Título artículo / Títol article:	**Synthesis and Leishmanicidal Activity of Cinnamic Acid Esters: Structure-Activity Relationship**
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Revista:	**Medicinal Chemistry Research**
Versión / Versió:	**Pre-print**
Cita bibliográfica / Cita bibliogràfica (ISO 690):	**OTERO, Elver, et al. Synthesis and leishmanicidal activity of cinnamic acid esters: Structure–activity relationship. Medicinal Chemistry Research, 2014, vol. 23, no 3, p. 1378-1386.**
url Repositori UJI:	http://hdl.handle.net/10234/127408

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. Cinnamic acids derivatives were synthesized via Knoevenagel reaction between substituted benzaldehydes and malonic acid in aqueous medium assisted by microwave heating. Structures of the products were elucidated by spectroscopic analysis. The synthesized compounds were evaluated for antileishmanial activity against L. panamensis amastigotes and cytotoxic activity against U-937 cells. The compounds **6**, **10-12** and **18**, were active against *Leishmania* parasite but toxic for mammalian cells. They are potential candidates for antileishmanial drug development.

Keywords Leishmaniasis, Antiprotozoal, Caffeic acid, Cinnamic acid esters

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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 2 million new cases occur yearly (Murray *et al.*, 2005) and 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 as the antimony derivative glucantine, 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 *et al.*, 2004; Ouellette *et al.*, 2004; Barrett *et al*,. 2002; Croft *et al.*, 2003; Faraut-Gambarelli *et al.*, 1997; Olliaro *et al*,. 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 *et al.*, 1994) antioxidant (Hung *et al.*, 2005; Noriaki *et al.*, 2005; Kikuzaki *et al*,. 2002; Son *et al.*, 2002; Rajan *et al.*, 2001) and anticarcinogenic effects (De et. al. 2011). Besides, some studies showed high leishmanicidal activity for these compounds (IC_{50} 4.4 nM to caffeic acid (Radtke *et al.*, 2003) and 2.0, 10 and 1.8 μM to 2, 3 and 4 esters (fig. 1) (Cabanillas *et al.*, 2010)). In the search of new therapeutic alternatives for the treatment of Leishmaniasis several cinnamic acid esters analogues were synthesized and their cytotoxic and leishmanicidal activities were determined.

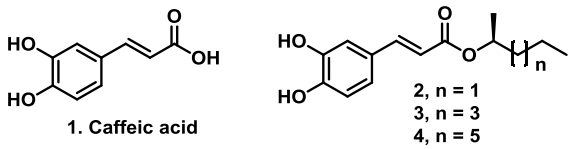
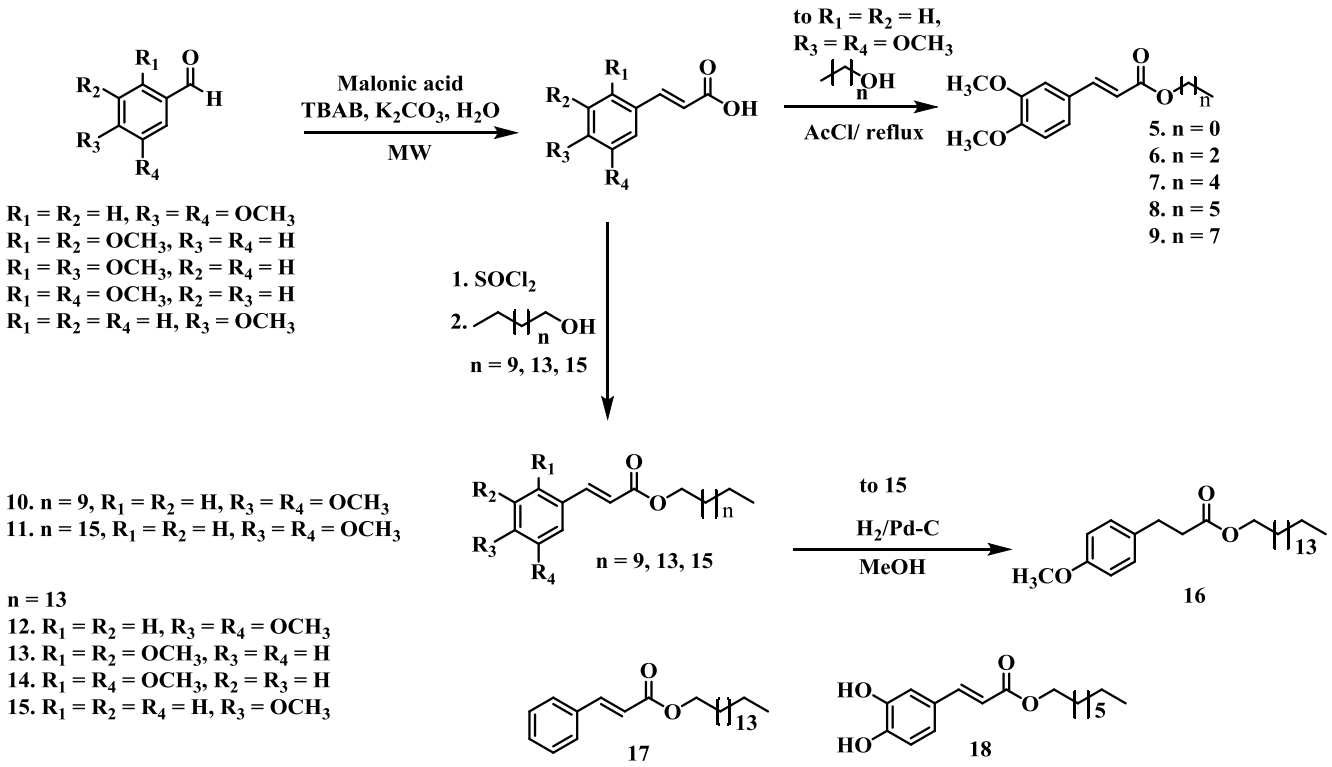


Fig. **1** Caffeic acid and its esters derivatives

Results and Discussion

Chemistry

Cinnamic acid esters were obtained via Knoevenagel condensation in water under microwave heating (Gupta *et al.*, 2007). These compounds were dissolved in thionyl chloride; the solution was stirred and refluxed by two hours. After evaporation, the acid chloride was added to a solution of cetyl alcohol in dichloromethane. Following evaporation and purification by column chromatography a total of six compounds were obtained. The cetyl alcohol was change by dodecyl alcohol or stearyl alcohol (Narasimhan *et al.*, 2004). 3,4-dimethoxycinnamic acid was dissolved in methanol, propanol, pentanol, hexanol and 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 were obtained (De Campos *et al.*, 2009). Compounds **17** and **18** were similarly obtained by starting from cinnamic and caffeic acid, respectively. Compound **16** was obtained by catalytic hydrogenation of compound **15** (Cardona *et al.*, 2006) (scheme 1).



Scheme 1. Synthetic pathway to cinnamic acid esters derivatives

Antileishmanial Activity

The leishmanicidal activity and cytotoxicity of the synthesized compounds as well as amphotericin B, used as control drug, were evaluated following the method previously reported in the literature (Varela *et al.*, 2009; Robledo *et al.*, 2005; Weninger *et al.*, 2001; Robledo *et al.*, 1999). The results were reported as EC_{50} and LC_{50} values of compounds and are shown in the tables 1 and 2.

Table 1. *In vitro* leishmanicidal activity against axenic amastigotes of *L. panamensis* and toxicity of Cinnamic acid esters.

Compound	Cytotoxicity U937 cells LC_{50} (µg/ml)[a]	Leishmanicidal Activity EC_{50}(µg/ml)[b]	SI[c]
5	162.6 ± 49.6	55.0± 14.7	3.0
6	85.3 ± 17.5	33.0 ± 0.2	2.6
7	49.8 ± 6.3	38.1± 9.7	1.3
8	54.7 ± 7.7	60.0 ± 4.0	0.9
9	79.9 ± 5.5	>100.0	<0.8
10	49.7 ± 18.1	59.0 ± 1.9	0.8
11	28.5 ± 1.9	108.2 ± 1.5	0.3
12	69.1 ± 6.7	55.4 ± 2.0	1.2
13	>200.0	>100.0	<2.0
14	>200.0	>100.0	<2.0
15	>200.0	>100.0	<2.0
16	>200.0	>100.0	<2.0
17	>200.0	>100.0	<2.0
18	9.9 ± 1.7	2.3 ± 0.5	4.3
Amphotericin B	29.6 ± 4.7	0.05 ± 0.01	592

[a] LC_{50} : Lethal Concentration 50
[b] EC_{50} : Effective Concentration 50;
[c] SI: selectivity index: LC_{50} / EC_{50};
[d] Cytotoxicity: LC_{50} <100 µg/ml; No Cytoxicity: LC_{50} >200 µg/ml; Active: EC_{50} <50 µg/ml; Moderately Active: EC_{50}<100 µg/ml; No Active: EC_{50} >100 µg/ml

According to the results shown in the table 1, only compounds **5**, **6**, **7**, **8**, **10**, **12** and **18** showed activity against axenic amastigotes of *L. panamensis* with EC_{50} lower than 100 µg/ml. The most active compound was **18** (EC_{50} = 2.3 µg/ml) followed by **6** and **7** exhibiting EC_{50} values of 33.0 and 38.1 µg/ml, respectively. Compounds **5**, **8**, **10** and **12** 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 **6** to **12** and **18**, with a LC_{50} < 100 µg/ml. The lower toxic activity was obtained with the compound **5** (EC_{50} = 162.6 µg/ml). No leishmanicidal activity and toxicity were detected for the compounds **13** to **17** (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 **18**, **5** and **6** with values of 4.3, 3.0 and 2.6, respectively. Compound **18** is structurally similar to the 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 are cytotoxic and highly active against Leishmania parasites. Differences in the Leishmania species and cell type used in the

assays could explain the different results obtained with compounds **18** and **4**. Thus, compound **4** was tested against axenic amastigotes of *L. amazonensis* and murine peritoneal macrophages (Cabanillas et al., 2010) whereas compound **18** was tested in this report against axenic amastigotes of *L. panamensis* and human macrophages.

Structure-activity relationship

There is a relationship between the leishmanicidal activity and compounds structure. The smaller the alkyl chains the higher the selectivity index (**5**, **6** vs **7-12**); the degree of oxygenation is essential for activity, primarily in positions 2, 3 and 4 (**12** vs **13-17**); hydroxyl groups increase both the activity and cytotoxicity (**9** vs **18**); The importance of the double bond is not conclusive since the compounds were inactive, which can be related to the degree of oxygenation (**16** vs **17**). However, if the action mechanism involves a Michael addition for nucleophilic amino acid residues, this would be crucial for the activity as has been reported for other α,β-unsaturated compounds such as lactones, chalcones and cumarins (De Fatima *et al.,* 2006; Buck *et al.,* 2003).

Table 2. *In vitro* activity of Cinnamic acid esters against intracellular amastigotes of *L. panamensis.*

Compound	Leishmanicidal Activity EC_{50}(µg/ml)	SI
6	60.2 ± 1.2	2.6
10	25.2 ± 2.3	2.0
11	18.3 ± 3.3	1.6
12	26.5 ± 2.0	2.7
18	3.2 ± 0.8	3.1
Amphotericin B	0.06 ± 0.01	592

The leishmanicidal activity against the intracellular forms of *Leishmania* parasites was also measured for all compounds. Only the compounds **6**, **10-12** and **18** were active (table 2). The most active compounds were **18** and **11** with EC_{50} of 3.2 and 18.3 µg/ml, respectively. The compound **6** had the lowest activity with EC_{50} 60.2 µg/ml. The best SI was observed for compounds **18**, **12** and **6** with values of 3.1, 2.7 and 2.6, respectively.

Overall, the compounds **6**, **10, 12** and **18** were apparently the most active compounds showing activity against both axenic and intracellular amastigotes of *L. panamensis,* while the compounds **5, 7** and **8** showed activity only on the axenic form of this *Leishamania* species.

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 the leishmanicidal activity and the cytotoxicity. The compounds **6**, **10-12** and **18**, active against *Leishmania* parasite but toxic for mammalian cells, are potential candidates for antileishmanial drug development. However, more studies on toxicity using other cell lines are needed in order to discriminate whether the toxicity shown by these compounds is against tumor or non-tumor cells.

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. The chemical shifts are expressed in δ ppm. High resolution mass spectra were run 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 (**5-9**)*

Cinnamic acid (10 mmol), acetyl chloride (0.5 mmol), and the alcohol (50 ml), 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 hours. 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 75-85%.

*Synthesis of cinnamic acid esters (**10-16**)*

Cinnamic acid (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 hours. The reaction mixture was concentrated on a rotatory evaporator, and the residue was added to a solution of cetyl alcohol (dodecyl or estearyl alcohol) in dichloromethane and the mixture was stirred and monitored by thin layer chromatography. The reaction was complete after about 4 hours. 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, 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 70-85%.

*Methyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**5**)*
Yield 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). MS: m/z 223.0972 (M + 1).

*Propyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**6**)*
Yield 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). MS: m/z 251.1283 (M + 1).

*Pentyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**7**)*
Yield 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). MS: m/z 279.1596 (M + 1).

*Hexyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**8**)*
Yield 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). MS: m/z 293.1753 (M + 1).

*Octyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**9**)*
Yield 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). MS: m/z 321.2023 (M + 1).

*Dodecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**10**)*
Yield 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). MS: m/z 377.2632 (M + 1).

*Hexadecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**11**)*
Yield 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). MS: m/z 433.3318 (M + 1).

*Octadecyl (2E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**12**)*
Yield 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). MS: m/z 461.3631 (M + 1).

*Hexadecyl (2E)-3-(2,3-dimethoxyphenyl)prop-2-enoate (**13**)*
Yield 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* = 7.1Hz), 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). MS: m/z 433.3318 (M + 1).

*Hexadecyl (2E)-3-(2,5-dimethoxyphenyl)prop-2-enoate (**14**)*
Yield 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). MS: m/z 433.3318 (M + 1).

*Hexadecyl (2E)-3-(4-methoxyphenyl)prop-2-enoate (**15**)*
Yield 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). MS: m/z 403.3212 (M + 1).

*Hexadecyl 3-(4-methoxyphenyl)propanoate (**16**)*
Yield 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* = 7.1Hz), 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). MS: m/z 405.3369 (M + 1).

*Hexadecyl (2E)-3-phenylprop-2-enoate (**17**)*
Yield 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). MS: m/z 373.3107 (M + 1).

*Octyl 3-(3,4-dihydroxyphenyl)prop-2-enoate (**18**)*

Yield 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). MS: m/z 293.1732 (M + 1).

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

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 Robledo et al. (2005). 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 lg/ml in duplicate. The cells were incubated at 37ºC with 5% CO_2 for 72 h in the presence of the compounds, and then the effect was determined using MTT assay, incubating at 37ºC for 3 h. The effect of the compounds was determined by measuring the activity of the mitochondrial dehydrogenase by adding 10 ll/well of MTT solution (0.5 mg/ml) and incubating at 37 ºC 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; Amphotericin B was used as cytotoxicity control. The results are expressed as the Lethal Concentration 50 (LC_{50}) calculated by the Probit method (Finney, 1971).

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., 2010). In short, parasites were cultivated in Schneider's medium pH 5.4 supplemented with 20% heat inactivated FBS for 3 days at 32ºC. Afterward, they were harvested, washed, and resuspended at 2 x 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 32ºC. After 72 h of incubation, the effect of drugs was determined by adding 10 µl/well of MTT and incubating at 32ºC 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 Amphotericin B were used as controls for leishmanicidal activity.

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 37ºC, after which they were infected with promastigotes of L. (V.) panamensis in stationary growth phase (day 5) in modified NNN medium, a proportion of 1:25 cell/parasite, after 3 h of incubation at 34ºC in 5% CO_2 non-internalized parasites were washed, and incubated again at 34ºC 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 34ºC 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 Varela et al. (2009). The results are expressed as the Effective Concentration 50 (EC50) calculated by the Probit statistical method. The data are the averages of three independent experiments conducted in duplicate. Infected but untreated cells were used as control of viability. In addition, infected cells exposed to Amphotericin B were used as leishmanicidal activity controls. The Selectivity Index (SI) was calculated by dividing the cytotoxic activity between the leishmanicidal activity ($SI = LC_{50}/EC_{50}$).

Acknowledgment The authors thank Dr. Javier Garcés for his help during the performance of this work. We acknowledge support by the Universidad de Antioquia (Estrategia de Sostenibilidad 2013-2014)

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