing amounts of MeOH (up to 100%), to give 8 fractions (A1–A8). Fraction A2 (3.6 g) was purified by silica gel column chromatography eluted with increasing amounts of EtOAc in CH_2Cl_2 (up to 100%) to afford 700 mg of **2**. Fraction A4 (590 mg) was subjected to silica gel column chromatography eluted with mixtures of CH_2Cl_2:EtOAc:MeOH in gradient form to give 12 fractions (B1–B12). Fraction B2 (142 mg) was purified by silica gel column chromatography eluted with CH_2Cl_2 containing increasing amounts of EtOAc (up to 100%) and with EtOAc containing increasing amounts of MeOH (up to 100%) to give 30 mg of **1**.

2.4. 5,7,4'-Trihydroxy-flavanone (naringenin-***1***)

^{1}H NMR (300 MHz, CD_3OD/DMSO-d_6) δ_H: 7.27 (d, J = 8.5 Hz, H-2'/H-6'), 6.82 (d, J = 8.5 Hz, H-3'/H-5'), 5.91 (s, H-6/H-8), 5.29 (dd, J = 12.9 and 2.8 Hz, H-2), 3.06 (dd, J = 17.1 and 12.9, H-3a), 2.68 (dd, J = 17.1 and 2.8 Hz, H-3b). ^{13}C NMR (75 MHz, CD_3OD/DMSO-d_6) δ_C: 197.3 (C-4), 167.9 (C-9), 164.9 (C-7), 164.3 (C-4'), 158.4 (C-5), 130.4 (C-1'), 130.4 (C-6), 128.7 (C-2'/C-6'), 116.2 (C-3'/C-5'), 103.2 (C-10), 96.7 (C-8), 80.0 (C-2), 43.8 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 272 (100), 254 (6), 229 (5), 207 (4), 179 (20), 166 (28), 153 (83), 120 (81), 107 (27), 91 (35), 69 (35). 32 (60).

2.5. 5,4'-Dihydroxy-7-methoxyflavanone (sakuranetin-***2***)

^{1}H NMR (300 MHz, CD_3OD) δ_H: 7.26 (d, J = 8.5 Hz, H-2'/H-6'), 6.83 (d, J = 8.5 Hz, H-3'/H-5'), 6.01 (s, H-6/H-8), 5.32 (dd, J = 13.0 and 3.0 Hz, H-2), 3.77 (s, OCH_3-7), 3.08 (dd, J = 17.2 and 13.0 Hz, H-3a), 2.73 (dd, J = 17.2 and 3.0 Hz, H-3b). ^{13}C NMR (75 MHz, CD_3OD) δ_C: 196.5 (C-4), 168.0 (C-4'), 163.6 (C-7), 163.0 (C-5), 157.4 (C-9), 129.0 (C-1'), 127.7 (C-2'/C-6'), 127.6 (C-6), 115.3 (C-3'/C-5'), 102.8 (C-10), 93.9 (C-8), 79.1 (C-2), 55.3 (OCH_3), 42.8 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 286 (67), 193 (33), 180 (39), 167 (100), 138 (24), 120 (44), 95 (38), 69 (25).

2.6. Preparation of sakuranetin-4'-methyl ether (***3***)

Compound **2** (100 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (10 mL) and subjected to a methylation reaction driven by phase transfer catalysis using methyl iodide (1.49 g, 9.9 mmol), potassium carbonate (1 g, 7.23 mmol), and cetyltrimethyl ammonium bromide (30 mg, 0.082 mmol). After 72 h at room temperature, the crude product was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent under reduced pressure followed by purification on CC using SiO_2 and n-hexane:EtOAc 3:2 as eluent afforded **3** (12 mg, 0.04 mmol).

2.7. 5-Hydroxy-7,4'-dimethoxyflavanone (sakuranetin 4'-methyl ether - ***3***)

^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 12.02 (s, OH-5), 7.37 (d, J = 8.7 Hz, H-1′/H-6′), 6.94 (d, J = 8.7 Hz, H-2′/H-5′), 6.06 (d, J = 2.3 Hz, H-6), 6.03 (d, J = 2.3 Hz, H-8), 5.35 (dd, J = 12.9 and 3.0 Hz, H-2), 3.82 (s, OCH_3-4′), 3.79 (s, OCH_3-7), 3.09 (dd, J = 17.1 and 13.0 Hz, H-3b), 2.77 (dd, J = 17.1 and 3.0 Hz, H-3a). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 196.0 (C-4), 168.0 (C-7), 164.1 (C-5), 162.9 (C-9), 160.0 (C-4′), 130.4 (C-1′), 127.7 (C-2′, 6′), 114.2 (C-3′, 5′), 103.1 (C-10), 95.1 (C-6), 94.2 (C-8), 79.0 (C-2), 55.7 (OCH_3-4′), 55.4 (OCH_3-7), 43.2 (C-3). LREIMS (70 eV) *m/z* (int. rel.): 300 (46), 282 (3), 207 (6), 193 (18), 166 (11), 134 (83), 121 (73), 108 (12), 91 (37), 65 (17), 32 (100).

2.8. Bioassays procedures

BALB/c mice and Golden hamsters were supplied by the animal breeding facility at the Instituto Adolfo Lutz, São Paulo, and maintained in sterilized cages under a controlled environment, receiving water and food *ad libitum*. Animal procedures were performed with the approval of the Research Ethics Commission, in agreement with the Guide for the Care and Use of Laboratory Animals from the National Academy of Sciences.

2.9. Parasite maintenance

Isolated promastigotes of *L.* (*L.*) *amazonensis* (WHO/BR/00/LT0016), *L.* (*V.*) *braziliensis* (MHO/BR/75/M2903), *Leishmania (L.) chagasi* (MHOM/BR/1972/LD) and *Leishmania (L.) major* (MHOM/1L/80/Fredlin) were maintained in M-199 medium supplemented with 10% calf serum and 0.25% hemin at 24 °C. *L.* (*L.*) *chagasi* (MHOM/BR/1972/LD) was maintained in hamsters (*Mesocricetus auratus*). Amastigotes were harvested from spleens of infected hamsters by differential centrifugation (Stauber, 1958). *T. cruzi* trypomastigotes (Y strain) were maintained in LLC-MK2 (ATCC CCL 7) cells using RPMI-1640 medium supplemented with 2% calf serum at 37 °C.

2.10. Mammalian cells

Peritoneal macrophages were collected from the peritoneal cavity of female BALB/c mice by washing with RPMI-1640 without phenol red, supplemented with 10% fetal bovine serum. THP-1 (human monocytes ATCC number TIB-202) and kidney *Rhesus* monkey cells (LLC-MK2) were maintained in RPMI-1640 medium without phenol red and supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 humidified incubator (Corrêa et al., 2011).

2.11. Determination of the activity against Leishmania spp. – promastigotes

To determine the 50% inhibitory concentration (IC_{50} value) against *Leishmania* promastigotes, compounds **1–3** were previously dissolved in MeOH and diluted with M-199 medium in 96-well microplates. Promastigotes were counted in a Neubauer hemocytometer and seeded at 1 × 10^6/well with a final volume of 150 μL. Controls with MeOH and without drugs were also performed. Pentamidine was used as a standard drug. Top concentrations were 200 μg/mL for compounds **1–3** and 1.5 μg/mL for pentamidine