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Trypanocidal tetrahydrofuran lignans from *Peperomia blanda*

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Abstract

Five tetrahydrofuran lignans and two known flavones were isolated from the aerial parts of *Peperomia blanda*. The structures of the isolated lignans were elucidated by interpretation of their spectroscopic data, including by gHMQC and gHMBC. The relative and absolute configurations of the isolates were determined from NOESY interactions and optical properties, respectively. Four of the lignans were diastereomeric whilst one was of mixed biosynthetic origin. All but one of the lignans exhibited high *in vitro* trypanocidal activity when assayed against epimastigotes of *Trypanosoma cruzi* strain Y.

Keywords: *Peperomia blanda*; Piperaceae; Tetrahydrofuran lignans; Structural elucidation; Trypanocidal activity

1. Introduction

Chagas disease is one of the most widespread of tropical diseases, being endemic in 21 countries, with some 16–18 million individuals infected and a further 100 million people at risk (Molfetta et al., 2005; Pozas et al., 2005).

Significant antiprotozoal activities have recently been reported for a number of lignans derived from members of the Piperaceae family (Martins et al., 2003; Luize et al., 2006). This family, which is included among the basal angiosperms and can be found in the tropics mainly as pioneer plants, has been the subject of numerous phytochemical investigations. The major classes of compounds described include phenylpropanoids (Orjala et al., 1993), lignan/neolignans (Monache and Compagnone, 1996; Parmar et al., 1997; Benevides et al., 1999; Martins et al., 2003), pyrones (Singh, 1992), aliphatic and aromatic amides (Alécio et al., 1998; Navickiene et al., 2000; Silva et al., 2002), alkaloids (Dodson et al., 2000), polyketides (Cheng et al., 2003), benzoic acid derivatives (Bergamo et al., 2005; Morandim et al., 2005) and chromenes (Moreira et al., 1998; Baldoqui et al., 1999; Lago et al., 2004; Morandim et al., 2005; Salazar et al., 2005).

Piper and *Peperomia* represent the most important genera of the Piperaceae family with 2000 (Wanke et al., 2006) and 1700 species (Quijano-Abril et al., 2006), respectively. Species of *Peperomia* are well known as ornamental plants and have found application in folk medicine for the treatment of inflammation, asthma and gastric ulcers, and as analgesic and antibacterial agents (Arrigon-Blank et al., 2004). The major classes of compounds present in the relatively few species of *Peperomia* that have been investigated are prenylated phenols and benzoic acids (Monache and Compagnone, 1996; Seeram et al., 1998; Tanaka et al., 1998; Salazar et al., 2005), and also dibenzylbutyrolactone and dibenzylbutanediol lignans (Li et al., 2006).

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Additionally, several lignans (Li et al., 2007) and unusual seco- and nor-lignans have also been described (Bayma et al., 2000; Xu et al., 2006; Wu et al., 2006). More recently, two new tetrahydrofuran lignans were isolated from *Peperomia pellucida* (Xu et al., 2006) and thus a phenylpropanoid-based lineage for the *Peperomia* species can be supported.

Peperomia blanda H.B. & K. is a perennial herb that typically grows in wet rock crevices (Guimarães and Giordano, 2004). At the present time, information is only available concerning the essential oil (Santos et al., 2001) and two chromenes isolated from the non-polar fraction of the aerial parts of the species (Velozo et al., 2006). In the present work, we report the isolation and structural elucidation of four new tetrahydrofuran lignan stereoisomers and a new tetrahydrofuran lignan of mixed biosynthetic origin. The antitripanosomal properties of these novel lignans have been determined.

2. Results and discussion

The EtOAc extract derived from aerial parts of *P. blanda* was resuspended in MeOH:H_2O (4:1) and partitioned against hexane, CH_2Cl_2 and EtOAc. The portion soluble in CH_2Cl_2 was fractionated chromatographically to yield five lignans **1–5**; (Fig. 1) and two flavones (**6** and **7**). The ^{1}H NMR spectra of compounds **1–4** were characteristic of non-symmetric tetrahydrofuran lignans since each exhibited a pair of doublets at δ 0.63 ± 0.05 (CH_3-9) and 1.04 ± 0.04 (CH_3-9′) corresponding to methyl groups, a second set of doublets at δ 5.03 ± 0.02 (H-7) and 4.32 ± 0.03 (H-7′) that were assignable to oxybenzyl methine groups, and multiplets at δ 2.18 ± 0.03 and 1.69 ± 0.03 associated, respectively, with the H8 and H8′ protons. The ^{13}C NMR spectroscopic data corroborated the presence of the tetrahydrofuran system in each case, and all signals were assigned accordingly based on HMBC data (Tables 1 and 2).

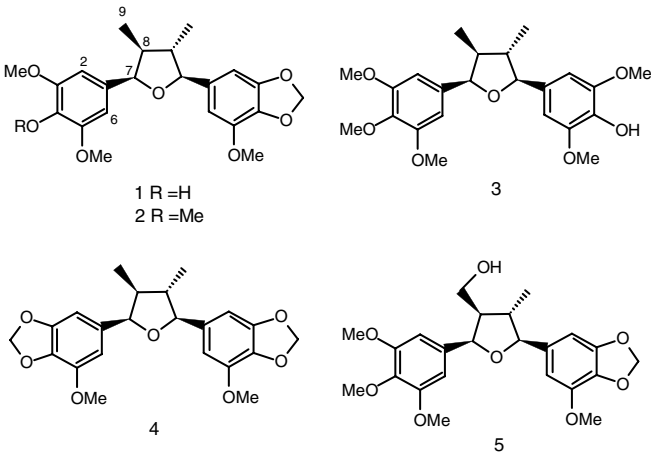

Fig. 1. Tetrahydrofuran lignans isolated from the aerial parts of *Peperomia blanda*.

As previously reported (Biftu and Stevenson, 1987; Barbosa-Filho et al., 1989), the coupling constants of 8.5 and 9.0 Hz for the doublet at δ 5.05, and of 4.33 Hz for H-7 and H-7′ indicate that these hydrogens are in a *trans* configuration with the adjacent H-8 and H-8′ in a tetrahydrofuran ring. Differential NOE experiments (Fig. 2) confirmed the relative stereochemistry of all of the lignans isolated. Thus, as for lignan 3, H-7 showed clear NOE correlations with H-7′ and H-8, H-7′ correlated with H-7, H-8 and H-9′, and H-8′ correlated with H-9 confirming the *trans* configuration of the adjacent H-8 and H-8′ and a *cis* configuration between H-7 and H-7′.

The differences observed in the NMR spectra of compounds **1–4** were due to different substitution patterns in the aromatic ring. The molecular formula of lignan 1 was established as $C_{22}H_{26}O_7$ by HRMS. The aromatic moieties were determined to be syringyl and 5-methoxy-3,4-methylenedioxyphenyl groups on the following basis: (i) two equivalent aromatic hydrogens at δ 6.49 (*s*, 2H) correlated to H7 in the HMBC spectrum, (ii) aromatic carbons C3 and C5 displayed correlations to OCH_3 (δ 3.80) and to H2/H6, and (iii) the second aromatic ring was not symmetrical and displayed two singlets at δ 6.61 (H2′) and 6.64 (H6′), respectively, which showed cross peaks to C-4′ and C-7′ in the HMBC spectrum. Thus, **1** was established as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4-hydroxy-4′,5′-methylenedioxy-3,5,3′-trimethoxy-7,7′-epoxylignan.

Compound **2** showed a molecular formula of $C_{23}H_{28}O_7$ by HRMS. ^{1}H NMR spectra indicated two equivalent aromatic hydrogens at δ 6.48 (*s*, 2H) correlating to H7 in the HMBC spectrum and aromatic carbons C3, C5 at δ 152.8 displayed correlations to OCH_3 (δ 3.77) and to H2/H6 and subsequently, C4 at δ 136.6 displayed correlations to OCH_3 (δ 3.76). The second aromatic ring was not symmetrical and displayed two singlets at δ 6.61 (H2′) and 6.64 (H6′), respectively, which showed cross peaks to C-4′ and C-7′ in the HMBC spectrum. Thus, **2** was identified as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4′,5′-methylenedioxy-3,4,5,3′-tetramethoxy-7,7′-epoxylignan, i.e., a methylated derivative of **1**.

The molecular formula of **3** was determined to be $C_{23}H_{30}O_7$ by HRMS, and the aromatic moieties were identified as 3,4,5-trimethoxyphenyl and 4-hydroxy-3,5-dimethoxyphenyl groups. The aromatic singlets at δ 6.49 and 6.67 were assigned to separate aromatic groups, since the HMBC showed correlation to H7 (δ 5.03) and H7′ (δ 4.34), respectively. The remaining correlations between C3/C3′, C4/C4′ and C5/C5′ perfectly matched those of H2/H2′and H6/H6′. Thus compound **3** was determined as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-7,7′-epoxylignan.

Compound **4** showed the molecular formula $C_{22}H_{24}O_7$ by HRMS, and both aromatic moieties were identified as 5-methoxy-3,4-methylenedioxyphenyl groups for the signals at δ 5.97 (*s*, 2H) and δ 5.96 (*s*, 2H) due to methylenedioxyphenyl groups connected to C4/C5 and C4′/C5′, respectively. At δ 3.93 (*s*, 3H) and δ 3.90 (*s*, 3H) are the resonances of two methoxyl groups correlated to C3 and C3′.

Table 1
^{1}H NMR spectroscopic data of the tetrahydrofuran lignans **1**–**5** isolated from *P. blanda* in $CDCl_3$

H	1	2	3	4	5
2	6.49 (*s*)	6.48 (*s*)	6.49 (*s*)	6.52 (*s*)	6.61 (*s*)
6	6.49 (*s*)	6.48 (*s*)	6.49 (*s*)	6.54 (*s*)	6.61 (*s*)
7	5.00 (*d*, 8.5)	5.02 (*d*, 8.5)	5.03 (*d*, 9.0)	5.05 (*d*, 8.5)	5.07 (*d*, 8.0)
8	2.15 (*m*)	2.16 (*m*)	2.18 (*m*)	2.20 (*m*)	2.31 (*m*)
9	0.59 (*d*, 7.0)	0.61 (*d*, 7.5)	0.62 (*d*, 7.0)	0.68 (*d*, 7.0)	3.40 (*d*, 6.0)
2′	6.61 (*s*)	6.61 (*s*)	6.67 (*s*)	6.69 (*s*)	6.59 (*d*, 1.5)
6′	6.64 (*s*)	6.65 (*s*)	6.67 (*s*)	6.69 (*s*)	6.63 (*d*, 1.5)
7′	4.29 (*d*, 9.5)	4.30 (*d*, 9.0)	4.34 (*d*, 9.5)	4.33 (*d*, 9.0)	4.29 (*d*, 8.5)
8′	1.67 (*m*)	1.68 (*m*)	1.71 (*m*)	1.72 (*m*)	1.93 (*m*)
9′	1.00 (*d*, 6.5)	1.01 (*d*, 6.5)	1.02 (*d*, 6.5)	1.06 (*d*, 6.5)	1.08 (*d*, 7.0)
O_2CH_2 (4,5)	–	–	–	5.97 (*s*)	–
O_2CH_2 (4′,5′)	5.89 (*s*)	5.89 (*s*)	–	5.96 (*s*)	5.91 (*s*, 2H)
OCH_3 (3)	3.80 (*s*)	3.77 (*s*)	3.76 (*s*)	3.93 (*s*)	3.80 (*s*)
OCH_3 (4)	–	3.76 (*s*)	3.77 (*s*)	–	3.77 (*s*)
OCH_3 (5)	3.80 (*s*)	3.77 (*s*)	3.76 (*s*)	–	3.86 (*s*)
OCH_3 (3′)	3.84 (*s*)	3.85 (*s*)	3.84 (*s*)	3.90 (*s*)	3.86 (*s*)
OCH_3 (4′)	–	–	–	–	–
OCH_3 (5′)	–	–	3.84 (*s*)	–	–

Table 2
^{13}C NMR spectroscopic data of the tetrahydrofuran lignans **1**–**5** isolated from *P. blanda* in $CDCl_3$

C	1	2	3	4	5
1	132.2	137.0	136.7	135.4	134.9
2	103.8	104.5	104.2	106.6	103.5
3	146.7	152.8	152.9	143.5	153.4
4	133.8	136.6	136.7	134.2	137.5
5	146.7	152.8	152.9	149.0	153.4
6	103.8	104.5	104.2	101.2	103.5
7	83.3	83.2	83.2	83.1	81.3
8	47.9	45.8	45.9	48.2	53.8
9	14.9	15.0	15.2	15.0	62.5
1′	135.6	135.4	131.9	135.8	135.0
2′	100.4	106.6	103.4	106.5	106.7
3′	143.5	143.4	147.0	143.2	143.5
4′	134.7	134.6	134.4	134.7	135.0
5′	149.0	149.0	147.0	148.6	149.1
6′	106.7	100.3	103.4	100.5	100.4
7′	87.4	87.3	87.5	87.4	87.6
8′	46.0	47.8	47.7	45.9	44.1
9′	15.1	14.8	14.8	14.9	16.2
O_2CH_2 (4,5)	–	–	–	101.3	–
O_2CH_2 (4′,5′)	101.4	101.3	–	101.4	101.4
OCH_3 (3)	56.3	56.0	56.0	56.6	56.1
OCH_3 (4)	–	60.7	60.8	–	60.9
OCH_3 (5)	56.3	56.0	56.0	–	56.1
OCH_3 (3′)	56.7	56.6	56.3	56.6	56.7
OCH_3 (5′)	–	–	56.3	–	–

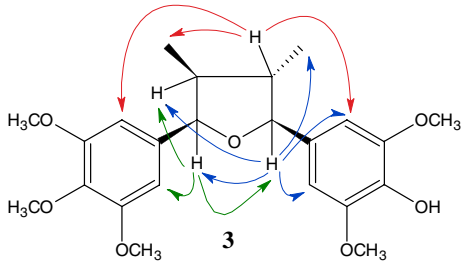

Fig. 2. The NOESY interactions observed for *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-7,7′-epoxylignan (**3**).

The identity of **4** was thus established as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4,5,4′,5′-dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan.

The molecular formula of **5** was established as $C_{23}H_{28}O_8$ by HRMS. The compound differed from lignans **1**–**4** in respect to a hydroxyl group linked to C-9. The presence of this group was deduced from various sources: the IR spectrum (absorption at 3450 cm^{-1}), from the ^{1}H NMR by signals at δ 3.40 (2H, m), from the ^{13}C NMR both from resonances at δ 62.5 and the presence of only one methyl group (δ 1.08; *d*, $J = 7.0$ Hz), and from the HMBC, which showed connectivity between the H9 protons and C7. Thus, **5** was determined as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-9-hydroxy-4′,5′-methylenedioxy-3,4,5,3′-tetramethoxy-7,7′-epoxylignan.

The known flavones 5-hydroxy-4′,7,8-trimethoxyflavone (**6**) and 5-hydroxy-3′,4′,7,8-tetramethoxyflavone (**7**) were also identified in the extract derived from the aerial parts of *P. blanda.* These compounds have been previously isolated from *Peperomia sui* (Cheng et al., 2003).

Compounds **1**–**5** were submitted to bioassay against epimastigotes of *Trypanosoma cruzi* strain Y and the respective IC_{50} values are shown in Table 3. All of the lignans exhibited potent trypanocidal activity. Compound **4**, bearing two methylenedioxyphenyl groups, was more

Table 3
Antiprotozoal activities of the tetrahydrofuran lignans **1**–**5** isolated from *Peperomia blanda* against *T. cruzi* epimastigotes

Compound	IC_{50} μg.mL^{-1} against epimastigotes
1	18.6
2	12.6
3	23.7
4	9.6
5	25.4
Benznidazole[a]	8.6

[a] Positive control.

active than other lignans possessing one or no methylenedioxyphenyl group.

2.1. Concluding remarks

In this work, we describe the isolation and structural elucidation of five new tetrahydrofuran lignans from the aerial part of *P. blanda*. The trypanocidal activity of these compounds was evaluated against epimastigotes of *T. cruzi* strain Y. All of the isolates showed potent activity when compared with the positive control benznidazole.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 spectrometer at 500 and 125 MHz, respectively, with $CDCl_3$ as solvent and TMS as reference. HREIMS were measured using a Bruker Daltronics model ultrOTOF$_Q$ ESI-TOF instrument. HPLC separations were performed on a Varian PrepStar model SD-1 LC/UV/VIS chromatograph equipped with a Supelcosil C-18 reversed phase column (250 × 21.2 mm; Supelco). IR spectra were measured in KBr pellets in a Perkin–Elmer Infrared spectrometer FTIR series 1600. Optical rotations were measured at $\lambda = 589$ nm in a digital polarimeter JASCO model DIP-370. UV spectra were recorded in MeOH using a HP 8452 A spectrophotometer. Separations by CC were carried out using silica gel (230–400 mesh; Merck). All solvents were redistilled prior to use.

3.2. Plant material

The aerial parts of *P. blanda* were collected at the Reserva da Ripasa, Ibaté – SP, Brazil in January of 2005 and identified by Dra. Elsie Franklin Guimarães. A voucher specimen (Kato-547) has been deposited at the Herbarium of the Instituto de Biociências, Universidade de São Paulo, São Paulo – SP, Brazil.

3.3. Isolation of compounds

Dried aerial parts (86 g) of *P. blanda* were milled, extracted with EtOAc (1.2 L) for 4 hours at room temperature, and the extract was concentrated in vacuo to yield a crude extract (8.5 g). This extract was resuspended in MeOH:H_2O (4:1) and partitioned against hexane, CH_2Cl_2 and EtOAc. The portion soluble in CH_2Cl_2 (1.95 g) was submitted to CC over silica gel eluted with a gradient of hexane–EtOAc to yield fractions 1-14. The precipitate present in fraction 3 (420 mg) was purified by recrystallisation in MeOH to yield compound **6** (6.3 mg), whilst the soluble part was submitted to flash CC over silica gel eluted with a gradient of hexane–EtOAc producing subfractions 3-1 to 3-60. Subfractions 3-20 (12 mg) and 3-43 (46 mg) afforded compounds **4** and **2**, respectively. Fraction 4 (220 mg) was submitted to flash CC over silica gel eluted with a gradient of hexane–EtOAc providing subfractions 4-1 to 4-37. Subfraction 4-23 (98 mg) was submitted to preparative HPLC eluted with MeOH:H_2O (72:28) to afford compound **1** (8.6 mg). The precipitate present in fraction 6 (103 mg) was purified by recrystallisation in MeOH to yield compound **7** (8.0 mg). Fraction 7 (350 mg) was subjected to flash CC over silica gel eluted with a gradient of hexane–EtOAc resulting in subfractions 7-1 to 7-167. Subfraction 7-140 afforded compound **5** (7 mg). Subfraction 7-79 (95.0 mg) was submitted to preparative HPLC eluted with MeOH:H_2O (2:3) to yield five fractions, the penultimate of which afforded compound **3** (17 mg).

3.4. rel-(7R,8S,7′S,8′S)-4-Hydroxy-4′,5′-methylenedioxy-3,5,3′-trimethoxy-7,7′-epoxylignan (*1*)

Pale yellow oil, $[\alpha]_D^{21}$ −52.8 (MeOH; *c* 0.125). UV λ_{max}^{MeOH} nm (log ε): 254 (4.1), 273 (3.7). IR υ_{max}^{KBr} cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091, 1031. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. HRMS/ESI-TOF *m/z* (rel. int.): 441.1276 $[M+K]^+$ (100), 425.1528 $[M+Na]^+$ (50), 403.1735 $[M+H]^+$ (22) (calcd for $C_{22}H_{26}O_7$, 402.4376). Found: C, 65.59; H, 6.48. $C_{22}H_{26}O_7$ requires: C, 65.66; H, 6.51%.

3.5. rel-(7R,8S,7′S,8′S)-4′,5′-Methylenedioxy-3,4,5,3′-tetramethoxy-7,7′-epoxylignan (*2*)

Pale yellow oil, $[\alpha]_D^{21}$ −30.7 (MeOH; *c* 0.225). UV λ_{max}^{MeOH} nm (log ε): 254 (4.1), 273 (3.7). IR υ_{max}^{KBr} cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091, 1031. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. HRMS/ESI-TOF *m/z* (rel. int.): 455.1418 $[M+K]^+$ (100), 439.1665 $[M+Na]^+$ (51), 417.1872 $[M+H]^+$ (13) (calcd for $C_{23}H_{28}O_7$, 416.4642). Found: C, 66.26; H, 6.81. $C_{23}H_{28}O_7$ requires: C, 66.33; H, 6.78%.

3.6. rel-(7R,8S,7′S,8′S)-4′-Hydroxy-3,4,5,3′,5′-pentamethoxy-7,7′-epoxylignan (*3*)

Pale yellow oil, $[\alpha]_D^{21}$ −31.2 (MeOH; *c* 0.125). UV λ_{max}^{MeOH} nm (log ε): 254 (4.1), 273 (3.7). IR υ_{max}^{KBr} cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091, 1031. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. HRMS/ESI-TOF *m/z* (rel. int.): 447.1564 $[M+K]^+$ (100), 441.1844 $[M+Na]^+$ (42), 419.2032 $[M+H]^+$ (6) (calcd for $C_{23}H_{30}O_7$, 418.4801). Found: C, 66.05; H, 7.16. $C_{23}H_{30}O_7$ requires: C, 66.01; H, 7.23%.

3.7. rel-(7R,8S,7′S,8′S)-4,5,4′,5′-Dimethylenedioxy-3,3′-dimethoxy-7,7′-epoxylignan (*4*)

Pale yellow oil, $[\alpha]_D^{21}$ −25.6 (MeOH; *c* 0.125). UV λ_{max}^{MeOH} nm (log ε): 254 (4.2), 277 (3.6). IR υ_{max}^{KBr} cm^{-1}: 2957, 2894, 1634, 1593, 1505, 1459, 1426, 1130, 1096, 1033. For ^{1}H

and ^{13}C NMR spectra, see Tables 1 and 2. HRMS/ESI-TOF *m/z* (rel. int.): 439.1106 $[M+K]^+$ (100), 423.1360 $[M+Na]^+$ (27), 401.1541 $[M+H]^+$ (11) (calcd for $C_{22}H_{24}O_7$, 400.4215). Found: C, 66.02; H, 5.96. $C_{22}H_{24}O_7$ requires: C, 65.99; H, 6.04%.

3.8. rel-(7R,8S,7′S,8′S)-9-Hydroxy-4′,5′-methylenedioxy-3,4,5,3′-tetramethoxy-7,7′-epoxylignan (5)

Pale yellow oil, $[\alpha]_D^{21}$ −29.1 (MeOH; *c* 0.200). UV λ_{max}^{MeOH} nm (log ε): 254 (4.1), 273 (3.7). IR υ_{max}^{KBr} cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091, 1031. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. HRMS/ESI-TOF *m/z* (rel. int.): 471.1408 $[M+K]^+$ (10), 455.1666 $[M+Na]^+$ (8) (calcd for $C_{23}H_{28}O_8$, 432.4636). Found: C, 63.71; H, 6.65. $C_{23}H_{28}O_8$ requires: C, 63.88; H, 6.52%.

3.9. In vitro bioassays

The biological assays with epimastigote forms of *T. cruzi* strain Y were performed as described previously (Bernacchi et al., 2002). Tetrahydrofuran lignans and benznidazole (Roche, Rio de Janeiro, Brazil) were stored as 1.0 mg ml^{-1} stock solutions in dimethyl sulphoxide (DMSO), and were serially diluted (1:3) prior to use with liver-infusion tryptose (LIT) medium to provide concentrations of 0.41, 1.23, 3.70, 11.1, 33.3, 100.0 and 300.0 μg ml^{-1}, each in triplicate. The number of viable protozoa was determined by counting in a Neubauer chamber after 72 h incubation at 28 °C. The 50% inhibitory concentration (IC_{50}) values were calculated by fitting a nonlinear regression curve (sigmoidal dose response, variable slope) and comparing with those of benznidazole, the positive control. A one-way ANOVA test was used to establish the significance level of the activity corresponding to each compound evaluated in the *in vitro* assay.

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