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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 1676–1682
Bioorganic & Medicinal Chemistry

Trypanocidal activity of a new pterocarpan and other secondary metabolites of plants from Northeastern Brazil flora

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Received 27 July 2007; revised 7 November 2007; accepted 8 November 2007
Available online 13 November 2007

Abstract—Two hundred fifteen compounds isolated from plants of Northeastern Brazil flora have been assayed against epimastigote forms of *Trypanosoma cruzi*, using the tetrazolium salt MTT as an alternative method. Eight compounds belonging to four different species: *Harpalyce brasiliana* (Fabaceae), *Acnistus arborescens* and *Physalis angulata* (Solanaceae), and *Cordia globosa* (Boraginaceae) showed significant activity. Among them, a novel and a known pterocarpan, a chalcone, four withasteroids, and a meroterpene benzoquinone were the represented chemical classes.

1. Introduction

The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas' disease (American trypanosomiasis) which affects 20 million people in Central and South America, and is responsible for the death of more than 45,000 patients per year.[1,2] In Brazil, there are around 4 million people infected.[3]

The overall efficacy of chemotherapy is uncertain because of variations in *T. cruzi* strain susceptibility—itself dependent on geographical factors—and the unreliability of methods to determine parasite eradication. The current chemotherapy for human Chagas' disease is based exclusively on nitroimidazoles, most of them developed more than 30 years ago. Benznidazole and nifurtimox are the most used drugs to treat Chagas' disease and both show low efficacy, particularly at the chronic stage of infection.[4] Besides, these drugs are toxic causing nausea and vomiting, bone marrow hypoplasia, dermatitis, and polyneuritis.[5] Despite the high incidence and mortality of the infection, there has been weak commercial interest in developing new trypanocidal compounds.[6]

Plants and their extracts have been used for many centuries as treatments for ailments, from headaches to parasite infections, but only in the past 20–30 years, have scientists seriously begun to determine whether plant-derived traditional remedies are effective, and, if so, their mode of action.[7] Searching for new drugs with high activity is very important, especially considering that in Brazil parasitic diseases constitute a serious public health problem.[8] The existing biodiversity is a potential source of many unknown bioactive molecules.[9] All these factors justify continued research for trypanocidal substances from plant origin.

In this study, the trypanocidal activity of compounds isolated from several plants of the Brazilian semi-arid region, called 'caatinga' (meaning 'clear forest' in the native language), has been assayed. Eight compounds from four species—*Harpalyce brasiliana* Benth. (Fabaceae), *Acnistus arborescens* Schltdl. and *Physalis angulata* L. (Solanaceae) and *Cordia globosa* (Jacq.) Kunth (Boraginaceae)—were the most active (Fig. 1).

Keywords: Pterocarpan; Trypanocidal activity; *Harpalyce brasiliana*; MTT; Brazilian caatinga.
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doi:10.1016/j.bmc.2007.11.027

The new pterocarpan (−)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (**1**), 2-geranyl-2,3′,4,4′-tetrahydroxychalcone (**2**), and 4′-dehydroxy-cabenegrin A–I (**3**) were isolated from *H. brasiliana*, a shrub called 'raiz-de-cobra' (Port. lit. 'snake root') and used in the Brazilian traditional medicine to treat snake bites. The structural characterization of the new compound (**1**) was accomplished by spectroscopic methods, and the spectral data are here described and discussed. The chalcone (**2**) was recently isolated from *Artocarpus nobilis*, and showed antifungal and radical scavenging properties.[10] 4′-Dehydroxy-cabenegrin A–I (**3**) was identified by Silva et al. from the ethanol extract of *H. brasiliana* roots, and there are no data describing any biological activity of this compound.[11]

Physalis angulata and *A. arborescens*, both belonging to the Solanaceae family, are known as prolific sources of withasteroids, C_{28}-steroidal lactones.[12,13] Trypanocidal activity against epimastigotes and trypomastigotes of the protozoan *T. cruzi* has been evaluated for withanolides isolated from *P. angulata*.[14,15] The physalin F (**4**) was isolated from aerial parts of *P. angulata*,[12] while the withaphysalins O (**5**), M (**6**), and N (**7**), were obtained from leaves of *A. arborescens*.[13]

The terpenoid benzoquinone (1aS*,1bS*,7aS*,8aS*)-4,5-dimethoxy-1a,7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta[1,2*b*]naphthalene-3,6-dione (**8**), isolated from roots of *C. globosa* a medicinal plant used to treat rheumatism, menstrual pains, and dyspepsia, and also as spasmolytic and vasodilator, was also evaluated.[16,17]

Some of these natural compounds have demonstrated cytotoxic and antitumor activities,[12–18] but there are no reports demonstrating the trypanocidal effects of the compounds described in this paper.

2. Results and discussion

Compound **1**, a white solid, showed m.p. 72.5–74.7 °C. The IR spectrum showed an absorption band at 3380 cm^{-1} related to the hydroxyl group. The ^1H NMR spectrum suggested a pterocarpan structure due to the splitting pattern of the protons at δ 5.21 (d, J = 7.1 Hz, H-11a), 3.83 (dd, J = 5.1, 10.9 Hz, H-6α), 3.48 (t, J = 10.9 Hz, H-6β), and δ 2.92 (m, H-6a), related to the oxymethylene protons of the heterocyclic ring B, and the bridging protons of rings B and C (H-6a and H-11a).[19] This spectrum also allowed the identification of a methylenedioxy group at δ 5.31 (2H-11′) and of a hydroxyl at δ 4.83 (OH-3). The presence of a geranyl group was suggested from the signals of the three methyl singlets at δ 1.68 (s, H-8′), 1.60 (s, H-10′), and 1.52 (s, H-9′), and the two olefinic protons at δ 5.41 (t, J = 7.2 Hz, H-2′) and 5.16 (d, J = 6.7 Hz, H-6′), besides the three methylenes at δ 3.32 (d, J = 7.2 Hz, 2H-1′), 2.02 (m, 2H-4′), and 2.12 (m, 2H-5′).[20] All proton couplings were confirmed through the ^1H,^1H-COSY spectrum, one of the most used 2D NMR experiments designed to establish proton–proton scalar coupling.[21] In addition, the four remaining aromatic singlets at δ 7.32 (s, H-1), 6.48 (s, H-10), 6.37 (s, H-4), and 6.34 (s, H-7) gave clear evidence of a 2,3,8,9-substitution pattern for the pterocarpan moiety.

The ^{13}C NMR spectral data of **1** (Table 1) along with the DEPT revealed 26 carbon atoms corresponding to 3 methyl, 5 methylene, 8 methine groups, and 10 non-hydrogenated carbons. The chemical shifts observed for the methyl at δ 18.0 (C-9′) and the methylene at δ 40.3 (C-4′) emphasize the geranyl, as suggested in the ^1H NMR, but not the neryl side chain.[22]

The HMQC spectrum (Heteronuclear Multiple Quantum Coherence, a 2D NMR pulse sequence, with detection in the hydrogen channel, designed to establish the connectivity of the protons to each carbon to which they are directly attached)[21] provided the assignment of all protonated carbons, as shown in Table 1. Several long-range heteronuclear correlations (depicted by arrows in Fig. 2) were used to assign the chemical shifts of the non-hydrogenated carbons of both aromatic rings. The assignment of the relative position of the geranyl moiety was defined in the HMBC spectrum (Heteronuclear Multiple Bond Correlation, also an inverse detected 2D NMR technique used to determine correlations of carbons to protons two, three or more bonds far way)[21] by the cross-peaks of the allylic methylene protons at δ 3.32 (2H-1′) with the carbons at δ 156.4 (C-3), 132.7 (C-1), 123.2 (C-2′), 122.1 (C-2), and 137.7 (C-3′), in addition to the correlations of the signal at δ 6.37 (H-4) with the carbon at δ 122.1 (C-2), and the signal at δ 7.32 (H-1) with the carbon at δ 29.2 (H-1′). These correlation data of the A-ring protons definitively established that the geranyl and OH groups are located at the C-2 and C-3 positions, respectively. Moreover, the correlations of the hydrogens of the aromatic D-ring at δ 6.34 (H-7) with the carbon at δ 149.0 (C-9), and at δ 6.48 (H-10) with the carbon at δ 142.4 (C-8), besides the concomitant correlations of the hydrogens of the methylenedioxy group at δ 5.31 (H-11) with the same carbons at δ 142.4 (C-8) and 149.0 (C-9), established that this group was, therefore, located at the C-8 and C-9 positions (Fig. 1). The above spectroscopic information led to the identification of compound **1** as the new (−)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan.

New potential trypanocidal drugs are generally screened in vitro against extracellular epimastigotes.[23] This stage has a different sensitivity in comparison with other stages, and thus it is useful to identify active compounds against the parasite.[24] For this reason, 215 isolated compounds were tested against epimastigotes of *T. cruzi*. The method most commonly used in the screening of drugs for the treatment of Chagas' disease is the microscopic counting of viable trypanosomes, which is time-consuming, labor-intensive, and dependent on the observer's ability. Therefore, we decided to carry out the colorimetric assay using the tetrazolium salt MTT. This method is rapid, simple, and reliable for the evaluation of trypanocidal activity.[25,26]

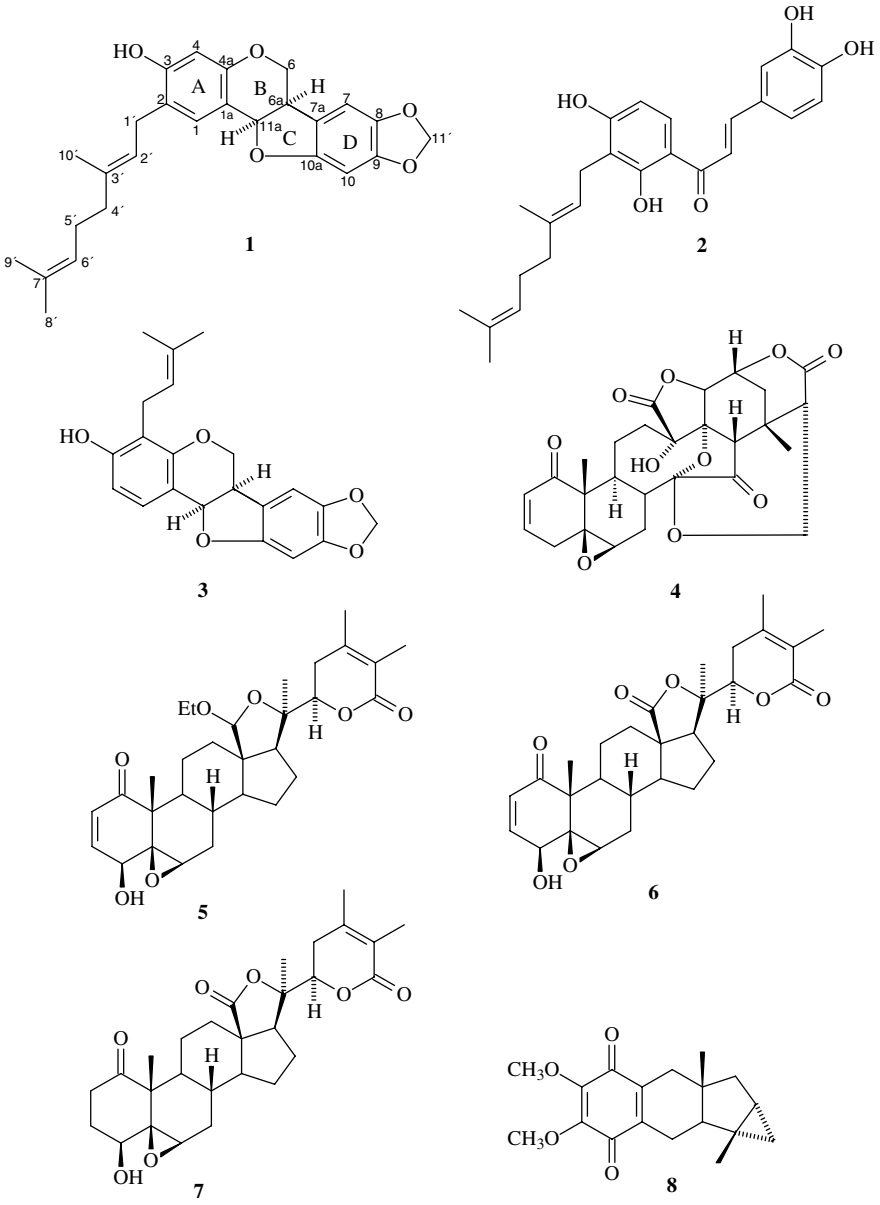

Figure 1. Chemical structures of the bioactive compounds **1**–**8**.

Eight compounds showed the greatest inhibitory activity (Table 2). The IC_{50} of all compounds were smaller than benznidazole $IC_{50} \sim 50$ μg/mL,[23] the reference drug used for the treatment of Chagas' disease. All compounds showed a concentration-dependent inhibitory effect on the in vitro growth of *T. cruzi* epimastigotes, and the effect of (−)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (**1**) is demonstrated in Figure 3. Despite their cytotoxic properties[12,13,17,18] these compounds have never been tested against parasites.

Chemical data and biological activities of the new pterocarpan (**1**) have not been reported in the literature, nor the biological activity of compound **3**. In addition to the important trypanocidal activity of compounds (**1**) and (**3**), they do not demonstrate cytotoxic effect in human peripheral blood cells (PMBC), presenting IC_{50} higher than 50 μg/mL (Table 3). Cytotoxicity on human cells is a very important criterion for assessing the selectivity of the observed biological activity.[27] Thus studies with these compounds should be continued in order to verify further biological activities and their mechanisms of action.

Compound **4** was previously tested by Magalhães et al. for antiproliferative activity in four tumor cell lines: B-16 (murine skin), HCT-8 (human colon), CEM, and HL-60 (leukemias) and it showed great activity, with IC_{50} values between 0.5 and 1.6 μg/mL.[12] Compounds **5**–**7** have also demonstrated toxicity to human lung cancer cells (Lu1), hormone-dependent human prostate cancer cells (LNCaP), and estrogen-dependent breast cancer cells (MCF-7).[13] Moreover, Rocha et al. demonstrated that these compounds have cytotoxic and anti-

Table 1. NMR data for (−)-2-geranyl- 3-hydroxy-8,9-ethylenedioxypterocarpan (**1**) (C_6D_6, 500/125 MHz)

Position	δC	δH, mult (*J* in Hz)
1a	113.3	
1	132.7	7.32, s
2	122.1	
3	156.4	
4	104.4	6.37, s
4a	155.9	
6	66.9	3.83 dd (5.1; 10.9) 3.48 t (10.9)
6a	41.1	2.92, m
7a	118.9	
7	105.4	6.34, s
8	142.4	
9	149.0	
10	94.3	6.48, s
10a	155.4	
11a	79.3	5.21, d (7.1)
1′	29.2	3.32, d (7.2)
2′	123.2	5.41, d (7.2)
3′	137.7	
4′	40.3	2.02, m
5′	27.2	2.12, m
6′	125.0	5.16, t (6.7)
7′	131.8	
8′	26.1	1.68, s
9′	18.0	1.52, s
10′	16.4	1.60, s
11′	101.5	5.31, s
OH		4.83, s

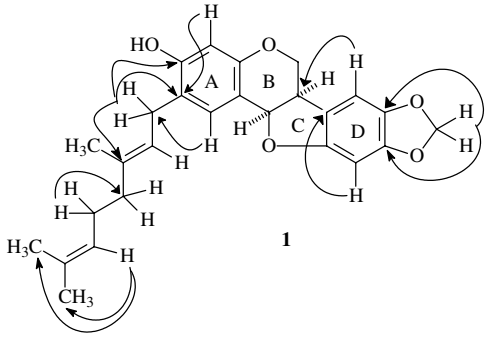

Figure 2. Key two- and three-bond carbon–hydrogen correlations (depicted by arrows) observed for compound **1** through the HMBC experiment.

Table 2. Trypanocidal activity (IC_{50}) of the isolated compounds **1**–**8**

Compounds	IC_{50} (μg/mL)
(−)-2-Geranyl- 3-hydroxy-8, 9-methylenedioxypterocarpan (**1**)	12.2
3-Geranyl-2,3′,4,4′-tetrahydroxychalcone (**2**)	10.6
4′-Dehydroxy-cabenegrin A–I (**3**)	13.3
Physalin F (**4**)	9.4
Withaphysalin O (**5**)	0.3
Withaphysalin M (**6**)	0.7
Withaphysalin N (**7**)	6.4
(1aS*,1bS*,7aS*,8aS*)-4,5-Dimethoxy-1a, 7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta [1,2*b*]naphthalene-3,6-dione (**8**)	1.3

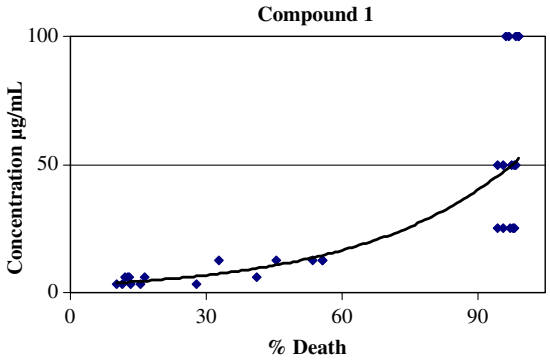

Figure 3. Mean graphic of the (−)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (**1**) effect on growth of epimastigote forms of *T. cruzi*.

Table 3. Effect of isolated compounds in human peripheral blood cells (PMBC)

Compounds	IC_{50} (μg/mL) CI 95%[a]
(−)-2-Geranyl-3-hydroxy-8, 9-methylenedioxypterocarpan (**1**)	>50
3-Geranyl-2,3′,4,4′-tetrahydroxychalcone (**2**)	23.04 18.92–28.06
4′-Dehydroxy-cabenegrin A–I (**3**)	>50
Physalin F (**4**)	4.23 2.84–6.29
(1aS*,1bS*,7aS*,8aS*)-4,5-Dimethoxy-1a, 7a-dimethyl-1,1a,1b,2,7,7a,8, 8a-octahydrocyclopropa [3,4]cyclopenta[1,2*b*]naphthalene-3,6-dione (**8**)	28.37 18.77–32.89
Doxorubicin[b]	0.97 0.52–1.80

[a] Data are presented as IC_{50} values and 95% confidence interval (CI 95%) from two independent experiments, performed in duplicate.
[b] Doxorubicin was used as the positive control.

proliferative effects in a dose-and-time-dependent manner on two leukemia cell lines (HL-60 and K562).[18]

Despite being toxic on tumor cell lines, the authors decided to verify whether or not the compounds showed a harmful effect against dividing normal cells. Interestingly, while doxorubicin was cytotoxic to both leukemia cell lines as well as to PHA-stimulated lymphocytes, no antiproliferative effect of withaphysalins (**5**–**7**) was found in PHA-stimulated lymphocytes.[18] It seems that structure requirements for cytotoxicity are far from being established, and so mechanisms implicated in activity against parasites may be different from those required against other cells.

A previous study demonstrated that compound **8** exhibited cytotoxic activity against five cancer cell lines with IC_{50} values in the range of 1.24–5.04 μg/mL after 72 h of incubation. Benzoquinones have been extensively studied as antitumor agents and many anti-cancer drugs of clinical and research interests contain the quinone nucleus. According to several authors, quinone-containing drugs generate reactive oxygen-free radicals that are implicated in drug cytotoxicity.[15]

Miltefosine, the unique oral treatment for leishmania parasite, is an example of a compound that was widely studied for its antiproliferative and cancerostatic properties, and is currently the best alternative for visceral leishmaniosis' treatment.[24] This fact can justify the effort to assay cytotoxic compounds against different kinds of parasites.

3. Experimental

3.1. General experimental procedures

Melting points were obtained on a Mettler FP82HT apparatus and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1000 FT-IR spectrophotometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. The mass spectra were obtained on a Hewlett–Packard 5971 mass spectrometer by electron impact ionization (70 eV). ^{1}H and ^{13}C NMR were recorded on a Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C); chemical shifts are given in ppm relative to residual $CHCl_3$ (7.24), and to the central peak of the triplet related to $CDCl_3$ carbon (77.0 ppm). Silica Gel 60 (Merck, ∅ μm 2–25) was used for analytical TLC. Silica gel 60 (Merck, ∅ μm 40–63) was used for column flash chromatography. All compounds were visualized on TLC by spraying vanillin/perchloric acid/EtOH, followed by heating.

3.2. Plant material

Plants were collected in the State of Ceará (CE), Northeast of Brazil, and authenticated by Prof. Edson P. Nunes of the Biology Department of the Federal University of Ceará, Brazil. The voucher specimens (*H. brasiliana* Benth.: 32525, *A. arborescens* Schltdl.: 30513, *P. angulata* L.: 33.576, and *C. globosa* (Jacq.) Kunth: 30005) have been deposited at the Prisco Bezerra Herbarium (EAC), at the Biology Department of the Federal University of Ceará, Brazil.

Roots of *H. brasiliana* were collected at the Araripe Plateau, Crato, CE. Leaves of *A. arborescens* were collected along the road margins at Pico Alto, Guaramiranga, CE. Aerial parts of *P. angulata* were collected during the flowering stage in Pentecoste, CE, and roots of *C. globosa* were collected in Acarape, CE.

3.3. Extraction and isolation

Roots of *H. brasiliana* (3.5 kg) were pulverized and extracted with EtOH at room temperature. The solvent was removed under reduced pressure to give a dark viscous extract (260.0 g).

Liquid–liquid partition of an aliquot of the EtOH extract (107.3 g) using hexane, $CHCl_3$, EtOAc, and *n*-BuOH as eluents yielded four fractions. Part of the $CHCl_3$ fraction (12.5 g) was further purified over Sephadex LH-20 by elution with MeOH to yield 23 fractions, which were combined in seven resulting fractions according to TLC analysis. Successive flash chromatography of fraction 4 (3.34 g), using $CHCl_3$, EtOAc, and MeOH as the binary mixture of increasing polarity, yielded 20 fractions according to TLC analysis. Flash chromatography of fraction 4 (8–28) (331.8 mg) by elution with hexane, CH_2Cl_2, and MeOH as a binary mixture of increasing polarity yielded (−)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (**1**) (31.2 mg) and 4′-dehydroxy-cabenegrin A–I (**3**) (63.0 mg). Flash chromatography of fraction 5 (989.2 mg) by eluting with mixtures of $CHCl_3$, AcOEt, and MeOH of increasing polarity yielded the 3′-geranyl-2′,3,4,4′-tetrahydroxychalcone (25.9 mg) (**2**).

Extraction, isolation, and NMR spectral data of physalin F (**4**) have been previously described in Magalhães et al.[12] Extraction and isolation of withaphysalins (**5**–**7**) were described by Rocha et al.[18] ^{1}H and ^{13}C NMR data were in accordance with previously published results.[13] Extraction, isolation, and NMR spectral data of the meroterpenoid benzoquinone (**8**) were published by Menezes et al.[17]

3.3.1. (−)-2-Geranyl-3-hydroxy-8,9-methylenedioxypterocarpan(1). White solid; $[\alpha]_D^{20}$ −232° (*c* 0.011, CH_2Cl_2); IR (KBr) ν_{max} 3380, 2922, 2847, 1624, 1505, 1474, 1379, 1328, 1261, 1185, 1145, 1114, 1094, 1032, 937 cm^{-1}. EIMS *m/z* (rel int.): 420 [$M^{\bullet+}$], 351, 335, 312, 297, 283, 267, 254, 239, 225, 213, 201, 189, 175, 162, 151, 133, 115, 107, 91, 77, 69, 43, 41. For ^{1}H and ^{13}C NMR spectral data, see Table 1.

3.4. Parasites

Epimastigote forms of *T. cruzi* (Berenice strain) were grown at 28 °C in liver infusion tryptose (LIT) supplemented with 5% heat-inactivated fetal calf serum (FCS) and antibiotics.[28]

3.5. Trypanocidal activity

Tests were performed based on Muelas-Serrano et al.[26] and Tempone et al.[29] with slight modifications. Briefly, compounds were dissolved in dimethylsulfoxide (DMSO) and diluted in LIT. The final concentration of DMSO did not exceed 1% and for each experiment there was a growth control with and without DMSO. No effect on toxicity attributable to DMSO was observed at the maximum concentration used of 1%. The experiments were performed in 96-well plates with compounds at initial concentrations of 100 μg/mL or 10 μg/mL. The inoculum consisted of 10^6 parasites per well in stationary phase. The cells were incubated at 28 °C for 72 h. Benznidazole (*N*-benzyl-2-nitro-1-imidazolacetamide, Roche Pharmaceuticals, Rio de Janeiro, Brazil) was used as the reference drug. Negative controls were performed with 10^6 parasites in LIT and culture medium alone. The viability of the epimastigotes was based on the cellular conversion of the soluble tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide—Sigma) into the insoluble formazan by mitochondrial enzymes. MTT (10 mg/mL) was dissolved in PBS, 20 μL/well, for 4 h at 37 °C. Formazan extraction was performed using 10% SDS (100 μL/well). The

number of living epimastigotes was determined spectrophotometrically at 540 nm. Assays were performed in quintuplicate.

3.6. Lymphocyte isolation

Peripheral venous blood (5 mL) was collected in heparinized vials from four normal, healthy donors, two women and two men, with no history of smoking/drinking or chronic use of medication. The blood was washed with PBS and lymphocytes were isolated using a gradient of Ficoll–Histopaque (7 blood:3 ficoll).

3.7. Alamar Blue assay

In order to investigate selectivity of compounds toward a normal proliferating cell, the Alamar Blue assay was performed with PBL after 72 h drug exposure. Briefly, PBL were plated in 96-well plates (2×10^4 cells/well in 100 μL of medium). After 24 h, tested compounds dissolved in DMSO were added to each well (using the HTS—high-throughput screening—biomek 3000—Beckman Coulter, Inc., Fullerton, Califórnia, EUA) and incubated for 72 h. Doxorubicin (0.009–5 μg/mL) was used as positive control. Control groups received the same amount of DMSO. Twenty four hours before the end of the incubation, 10 μL of stock solution (0.312 mg/mL) of the Alamar Blue (resazurin, Sigma Aldrich Co., St. Louis, MO, USA) was added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, Califórnia, EUA). The drug effect was quantified as the percentage of control absorbance at 570 nm and 595 nm. The absorbance of the medium alone is subtracted from the absorbance of medium plus Alamar Blue at the higher and lower wavelengths. These values are called AO_{HW} and AO_{LW}, respectively. A correction factor R_0 can be calculated from AO_{HW} and AO_{LW}, where $R_0 = AO_{LW}/AO_{HW}$. The percent Alamar Blue reduced is then expressed as follows: % Reduced $= AO_{LW} - (AO_{HW} \times R_0) \times 100$. Data are presented as IC_{50} values and 95% confidence interval from two independent experiments, performed in duplicate. Doxorubicin was used as the positive control.

3.8. Statistical analysis

For trypanocidal assay, IC_{50} values were determined by non linear regression analysis of data in MS Excel.

For cytotoxicity assays, IC_{50} values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression using GRAPHPAD PRISM software (Intuitive Software for Science, San Diego, CA, USA).

Acknowledgments

The authors wish to thank CNPq, CAPES, FUNCAP, FINEP, and PRONEX, Brazilian governmental agencies, for the financial support in the form of grants and fellowship awards. In particular Dr. Letícia Costa-Lotufo for helping with the cytotoxicity assay in human cells.

References and notes

1. Leite, A. C. L.; Lima, R. S.; Moreira, D. R. M.; Cardoso, M. V. O.; Brito, A. C. G.; Santos, L. M. F.; Hernandes, M. Z.; Kiperstok, A. C.; Lima, R. S.; Soares, M. B. P. *Bioorg. Med. Chem.* **2006**, *14*, 3749–3757.
2. WHO Tropical Disease Research: Progress 1997–98, World Health Organization: Geneva, 1999.
3. Vinhaes, M. C.; Dias, J. C. P. *Cad. Saude Publica* **2000**, *16*, 7–12.
4. Senior, K. *Lancet Infect. Dis.* **2007**, *7*, 572.
5. Umezawa, E. S.; Stolf, A. M. S.; Corbett, C. E. P.; Shikanai-Yasuda, M. A. *Lancet* **2001**, *357*, 797–799.
6. Trouiller, P.; Olliaro, P.; Torreele, E.; Orbinski, J. L. R.; Ford, N. *Lancet* **2002**, *359*, 2188–2194.
7. Anthony, J-P.; Fyfe, L.; Smith, H. *Trends Parasitol.* **2005**, *21*, 462–468.
8. De Mesquita, M. L.; Grellier, P.; Blond, A.; Brouard, JP.; de Paula, J. E.; Espindola, L. S.; Mambu, L. *Bioorg. Med. Chem.* **2005**, *13*, 4499–4506.
9. Truiti, M. C. T.; Ferreira, I. C. P.; Zamuner, M. L. M.; Nakamura, C. V.; Sarragiotto, M. H.; Souza, M. C. *Braz. J. Med. Biol. Res.* **2005**, *38*, 1873–1878.
10. Jayasinghe, L.; Balasooriya, B. A.; Padmini, W. C.; Hara, N.; Fujimoto, Y. *Phytochemistry* **2004**, *65*, 1287–1290.
11. Silva, G. L.; Machado, M. I. L.; Matos, F. J. A.; Braz-Filho, R. *J. Braz. Chem. Soc.* **1999**, *10*, 438–442.
12. Magalhães, H. I. F.; Veras, M. L.; Pessoa, O. D. L.; Silveira, E. R.; de Moraes, M. O.; Pessoa, C.; Costa-Lotufo, L. V. *Lett. Drug. Des. Discovery* **2006**, *3*, 625–632.
13. Veras, M. L.; Bezerra, M. Z. B.; Lemos, T. L. G.; Uchoa, D. E. A.; Braz-Filho, R.; Chai, H. B.; Cordell, G. A.; Pessoa, O. D. *J. Nat. Prod.* **2004**, *67*, 710–713.
14. Nagafuji, S.; Okabe, H.; Akahane, H.; Abe, F. *Biol. Pharm. Bull.* **2004**, *27*, 1226–1228.
15. Abe, F.; Nagafuji, S.; Okawa, M.; Kinjo, J. *Chem. Pharm. Bull.* **2006**, *54*, 1226–1228.
16. Silva, S. A. S.; Rodrigues, M. S. L.; Agra, M. F. A.; Cunha, E. V. L.; Barbosa-Filho, J. M.; Silva, M. S. *Biochem. Syst. Ecol.* **2004**, *32*, 359–361.
17. Menezes, J. E. S. A.; Lemos, T. L. G.; Pessoa, O. D. L.; Braz-Filho, R.; Montenegro, R. C.; Wilke, D. V.; Costa-Lotufo, L. V.; Pessoa, C.; de Moraes, M. O.; Silveira, E. R. *Planta Med.* **2005**, *71*, 54–58.
18. Rocha, D. D.; Militão, G. C. G.; Veras, M. L.; Pessoa, O. D. L.; Silveira, E. R.; Alves, A. P. N. N.; Moraes, M. O.; Pessoa, C.; Costa-Lotufo, L. V. *Life Sci.* **2006**, *79*, 1692–1701.
19. Pachler, K. G. R.; Underwood, W. G. E. *Tetrahedron* **1967**, *23*, 1817–1826.
20. Kozawa, M.; Morita, N.; Baba, K.; Hata, K. *Chem. Pharm. Bull.* **1977**, *25*, 515–516.
21. Berger, S.; Braun, S. In 200 and More NMR Experiments: A Practical Course. Ed.; Wiley-VCH: Verlag GmbH & Co. KGaA, Weinheim, Germany, 2004, 838p.
22. Dagne, E.; Bekele, A.; Waterman, P. G. *Phytochemistry* **1989**, *28*, 1897–1900.
23. Lirussi, D.; Li, J.; Prieto, J. M.; Gennari, M.; Buschiazzo, H.; Rios, J. L.; Zaidenberg, A. *Fitoterapia* **2004**, *75*, 718–723.
24. Fichoux, Y.; Rousseau, D.; Ferrua, B.; Ruette, S.; Leliévre, A.; Grousson, D.; Kubar, J. *Antimicrob. Agents Chemother.* **1998**, *42*, 654–658.
25. Ellis, J. A.; Fish, W. R.; Sileghem, M.; McOdimba, F. *Vet. Parasitol.* **1993**, *50*, 143–149.
26. Muelas-Serrano, S.; Nogal-Ruiz, J. J.; Gómez-Barrio, A. *Parasitol. Res.* **2000**, *86*, 999–1002.
27. Ferreira, V. F.; Jorqueira, A.; Souza, A. M. T.; da Silva, M. N.; de Souza, M. C. B. V.; Gouvêa, R. M.; Rodrigues, C. R.; Pinto, A. V.; Castro, H. C.; Santos, D. O.; Araújo,

H. P., ; Bourguignon, S. C. *Bioorg. Med. Chem.* **2006**, *14*, 5459–5466.
28. Espindola, L. S.; Junior, J. R. V.; de Mesquita, M. L.; Marquie, P.; de Paula, J. E.; Mambu, L.; Santana, J. M. *Planta Med.* **2004**, *70*, 1093–1095.
29. Tempone, A. G.; Borborema Treiger, S. E.; de Andrade, H. F., Jr.; de Amorim Gualda, N. C.; Yogi, A.; Salerno Carvalho, C.; Bachiega, D.; Lupo, F. N.; Bonotto, S. V.; Fischer, D. C. H. *Phytomedicine* **2005**, *12*, 382–390.