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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and leishmanicidal activity of cinnamic acid esters: structure–activity relationship

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Abstract Several cinnamic acid esters were obtained via Fischer esterification of cinnamic acids derivatives with aliphatic alcohols. Structures of the products were elucidated by spectroscopic analysis. The synthesized compounds were evaluated for antileishmanial activity against *L. (V) panamensis* amastigotes and cytotoxic activity was evaluated against mammalian U-937 cells. The compounds **11**, **15**–**17,** and **23**, were active against *Leishmania* parasite and although toxic for mammalian cells, they still are potential candidates for antileishmanial drug development. A SAR analysis indicates that first, while smaller alkyl chains lead to higher selectivity indices (**10**, **11** vs. **12**–**17**); second, the degree of oxygenation is essential for activity, primarily in positions 3 and 4 (**17** vs. **18**–**20** and **22**); and third, hydroxyl groups increase both activity and cytotoxicity (**14** vs. **23**). On the other hand, the presence of a double bond in the side chain is crucial for cytotoxicity and leishmanicidal activity (**12** vs. **21**). However, further studies are required to optimize the structure of the promising molecules and to validate the in vitro activity against *Leishmania* demonstrated here with in vivo studies.

Keywords Leishmaniasis · Antiprotozoal · Caffeic acid · Cinnamic acid ester

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Introduction

Leishmaniasis is one of the world's most neglected diseases, affecting largely the poorest of the poor, mainly in developing countries; 350 million people are considered at risk of contracting leishmaniasis, and some two million new cases occur yearly (Murray *et al.,* 2005); the WHO has classified leishmaniasis as a category 1 disease, i.e., emerging and uncontrolled (http://www.who.int/topics/leishmaniasis/en/). This disease has symptoms from skin lesions to fatal systemic infection caused by protozoan parasites of the *Leishmania* species (Handman, 1999). Recently, a dramatic increase in the number of cases of leishmaniasis has been observed in patients with compromised T cell function, such as those infected with the human immunodeficiency virus (Wolday *et al.,* 1999). Drugs currently in use, such as the antimony derivative glucantime, the bis-amidines, pentamidine, and stilbamidine or the glycomacrolide amphotericin B, display high liver and heart toxicities, develop clinical resistance after a few weeks of treatment, and moderate and severe side effects (Desjeux, 2004; Ouellette *et al*., 2004; Barrett and Gilbert, 2002; Croft and Coombs, 2003; Faraut-Gambarelli *et al*., 1997; Olliaro and Bryceson, 1993). For these reasons, it becomes necessary to discover new, more potent, and selective agents for treating this increasing parasitosis.

Caffeic acid (**1**), 3,4-dihydroxy cinnamic acid, and its esters derivatives exhibit a broad spectrum of biological activities, including anti-inflammatory (Jayaprakasam *et al*., 2006; Da Cunha *et al*., 2004), antimicrobial (Almajano *et al*., 2007; Noriaki *et al*., 2005; King *et al*.,1999; Valenta *et al*., 1998; Bowles and Miller, 1994) antioxidant (Hung *et al*., 2005; Noriaki *et al*., 2005; Kikuzaki *et al*., 2002; Son and Lewis, 2002; Rajan *et al*., 2001), and anticarcinogenic effects (De *et al*., 2011). In addition, some studies showed that these compounds have high leishmanicidal activity and high cytotoxicity (Cabanillas *et al*., 2010; Radtke *et al*., 2003) (Fig. 1).

In the search for active compounds with low toxicity for the treatment of leishmaniasis and based on the fact that the biological activity (including leishmaninicidal activity and cytotoxicity) of molecules can be modified by the methylation of the aromatic hydroxyl group (Aponte *et al*., 2010; Brenzan *et al*., 2008), several cinnamic acid esters analogs were synthesized and their cytotoxic and leishmanicidal activities were determined.

Results and discussion

Chemistry

The design of the compounds were based on both electronic and steric modifications, which modify the reactivity of the molecule. Cinnamic acids **5–9** were obtained via Knoevenagel condensation between malonic acid and different benzaldehydes in water under microwave heating (Gupta and Purnima, 2007). These compounds were dissolved in thionyl chloride, the solution was stirred and refluxed during 4 h. Then, the mixture was concentrated under vacuum and the residue, the respective acid chloride, was added to a solution of cetyl alcohol (dodecyl or estearyl alcohol) in dichloromethane, the mixture was stirred and monitored by thin layer chromatography (Narasimhan *et al*., 2004). After evaporation and purification by column chromatography, a total of six compounds **15–20** were obtained. Compound **22** was similarly obtained by starting from cinnamic acid. On the other hand, 3,4-dimethoxycinnamic acid **5** was dissolved in methanol (propanol, pentanol, hexanol or octanol), acetyl chloride was added and the solution was stirred under reflux to complete the reaction. Following evaporation and purification by column chromatography a total of five compounds **10–14** were obtained (De Campos *et al*., 2009). Compound **23** was similarly obtained by starting from caffeic acid. Compound **21** was obtained by catalytic hydrogenation of compound **12** (Cardona *et al*., 2006) (Scheme 1).

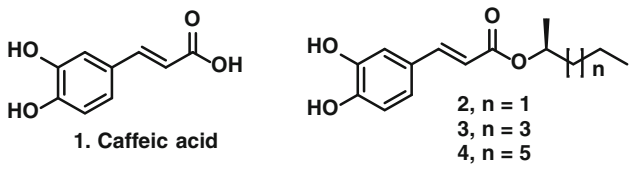


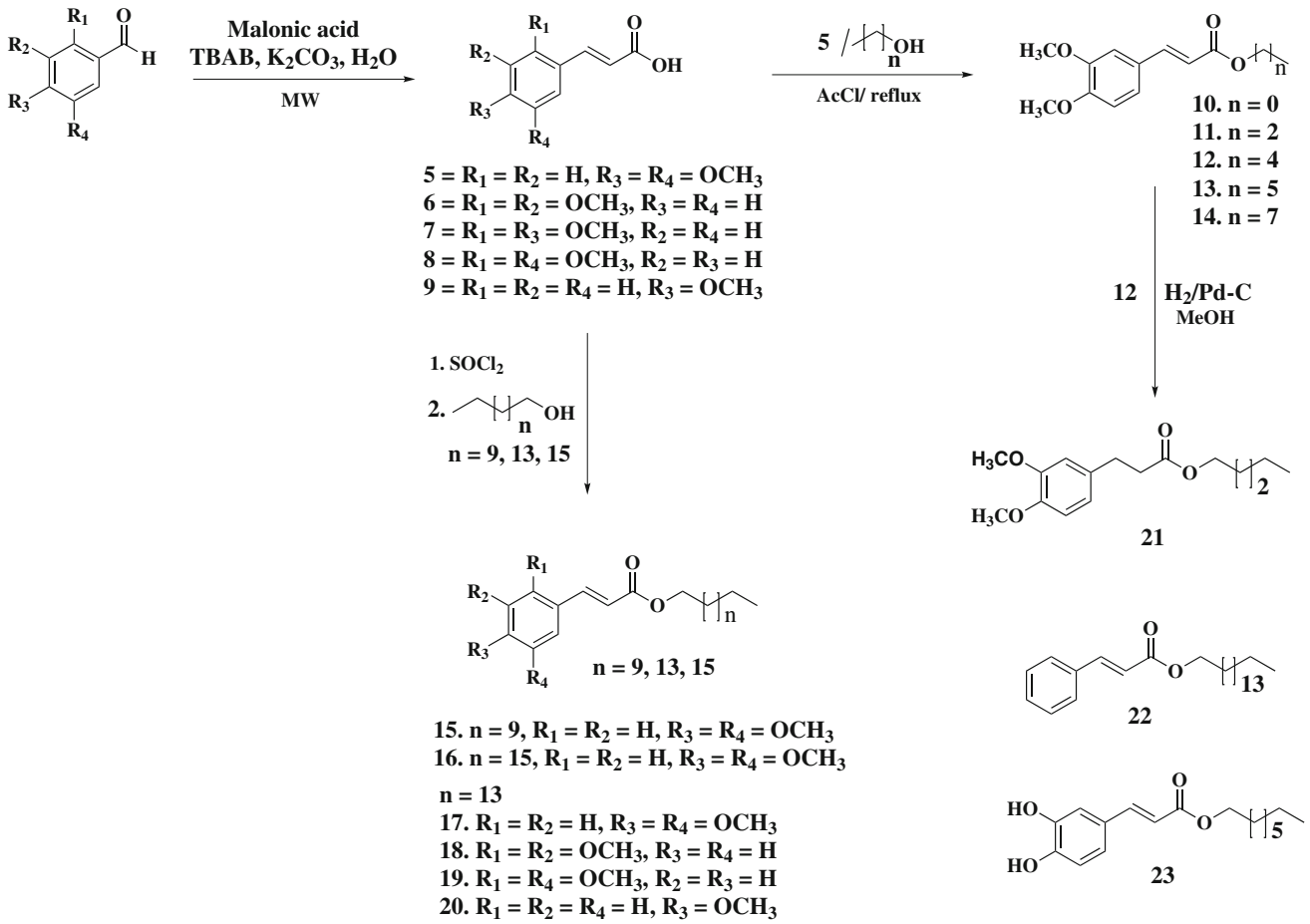
Fig. 1 Caffeic acid and its esters derivatives

Antileishmanial and cytotoxic activities

The leishmanicidal activity and cytotoxicity of the synthesized compounds were evaluated following the method previously reported in the literature (Pulido *et al*., 2012; Taylor *et al*., 2011, 2010;Varela *et al*., 2009; Robledo *et al*., 2005, 1999; Weninger *et al*., 2001). The leishmanicidal activity and cytotoxicity were reported as effective concentration (EC_{50}) and lethal concentration (LC_{50}) values of compounds respectively, and are shown in Tables 1 and 2.

According to the results shown in Table 1, only compounds **10–13**, **15**, **17**, and **23** showed activity against axenic amastigotes of *L. (V) panamensis* with EC_{50} lower than 100 μg/ml. The most active compound was **23** (EC_{50} = 2.3 μg/ml) followed by **11** and **12** exhibiting EC_{50} values of 33.0 and 38.1 μg/ml, respectively. Compounds **10**, **13**, **15**, and **17** showed a moderate leishmanicidal activity with an EC_{50} ranging between 55.0 and 60.0 μg/ml. On the other hand, a high toxicity activity was measured for compounds **11–17** and **23**, with LC_{50} <100 μg/ml. The lower toxic activity was obtained for compound **10** (EC_{50} = 162.6 μg/ml). No leishmanicidal activity or toxicity was detected for compounds **18–22** (EC_{50} values higher than 100 μg/ml and LC_{50} higher than 200 μg/ml, respectively). The best selectivity index was observed for compounds **23**, **10**, and **11** with values of 4.3, 3.0, and 2.6, respectively. Compound **23** is structurally similar to compound **4** reported by Cabanillas *et al*. 2010. Although the EC_{50} and LC_{50} values reported previously for compound **4** were slightly different, (EC_{50} = 0.55 μg/mL and LC_{50} 3.9 μg/mL), both compounds remain highly active against *Leishmania* parasites. Differences in the *Leishmania* species and cell type used in the assays may explain the different results obtained for compounds **23** and **4**. Thus, compound **4** was tested against axenic amastigotes of *L. (L) amazonensis* and murine peritoneal macrophages (Cabanillas et al., 2010) whereas compound **23** was tested in this report against axenic amastigotes of *L. (V) panamensis* and human macrophages.

The leishmanicidal activity against the intracellular forms of *L. (V) panamensis* was also measured for those compounds that were active against axenic forms. Thus, compounds **14**, and **18–22** were not evaluated against intracellular amastigotes. The compounds **11**, **15–17**, and **23** were active (Table 2); while in compounds **10**, **12** and **13,** the leishmanicidal activity was lower than the cytotoxicity and therefore the EC_{50} values



Scheme 1 Synthetic pathway to cinnamic acid ester derivatives

for these compounds (Table 2) were higher than the LC_{50} values (Table 1). The most active compounds for intracellular parasites were **23** and **16** with EC_{50} of 3.2 and 18.3 μg/ml, respectively (Table 2), followed by compounds **15** and **17** with EC_{50} values of 25.2 and 26.5 μg/ml. Compound **11** had the lowest activity with EC_{50} 60.2 μg/ml. The best SI was observed for compounds **23**, **17**, and **11** with values of 3.1, 2.7, and 2.6, respectively. As expected, meglumine antimoniate was only active against intracellular amastigotes of *L. (V) panamensis* (Table 2) but did not show activity against axenic amastigotes that is an extracellular form of this parasite (Table 1). In turn, amphotericine B was active against both intracellular and axenic amastigotes (Table 1 and 2).

Overall, compounds **11**, **15, 17** and **23** were apparently the most active, showing activity against both axenic and intracellular amastigotes of *L. (V) panamensis,* while compounds **10, 12**, and **13** showed activity only on the axenic form of this *Leishamania* species.

Meglumine Antimoniate and Amphotericin B, drugs currently used for the treatment of Leishmaniasis had SI values greater than 1, leading to greater activity against the parasite than the toxicity against the host cell. Cinnamic acid esters **10–12**, **17**, and **23** have also SI values greater than 1. The in vitro activity of these molecules could be improved in further studies by modifying their structure. Further studies are also required to validate the in vitro results with in vivo studies.

In general, as observed in other studies, no correlation was found between the activity shown by the compounds against intracellular and extracellular (axenic) forms of *L. (V) panamensis* parasite. This lack of correlation may be related to the fact that the activity of a compound may be due to direct action of the compound on the parasite (which can be detected in the in vitro system using axenic amastigotes) or the formation of a derivative product of metabolism that the compound suffers when it is internalized by the host cell (activity observed in the in vitro system using intracellular amastigotes).

Structure–activity relationship

A relationship between the leishmanicidal activity against axenic amastigotes and compounds structure was observed (see Table 1). First, while smaller alkyl chains lead to

Table 1 In vitro toxicity and leishmanicidal activity against axenic amastigotes of *L. (V) panamensis* of Cinnamic acid ester

Compound	Cytotoxicity U937 cells LC_{50} (μg/ml)[a]	Leishmanicidal Activity EC_{50}(μg/ml)[b]	SI
10	162.6 ± 49.6	55.0 ± 14.7	3.0
11	85.3 ± 17.5	33.0 ± 0.2	2.6
12	49.8 ± 6.3	38.1 ± 9.7	1.3
13	54.7 ± 7.7	60.0 ± 4.0	0.9
14	79.9 ± 5.5	>100.0	<0.8
15	49.7 ± 18.1	59.0 ± 1.9	0.8
16	28.5 ± 1.9	108.2 ± 1.5	0.3
17	69.1 ± 6.7	55.4 ± 2.0	1.2
18	>200.0	>100.0	<2.0
19	>200.0	>100.0	<2.0
20	>200.0	>100.0	<2.0
21	>200.0	>100.0	<2.0
22	>200.0	>100.0	<2.0
23	9.9 ± 1.7	2.3 ± 0.5	4.3
Meglumine antimoniate	495,9 + 55,6	>200.0	<2.3
Amphotericin B	29.6 ± 4.7	0.05 ± 0.01	592

LC_{50} Lethal concentration 50

EC_{50} Effective concentration 50

SI selectivity index: LC_{50}/EC_{50}

[a] Degree of toxicity: Highly toxic: $LC_{50} < 10$ μg/ml, toxic: $LC_{50} > 10$ to < 50 μg/ml; Moderately toxic: $LC_{50} > 50$ to < 200 μg/ml and potentially non toxic: $LC_{50} > 200$ μg/ml

[b] Degree of activity: Highly Active: $EC_{50} < 10$ μg/ml; Active: > 10 to < 50 μg/ml; Moderately active: $EC_{50} > 50$ to < 100 μg/ml; No Active: $EC_{50} > 100$ μg/ml

Table 2 In vitro activity of cinnamic acid ester against intracellular amastigotes of *L. (V) panamensis*

Compound	Leishmanicidal activity EC_{50}(μg/ml)	SI
10	>162.6 ± 49.6	<1.0
11	60.2 ± 1.2	2.6
12	>49.8 ± 6.3	<1.0
13	>54.7 ± 7.7	<1.0
15	25.2 ± 2.3	2.0
16	18.3 ± 3.3	1.6
17	26.5 ± 2.0	2.7
23	3.2 ± 0.8	3.1
Meglumine antimoniate	6.3 + 0.9	78.7
Amphotericin B	0.06 ± 0.01	592

EC_{50} Effective concentration 50

SI selectivity index: LC_{50}/EC_{50}

Active: $EC_{50} < 50$ μg/ml; Moderately active: $EC_{50} < 100$ μg/ml; No active: $EC_{50} > 100$ μg/ml

higher selectivity indices (**10**, **11** vs. **12–17**); second, the degree of oxygenation is essential for activity, primarily in positions 3 and 4 (**17** vs. **18–20** and **22**); and third, hydroxyl groups increase both activity and cytotoxicity (**14** vs. **23**), This result is in agreement with the reports by Aponte *et al*. 2010 and by Brezan *et al*. 2008, in which the leishmanicidal activity and cytotoxicity of chalcones and coumarins was found to be enhanced by the presence of polar hydroxyl groups. On the other hand, the presence of a double bond in the side chain is crucial for cytotoxicity and leishmanicidal activity (**12** vs. **21**). The action of these compounds may be explained by conjugated addition of nucleophilic amino acid residues present in biomolecules of the natural receptors, Michael type mechanism. This mechanism has been reported for other α, β-unsaturated compounds such as lactones, chalcones and coumarins (De Fatima *et al*., 2006; Buck *et al*., 2003; Cardona and Saez, 2011).

Conclusion

The design, synthesis, and antileishmanial screening of fourteen cinnamic acid esters was reported. Several of the reported compounds have potential as leishmanicidal drugs, as determined by both leishmanicidal activity and cytotoxicity. A structure–activity relationship was observed for these compounds where the length of the alkyl chains, the degree of oxygenation or the presence of hydroxyl groups may modify the leishmanicidal activity but also the cytotoxicity. Overall, the compounds **11**, **15–17**, and **23** are potential candidates for antileishmanial drug development based on their activity against *Leishmania* parasite. Although toxic for U-937 cells, more studies on toxicity using other cell types are needed in order to discriminate whether the toxicity shown by these compounds is against tumor or non-tumor cells. In addition, further studies are required to optimize the structure of these promising molecules and to validate the in vitro activity against *Leishmania* demonstrated here with in vivo studies.

Experimental procedures

Chemistry

IR spectra were recorded on a Perkin-Elmer Spectrum RX I FT-IR system in a KBr disk. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity 500 MHz spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. Chemical shifts are expressed in δ ppm. High resolution mass spectra were recorded by the electron impact mode (EIMS, 70 eV) on VG AutoSpec spectrometer. Silica gel 60

(Merck 0.063–0.200 mesh) was used for column chromatography, and precoated silica gel plates (Merck 60 F254 0.2 mm) were used for TLC.

*Synthesis of cinnamic acid esters (**10–14**)*

3,4-dimethoxycinnamic acid (10 mmol), acetyl chloride (0.5 mmol), and 50 mL of alcohol (methanol, propanol, pentanol, hexanol or octanol), were placed in a 250 ml 3-neck round-bottom flask equipped with a magnetic stirring bar. The mixture was stirred, heated to reflux for a period of 4 h. The reaction mixture was concentrated on a rotatory evaporator, and the residue was purified by chromatographic column over silica gel eluted with a mixture hexane–ethyl acetate at different ratios to obtain the cinnamic acid esters in yield between 75 and 85 %.

*Synthesis of cinnamic acid esters (**15–21**)*

Cinnamic acid, **5–9** (10 mmol) and thionyl chloride (10 mL) were placed in a 50 ml 3-neck round-bottom flask equipped with a magnetic stirring bar. The mixture was stirred, heated to reflux for a period of 4 h. The reaction mixture was concentrated on a rotatory evaporator, and the residue was added to a solution of cetyl alcohol (dodecyl or stearyl alcohol) in dichloromethane and the mixture was stirred and monitored by thin layer chromatography. The reaction was complete after about 4 h. The mixture was transferred to a separatory funnel and then quenched by addition of a solution of 20 mL of potassium carbonate; the organic layer was washed with water, separated, dried on anhydrous sodium sulfate, and filtered and concentrated under reduced pressure. The residue was chromatographed over silica gel (hexane–ethyl acetate, different ratios) to obtain the cinnamic acid esters in yield between 70 and 85 %.

*Methyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**10**)*

Yield: 7.4 mmol, 1.643 g, 74 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2945 (C–H), 1700 (C=O), 1627 (C=C), 1510 ($C{=}C_{Ar}$), 1270 (C–O–C), 1178 ((C=O)–O), 857 ($C{-}H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.78 (3H, s, OCH_3), 3.90 (6H, s, OCH_3), 6.30 (1H, d, $J = 15.9$ Hz, –CO–CH=), 6.86 (1H, d, $J = 8.0$ Hz, Ar–H), 7.04 (1H, d, $J = 1.6$ Hz, Ar–H), 7.10 (1H, dd, $J = 8.0$, 1.6 Hz, Ar–H), 7.63 (1H, d, $J = 15.9$ Hz, Ar–CH = C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 51.46 (OCH_3), 55.83 (OCH_3), 55.91 (OCH_3), 109.50, 110.88, 115.51 (=C–CO–),122.57, 127.38, 144.70 (Ar–C=), 149.18, 151.09, 167.40 (C=O). EIMS: *m/z* 223.0972 (M+1), Calcd. for $C_{12}H_{14}O_4$: 222,09.

*Propyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**11**)*

Yield: 7.0 mmol, 1.751 g, 70 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2967 (C–H), 1706 (C=O), 1635 (C=C), 1513 ($C{=}C_{Ar}$), 1260 (C–O–C), 1177 ((C=O)–O), 808 ($C{-}H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.97 (3H, t, $J = 7.4$ Hz), 1.70 (2H, m), 3.88 (6H, s, OCH_3), 4.13 (2H, t, $J = 6.9$ Hz), 6.29 (1H, d, $J = 16.0$ Hz, –CO–CH=), 6.83 (1H, d, $J = 8.3$ Hz, Ar–H), 7.03 (1H, d, $J = 1.8$ Hz, Ar–H), 7.07 (1H, dd, $J = 8.3$, 1.6 Hz, Ar–H), 7.60 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.37 (CH_3), 22.02 (CH_2), 55.79 (OCH_3), 55.86 (OCH_3), 65.94 (–OCH_2–), 109.59, 110.98, 115.86 (=C–CO–), 122.44, 127.35, 144.37 (Ar–C=), 149.10, 150.95, 167.15 (C=O). EIMS: *m/z* 251.1283 (M+1), Calcd. for $C_{14}H_{18}O_4$: 250,12.

*Pentyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**12**)*

Yield: 8.5 mmol, 2.364 g, 85 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2958 (C–H), 1706 (C=O), 1635 (C=C), 1513 ($C{=}C_{Ar}$), 1259 (C–O–C), 1160 ((C=O)–O), 807 ($C{-}H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.92 (3H, t, $J = 7.3$ Hz), 1.37 (4H, m), 1.70 (2H, m), 3.90 (6H, s, OCH_3), 4.19 (2H, t, $J = 6.8$ Hz), 6.30 (1H, d, $J = 16.0$ Hz, –CO–CH =), 6.85 (1H, d, $J = 8.4$ Hz, Ar–H), 7.05 (1H, d, $J = 1.6$ Hz, Ar–H), 7.09 (1H, dd, $J = 8.4$, 1.6 Hz, Ar–H), 7.61 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.79 (CH_3), 22.28 (CH_2), 27.93 (CH_2), 28.35 (CH2), 55.80 (OCH_3), 55.87 (OCH_3), 65.31 (–OCH_2–), 109.55, 110.06, 115.78 (=C–CO–), 122.37, 127.29, 144.25 (Ar–C=), 149.12, 151.01, 167.24 (C=O). EIMS: *m/z* 279.1596 (M+1), Calcd. for $C_{16}H_{22}O_4$: 278,15.

*Hexyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**13**)*

Yield: 8.3 mmol, 2.364 g, 83 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2957 (C–H), 1704 (C=O), 1600 (C=C), 1512 ($C{=}C_{Ar}$), 1270 (C–O–C), 1173 ((C=O)–O), 808 ($C{-}H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.91 (3H, t, $J = 7.0$ Hz), 1.33 (4H, m), 1.41 (2H, m), 1.70 (2H, m), 3.91 (6H, s, OCH_3), 4.19 (2H, t, $J = 6.8$ Hz), 6.31 (1H, d, $J = 15.9$ Hz, –CO–CH=), 6.86 (1H, d, $J = 8.2$ Hz, Ar–H), 7.06 (1H, d, $J = 1.8$ Hz, Ar–H), 7.10 (1H, dd, $J = 8.2$, 1.8 Hz, Ar–H), 7.62 (1H, d, $J = 15.9$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.85 (CH_3), 22.43 (CH_2), 22.59 (CH_2), 28.74 (CH_2), 31.46 (CH_2), 55.84 (OCH_3), 55.91 (OCH_3), 64.76 (–OCH_2–), 109.56, 110.92, 115.97 (=C–CO–), 122.44, 127.33, 144.35 (Ar–C=), 149.07, 151.03, 167.07 (C=O). EIMS: *m/z* 293.1753 (M+1), Calcd. for $C_{17}H_{24}O_4$: 292,17.

*Octyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**14**)*

Yield: 8.0 mmol, 2.425 g, 80 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2956 (C–H), 1718 (C=O), 1633 (C=C), 1514 (C=C_{Ar}), 1271 (C–O–C), 1176 ((C=O)–O), 802 (C–H_{Ar}); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.81 (3H, t, $J = 6.6$ Hz), 1.09–1.38 (10H, m), 1.61 (2H, m), 3.84 (6H, s, OCH_3), 4.12 (2H, t, $J = 6.7$ Hz), 6.24 (1H, d, $J = 16.6$ Hz, –CO–CH=), 6.79 (1H, d, $J = 8.4$ Hz, Ar–H), 6.99 (1H, d, $J = 1.3$ Hz, Ar–H), 7.03 (1H, dd, $J = 8.4$, 1.3 Hz, Ar–H), 7.55 (1H, d, $J = 16.6$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.52 (CH_3), 23.09 (CH_2), 26.41 (CH_2), 29.15 (CH_2), 29.61 ($2CH_2$), 32.20 (CH_2), 56.27 (OCH_3), 56.36 (OCH_3), 65.21 (–OCH_2–), 109.88, 111.39, 112.69 (3CH), 116.29 (=C–CO–), 123.01, 144.95 (Ar–C=), 167.68 (C=O). EIMS: *m/z* 321.2023 (M+1), Calcd. for $C_{19}H_{28}O_4$: 320,20.

*Dodecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**15**)*

Yield: 7.5 mmol, 2.822 g, 75 %; m.p. 55–58 °C; IR (KBr, cm^{-1}): ν_{max} 2939 (C–H), 1697 (C=O), 1625 (C=C), 1512 (C=C_{Ar}), 1251 (C–O–C), 1161 ((C=O)–O), 813 (C–H_{Ar}); ^{1}H NMR (CDCl3, 500 MHz): δ 0.85 (3H, t, $J = 6.9$ Hz), 1.15–1.35 (18H, m), 1.53 (2H, m), 3.61(2H, t, $J = 6.67$ Hz), 3.88 (6H, s, OCH_3), 6.28 (1H, d, $J = 16.4$ Hz, –CO–CH=), 6.84 (1H, d, $J = 8.3$ Hz, Ar–H), 7.02 (1H, d, $J = 1.1$ Hz, Ar–H), 7.08 (1H, dd, $J = 8.3$, 1.1 Hz, Ar–H), 7.61 (1H, d, $J = 16.4$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.65 (CH_3), 23.06 (CH_2), 26.14 (CH_2), 29.75–32.02 ($6CH_2$), 32.28 (CH_2), 33.27 (CH_2), 56.28 (OCH_3), 56.36 (OCH_3), 63.45 (–OCH_2–), 109.86, 111.39, 113.34, 115.85 (=C–CO–), 122.88, 125.87, 137.42, 145.22 (Ar–C=), 179.05 (C=O). EIMS: *m/z* 377.2632 (M+1), Calcd. for $C_{23}H_{36}O_4$: 376,26.

*Hexadecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**16**)*

Yield: 8.7 mmol, 3.761 g, 87 %; m.p. 48–50 °C; IR (KBr, cm^{-1}): ν_{max} 2918 (C–H), 1719 (C=O), 1635 (C=C), 1500 (C=C_{Ar}), 1271 (C–O–C), 1180 ((C=O)–O), 802 (C–H_{Ar}); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89 (3H, t, $J = 7.1$ Hz), 1.15–1.47 (26H, m), 1.70 (2H, m), 3.91 (6H, s, OCH_3), 4.20 (2H, t, $J = 6.74$ Hz), 6.31 (1H, d, $J = 16.0$ Hz, –CO–CH=), 6.87 (1H, d, $J = 8.3$ Hz, Ar–H), 7.06 (1H, d, $J = 1.6$ Hz, Ar–H), 7.11 (1H, dd, $J = 8.3$, 1.6 Hz, Ar–H), 7.63 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.08 (CH_3), 22.64 (CH_2), 25.70 (CH_2), 28.18-29.85 ($10CH_2$), 31.91(CH_2), 32.79 (CH_2), 55.84 (OCH_3), 55.93 (OCH_3), 64.59 (–OCH_2–), 109.60, 110.90, 116.02 (=C–CO–), 122.39, 127.45, 144.37 (Ar–C=), 150.95, 151.03, 167.14 (C=O). EIMS: *m/z* 433.3318 (M+1), Calcd. for $C_{27}H_{44}O_4$: 432,32.

*Octadecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**17**)*

Yield: 7.0 mmol, 3.761 g, 70 %; m.p. 154–158 °C; IR (KBr, cm^{-1}): ν_{max} 2915 (C–H), 1708 (C=O), 1620 (C=C), 1506 (C=C_{Ar}), 1274 (C–O–C), 1163 ((C=O)–O), 811 (C–H_{Ar}); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87 (3H, t, $J = 7.1$ Hz), 1.18–1.39 (30H, m), 1.55 (2H, m), 3.62 (2H, t, $J = 6.6$ Hz), 3.92 (6H, s, OCH_3), 6.38 (1H, d, $J = 16.0$ Hz, –CO–CH=), 6.88 (1H, d, $J = 8.3$ Hz, Ar–H), 7.07 (1H, d, $J = 1.8$ Hz, Ar–H), 7.15 (1H, dd, $J = 8.3$, 1.8 Hz, Ar–H), 7.78 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.06 (CH_3), 22.60 (CH_2), 25.70 (CH_2), 29.30 (CH_2), 29.40–29.80 ($11CH_2$), 31.89 (CH_2), 32.77 (CH_2), 55.90 (OCH_3), 55.98 (OCH_3), 63.03 (–OCH_2–), 109.96, 111.08, 114.35 (=C–CO–), 123.55, 126.74, 148.47 (Ar–C=), 149.33, 151.95, 162.84 (C=O). EIMS: *m/z* 461.3631 (M+1), Calcd. for $C_{29}H_{48}O_4$: 460,36.

*Hexadecyl (2E)-3-(2,3-dimethoxyphenyl)prop-2-enoate (**18**)*

Yield: 6.0 mmol, 2.594 g, 60 %; m.p. 41–43 °C; IR (KBr, cm^{-1}): ν_{max} 2920 (C–H), 1719 (C=O), 1635 (C=C), 1515 (C=C_{Ar}), 1270 (C–O–C), 1180 ((C=O)–O), 802 (C–H_{Ar}); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89 (3H, t, $J = 7.1$ Hz), 1.13–1.50 (26H, m), 1.72 (2H, m), 3.87 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.21 (2H, t, $J = 6.7$ Hz), 6.49 (1H, d, $J = 16.2$ Hz, –CO–CH =), 6.94 (1H, d, $J = 8.0$ Hz, Ar–H), 7.06 (1H, t, $J = 8.0$ Hz, Ar–H), 7.16 (1H, d, $J = 8.0$ Hz, Ar–H), 8.00 (1H, d, $J = 16.2$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.04 (CH_3), 22.68 (CH_2), 25.95 (CH_2), 28.74 (CH_2), 29.04–29.90 ($10CH_2$), 31.86 (CH_2), 55.88 (OCH_3), 61.26 (OCH_3), 64.66 (–OCH_2–), 113.85 (=C–CO–), 119.21, 119.65, 124.11, 128.69, 139.29 (Ar–C=), 148.50, 153.15, 167.19 (C=O). EIMS: *m/z* 433.3318 (M+1), Calcd. for $C_{27}H_{44}O_4$: 432,32.

*Hexadecyl (2E)-3-(2,5-dimethoxyphenyl)prop-2-enoate (**19**)*

Yield: 8.5 mmol, 3.675 g, 85 %; m.p. 63–65 °C; IR (KBr, cm^{-1}): ν_{max} 2918 (C–H), 1710 (C=O), 1630 (C=C), 1495 (C=C_{Ar}), 1219 (C–O–C), 1177 ((C=O)–O), 802 (C–H_{Ar}); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89 (3H, t, $J = 7.0$ Hz), 1.22–1.44 (26H, m), 1.72 (2H, m), 3.80 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.20 (2H, t, $J = 6.81$ Hz), 6.50 (1H, d, $J = 16.2$ Hz, –CO–CH=), 6.85 (1H, d, $J = 9.0$ Hz, Ar–H), 6.92 (1H, dd, $J = 9.0$, 3.0 Hz), 7.06 (1H, d, $J = 3.0$ Hz, Ar–H), 7.97 (1H, d, $J = 16.2$ Hz, Ar–CH=C); ^{13}C NMR

($CDCl_3$, 125 MHz): δ 14.08 (CH_3), 22.68 (CH_2), 25.96 (CH_2), 28.77 (CH_2), 29.17–29.97 ($10CH_2$), 31.90 (CH_2), 55.09 (OCH_3), 55.77 (OCH_3), 64.56 (-OCH_2-), 112.46, 113.24, 116.99, 119.04, 124.07 (=C–CO–), 139.67 (Ar–C=), 152.77, 153.56, 167.37 (C=O). EIMS: *m/z* 433.3318 (M+1), Calcd. for $C_{27}H_{44}O_4$: 432,32.

*Hexadecyl (2E)-3-(4-methoxyphenyl)prop-2-enoate (**20**)*

Yield: 7.8 mmol, 3.138 g, 78 %; m.p. 50–55 °C; IR (KBr, cm^{-1}): ν_{max} 2915 (C–H), 1707 (C=O), 1638 (C=C), 1517 ($C=C_{Ar}$), 1268 (C–O–C), 1182 ((C=O)–O), 826 ($C–H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89 (3H, t, $J = 7.1$ Hz), 1.19–1.46 (26H, m), 1.70 (2H, m), 3.84 (3H, s, OCH_3), 4.20 (2H, t, $J = 6.9$ Hz), 6.32 (1H, d, $J = 16.0$ Hz, –CO–CH=), 6.92 (2H, d, $J = 8.7$ Hz, Ar–H), 7.48 (2H, d, $J = 8.7$ Hz, Ar–H), 7.64 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.06 (CH_3), 22.64 (CH_2), 25.97 (CH_2), 28.75 (CH_2), 29.20-29.82 ($10CH_2$), 31.89 (CH_2), 55.32 (OCH_3), 64.58 (–OCH_2–), 114.24, 115.79 (=C–CO–), 127.24, 129.59, 144.11 (Ar–C=), 161.31, 167.33 (C=O). EIMS: *m/z* 403.3212 (M+1), Calcd. for $C_{26}H_{42}O_3$: 402,31.

*Hexadecyl 3-(4-methoxyphenyl)propanoate (**21**)*

Yield: 7.0 mmol, 2.830 g, 70 %; yellow pale oil; IR (KBr, cm^{-1}): ν_{max} 2929 (C–H), 1735 (C=O), 1515 ($C=C_{Ar}$), 1247 (C–O–C), 1175 ((C=O)–O), 835 ($C–H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.90 (3H, t, $J = 7.1$ Hz), 1.23–1.35 (26H, m), 1.60 (2H, m), 2.60 (2H, t, $J = 7.8$), 2.90, (2H, t, $J = 7.8$), 3.79 (3H, s, OCH_3), 4.07 (2H, t, $J = 7.1$ Hz), 6.83 (2H, d, $J = 8.3$ Hz, Ar–H), 7.13 (2H, d, $J = 8.3$ Hz, Ar–H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.10 (CH_3), 22.60 (CH_2), 25.87 (CH_2), 28.60 (CH_2), 29.01–30.45 ($11CH_2$), 31.94 (CH_2), 36.16 (CH_2), 55.14 (OCH_3), 64.54 (-OCH_2-), 113.83, 129.18, 132.32, 158.04, 172.97 (C=O). EIMS: *m/z* 405.3369 (M+1), Calcd. for $C_{26}H_{44}O_3$: 404,33.

*Hexadecyl (2E)-3-phenylprop-2-enoate (**22**)*

Yield: 9.4 mmol, 3.500 g, 94 %; m.p. 35–36 °C; IR (KBr, cm^{-1}): ν_{max} 2952 (C–H), 1714 (C=O), 1640 (C=C), 1475 ($C=C_{Ar}$), 1177 ((C=O)–O), 801 ($C–H_{Ar}$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.89 (3H, t, $J = 7.0$ Hz), 1.19–1.48 (26H, m), 1.72 (2H, m), 4.21 (2H, t, $J = 6.8$ Hz), 6.35 (1H, d, $J = 16.0$ Hz, –CO–CH=), 7.39 (3H, m, Ar–H), 7.53 (2H, dd, $J = 7.0$, 2.2 Hz, Ar–H), 7.69 (1H, d, $J = 16.0$ Hz, Ar–CH=C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.06 (CH_3), 22.66 (CH_2), 26.01 (CH_2), 28.72 (CH_2), 29.22-29.91 ($10CH_2$), 31.89 (CH_2), 64.66 (–OCH_2–), 118.32 (=C–CO–), 127.97, 128.82, 130.10, 134.45, 144.47 (Ar–C =), 167 (C=O). EIMS: *m/z* 373.3107 (M+1), Calcd. for $C_{25}H_{42}O_2$: 374,32.

*Octyl 3-(3,4-dihydroxyphenyl)prop-2-enoate (**23**)*

Yield: 7.2 mmol, 2.104 g, 72 %; m.p. 101–104 °C; IR (KBr, cm^{-1}): ν_{max} 3318 (OH), 2920 (C–H), 1683 (C=O), 1604 (C=C), 1442 ($C=C_{Ar}$), 1282 (C–O–C), 1178 ((C=O)–O), 815 ($C–H_{Ar}$); ^{1}H NMR (DMSO-D_6, 500 MHz): δ 0.79 (3H, t, $J = 6.5$ Hz), 1.03-1.31 (10H, m), 1.53 (2H, m), 4.01 (2H, t, $J = 6.0$ Hz), 4.43 (2H, m, OH), 6.17 (1H, d, $J = 15.9$ Hz, –CO–CH=), 6.75 (1H, d, $J = 8.2$ Hz, Ar–H), 6.83 (1H, d, $J = 8.2$ Hz, Ar–H), 7.02 (1H, s, Ar–H), 7.42 (1H, d, $J = 15.9$ Hz, Ar–CH=C); ^{13}C NMR (DMSO-D_6, 125 MHz): δ 13.65 (CH_3), 22.35 (CH_2), 25.73 (CH_2), 28.50 (CH_2), 28.98 (CH_2), 31.41 (CH_2), 45.96 (CH_2), 63.98 (–OCH_2–), 111.65, 121.61 (=C–CO–), 125.93 (2C), 145.21 (2C), 148.20 (Ar–C=C), 167.68, 167.01 (C=O). EIMS: *m/z* 293.1732 (M+1), Calcd. for $C_{17}H_{24}O_4$: 294,18.

Solubility Compounds **10–22** exhibited low polarity which makes them soluble in solvents such as dichloromethane, ether and ethyl acetate. Compound **23** is soluble in more polar solvents such as DMF and DMSO, due to the hydrophilicity provided by hydroxyl groups.

Biological activity assays

The compounds were subjected to in vitro cytotoxic activity on mammalian cells and leishmanicidal activity on axenic and intracellular amastigotes of *L. panamensis*.

In vitro cytotoxic activity in mammalian cells

The cytotoxic activity of the compounds was assessed based on the viability of the human promonocytic cell line U937 (ATCC CRL-1593.2^{TM}) evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as described by Taylor *et al*. 2010, 2011). In brief, cells were grown in 96-well cell-culture dishes at a concentration of 100,000 cells/mL in RPMI-1640 supplemented with 10 % FBS and the corresponding concentrations of the compounds, starting at 200 μg/mL in duplicate. The cells were incubated at 37 °C with 5 % CO_2 for 72 h in the presence of the compounds, and then the effect was determined using MTT assay, incubating at 37 °C for 3 h. The effect of the compounds was determined by measuring the activity of the mitochondrial dehydrogenase by adding 10 μL/well of MTT solution (0.5 mg/mL) and incubating at 37 °C for 3 h. The reaction was stopped by adding a 50 % isopropanol solution with 10 % sodium dodecyl sulfate for 30 min. Cell viability was determined based on the quantity of formazan produced, which was measured at 570 nm in a Bio-Rad ELISA. Cultured cells in the absence of extracts were used as viability controls; meglumine

antimoniate and amphotericin B were used as cytotoxicity controls. Assays were performed twice with three replications per each concentration tested. The results are expressed as lethal concentration 50 (LC_{50}), calculated by the Probit method (Finney, 1971). The degree of toxicity was established according to the LC_{50} values, using the following scale: Highly toxic: LC_{50} <10 μg/mL, toxic: LC_{50} >10 to <50 μg/mL; Moderately toxic: LC_{50} >50 to <200 μg/mL and potentially non toxic: LC_{50} >200 μg/mL.

In vitro leishmanicidal activity on axenic and intracellular amastigotes

Axenic and intracellular amastigotes of GFP-transfected L. *(V.) panamensis* strain (MHOM/CO/87/UA140epirGFP) were used for the in vitro testing of leishmanicidal activity of the cinnamic acid esters derivatives.

Activity against axenic amastigotes

The respective ability of the cinnamic acid esters to kill axenic amastigotes of *L. (V.) panamensis* was determined based on the viability of the parasites evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as described previously (Taylor *et al*., 2011; 2010). In short, parasites were cultivated in Schneider's medium pH 5.4 supplemented with 20 % heat inactivated FBS for 3 days at 32 °C. Afterward, they were harvested, washed, and resuspended at 2×10^6 axenic amastigotes/mL in fresh medium. Each well of a 96-well plate was seeded with 100 μL of each parasite suspension (in duplicate), and 100 μL of each concentration of the test compound was added, starting at 100 μg/mL. Plates were incubated at 32 °C. After 72 h of incubation, the effect of drugs was determined by adding 10 μL/well of MTT and incubating at 32 °C for 3 h. The reaction was stopped, and the quantity of formazan produced was measured with a Bio-Rad ELISA reader set at 570 nm. Parasites cultivated in the absence of the compound but maintained under the same conditions were used as controls for growth and viability. Parasites cultivated in the presence of meglumina antimoniate and amphotericin B were used as controls for leishmanicidal activity. Assays were performed at least twice with three replicates per each concentration tested. The results are expressed as effective concentration 50 (EC_{50}) calculated by the Probit method (Finney, 1971). The degree of leishmanicidal activity was established according to the EC_{50} values, using the following scale: Highly active: EC_{50} <10 μg/mL, active: EC_{50} >10 to <50 μg/mL; Moderately active: EC_{50} >50 to <200 μg/mL and potentially non active: EC_{50} >200 μg/mL.

The Selective Index (SI), also known as Therapeutic Index (TI) was calculated by dividing the cytotoxic activity and the leishmanicidal activity using the following formula: IS, IT = CL_{50}/CE_{50}.

Activity against intracellular amastigotes

The effects of cinnamic acid esters against intracellular amastigotes of *L. (V.) panamensis* were evaluated by flow cytometry using the methodology described by Varela *et al*. (2009). In brief, U937 cells were dispensed in 24-well plates at a concentration of 300,000 cells/well, which were treated with 1 μM of Phorbol Myristate Acetate (PMA) for 48 h at 37 °C, after which they were infected with promastigotes of *L. (V.) panamensis* in stationary growth phase (day 5) in modified NNN medium, at ratio of 1:25 cell/parasite, after 3 h of incubation at 34 °C in 5 % CO_2 non-internalized parasites were washed, and incubated again at 34 °C and 5 % CO_2 to allow differentiation to amastigotes form. After 24 h of incubation, the compounds with the appropriate dilution, not exceeding the LC_{50}, were added. Infected and treated cells were maintained at 34 °C and 5 % CO_2 for 72 h. The leishmanicidal effect was measured in a flow cytometer at 488 nm of excitation and 525 nm of emission, and determined as described by Pulido *et al*. (2012), Taylor *et al*. (2011) and Varela *et al*. (2009). Infected cells cultivated in the presence of meglumina antimoniate and amphotericin B were used as control for leishmanicidal activity. In addition, infected but untreated cells were used as control of viability. Assays were performed at least twice with 3 replicates per each concentration tested. The results were also expressed as the EC_{50} calculated by the Probit method as above (Finney, 1971). The degree of leishmanicidal activity was established according to the EC_{50} values, using the same scale previously described. SI was calculated as described above.

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