Thieme

Antiparasitic Activity of Diterpenoids Against *Trypanosoma cruzi*

Authors

Sergio Alegre-Gómez[1], Paula Sainz[1], M. Fátima Simões[2,3], Patrícia Rijo[2,3], Cristina Moiteiro[4], Azucena González-Coloma[1], Rafael A. Martínez-Díaz[5]

Affiliations

1 Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Madrid, Spain
2 Universidade Lusófona Research Center for Biosciences & Health Technologies (CBIOS), Campo Grande, Lisboa, Portugal
3 Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Lisboa Portugal
4 Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, Portugal
5 Departamento de Medicina Preventiva, Salud Pública y Microbiología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

Key words

Plectranthus spp., *Aeollanthus rydingianus*, Lamiaceae, diterpenoids, antitrypanosomal activity, intracellular amastigotes

received April 11, 2016
revised August 8, 2016
accepted August 16, 2016

Bibliography

DOI http://dx.doi.org/10.1055/s-0042-115646
Published online September 6, 2016 | Planta Med 2017; 83: 306–311
 | ISSN 0032-0943

Correspondence

Rafael A. Martínez-Díaz
Departamento de Medicina Preventiva, Salud Pública y Microbiología, Facultad de Medicina, Universidad Autónoma de Madrid
C/Arzobispo Morcillo S/N, 28029 Madrid, Spain
Phone: + 34 9 14 97 53 11
rafael.martinez@uam.es

Supporting information available online at http://www.thieme-connect.de/products

ABSTRACT

Twenty-seven diterpenes, including abietanes, labdanes, abeoabietanes, halimanes, and pimaranes, have been evaluated against epimastigote and intracellular amastigote forms of *Trypanosoma cruzi* and also against LC5 and NCTC cell lines. Royleanones (**3, 4**, and **5**) and a further abietane (**12**), obtained by purification of *Plectranthus* spp. extracts, were the most active compounds on epimastigotes, showing IC_{50} values similar (1.73 µg/mL, **12**) or even lower (0.39, 0.99, and 1.20 µg/mL, **3, 4**, and **5** respectively) than the positive control nifurtimox (2.3 µg/mL). On intracellular amastigotes, abietanes **3, 4**, and **5** showed a significant activity with IC_{50} values of 0.83, < 0.31, and 0.62 µg/mL, respectively, but were less potent than the positive control nifurtimox (IC_{50} < 0.16 µg/mL). Compounds **3, 4**, and **5** were not cytotoxic to LC5 and NCTC 929 cells at 1 µg/mL.

Introduction

Trypanosoma cruzi is the aethiologic agent of the Chagas disease, a frequently fatal illness affecting the heart and gastrointestinal systems. An estimated 8 million people in Latin America are infected with this pathogen and it is also spreading to the USA, Canada, many parts of Europe, and the Western Pacific as a result of migratory flows [1]. Only two drugs, nifurtimox and benznidazole, are in use against chronic infections and both have limitations due to the need of a large number of doses over a long time period, side effects, and a lack of effectiveness against all stages of the disease and all strains of the parasite. Moreover, problems are encountered in their production and distribution [2]. In the United States, these drugs are not FDA approved and are available only from Centers for Disease Control and Prevention under investigational protocols. Nifurtimox is licensed for use only in Argentina and Germany. Therefore, there is still a need to find more efficient and less toxic drugs that act specifically against the pathogen.

The genus *Plectranthus* (Lamiaceae) contains about 300 species distributed in tropical and subtropical Africa, Asia, and Australia [3]. *Plectranthus* species have medicinal properties, [4] including antiplasmodial activity against *Plasmodium falciparum* [4, 5], antileishmanial activity against *Leishmania chagasi* [6] and *L. infantum* [5], and trypanocidal activity against *Trypanosoma cruzi* and *T. brucei* [5]. The antiplasmodial activity of *Plectranthus* species has been attributed to the presence of abietane diterpenes [7].

Abietane diterpenes have been described as possessing cytotoxic, antibacterial and antiprotozoal effects [7–13], as inhibitors of T- and B-lymphocyte proliferation [14], and also as inhibitors of the growth of various human tumor cell lines [15, 16]. Diterpenes from the *Plectranthus* genus have demonstrated antimicrobial activity against *Mycobacterium tuberculosis, Staphylococcus aureus,* and *Enterococcus* species [17, 18]. These diterpenes have also shown antiprotozoal activity against *P. falciparum* [7]. Some abietane diterpenoids isolated from *P. barbatus* (dehydroabietane; 5,6-didehydro-7-hydroxy-taxodone; taxodione; 20-deoxocarnosol; and 6α,11,12,-trihydroxy-7β,20-epoxy-8,11,13-abietatriene) demonstrated activity on the parasitic protozoa *P. falciparum, L. infantum, T. cruzi*, and *T. brucei* [19]. Previous studies of *Aeollanthus* species (Lamiaceae) have shown antifungal, anticonvulsant

and antimicrobial activities related with the presence of abietane and pimarane diterpenoids [20, 21].

The aim of this work is to study the trypanocidal effects of seventeen natural and ten semisynthetic diterpenoids against *Trypanosoma cruzi* epimastigotes. Ten compounds, including abietanes, labdanes, and a halimane, were isolated from *Plectranthus* spp. while seven pimaranes were obtained from *Aeollanthus rydingianus*. A set of ten semisynthetic royleanone and halimane derivatives (**2–8, 14, 17**, and **18**) were prepared from compounds **1, 12**, and **16**, respectively. Active diterpenes were selected for further evaluation on amastigote *T. cruzi* forms. Additionally, their cytotoxicity has been tested on LC5 (human lung fibroblasts) and NCTC clone 929 (mouse subcutaneous fibroblasts) cell lines.

Results and Discussion

The activity of the diterpenoids studied on epimastigote forms of *T. cruzi* is shown in ▶ **Table 1**. Royleanone abietane **1** and its semisynthetic derivatives **2–8** showed activities near 100% at 100 µg/mL. Royleanones **2–6** and **8** showed activity levels higher than 50% at 10 µg/mL, with compounds **3, 4**, and **5** being active at the lowest concentration tested (1 µg/mL). Among abietanes **9–13**, compound **12** showed an activity of 93.9% at 10 µg/mL, and 52.3% at 1 µg/mL. Abeoabietane **14** and halimanes **17** and **18** were close to 100% activity at 100 µg/mL, but fell below 40% at 10 µg/mL. Pimaranes **21, 24, 25**, and **27** showed values close to 100% at 100 µg/mL, but only **24** and **25** exceeded 50% at 10 µg/mL. Therefore, the most active compounds **3, 4, 5**, and **12**, with IC_{50} values of 0.39, 0.99, 1.20, and 1.73 µg/mL, respectively (nifurtimox: 2.3 µg/mL), were selected for a further study of their trypanocidal effects (▶ **Table 2**, and **Table 1S, Figs. 1S–4S**, Supporting Information).

Overall, abietanes were the most active class of diterpenes. Especially potent were structures with lipophilic bulky (phenolic or propionate) substituents at C-2 (**12**), C-6 and/or C-12 (**3–6, 8**).

▶ **Table 3** shows the cytotoxicity of compounds **3, 4, 5**, and **12** on the cell lines LC5 (human lung fibroblasts) and NCTC 929 (mouse fibroblasts). NCTC 929 cells were more sensitive than LC5 cells to these diterpenes and nifurtimox. Among these compounds, **3, 4**, and **5** were not cytotoxic to NCTC 929 cells at 1 µg/mL. Therefore, these compounds were selected to test their effects on *T. cruzi* amastigotes, while compound **12** was not included in the secondary testing due to its high cytotoxicity at 100 and 10 µg/mL, with a toxicity level of 42.6% at 1 µg/mL (▶ **Table 3**).

▶ **Table 4** shows the effects of **3, 4**, and **5** on amastigotes. Abietanes **3** and **5** were active up to 1.25 µg/mL, with IC_{50} values of 0.83 and 0.62 µg/mL, respectively. Abietane **4** showed 100% activity at 0.6 µg/mL and 58.5% at 0.3 µg/mL, with an IC_{50} < 0.31 µg/mL, (**Table 2S** and **Figs. 5S–7S**, Supporting Information) while nifurtimox had an IC_{50} < 0.16 µg/mL. Therefore, derivative **4** showed promising trypanocidal effects. In short, royleanone abietanes **3, 4**, and **5** have shown great activity at very low concentrations against both epimastigotes and amastigotes of *T. cruzi*. Furthermore, these compounds were not cytotoxic to the host cells at concentrations with antiparasitic effect.

▶ **Table 1** Activity of natural and semisynthetic diterpenoids from *Plectranthus* spp. and *Aeollanthus rydingianus* on *T. cruzi* epimastigotes at concentrations of 100, 10, and 1 µg/mL. All assays were carried out in quadruplicate and were repeated three times (n = 3). Data are expressed as % growth inhibition ± standard deviation relative to untreated controls.

Compound	% growth inhibition [mean ± SD]		
	100 µg/mL	10 µg/mL	1 µg/mL
1	100.0	36.2 ± 22.0	1.8 ± 0.8
2	100.0	88.2 ± 10.5	5.2 ± 16.0
3	97.5 ± 2.3	90.7 ± 4.8	70.6 ± 11.3
4	100.0	99.8 ± 1.0	54.7 ± 31.2
5	100.0	100.0	54.7 ± 25.2
6	100.0	59.4 ± 37.2	11.1 ± 1.7
7	99.8 ± 0.3	32.5 ± 9.2	8.6 ± 0.2
8	100.0	92.1 ± 0.2	0.3 ± 4.7
9	93.9 ± 7.9	25.1 ± 4.4	9.7 ± 12.0
10	0.0	6.6 ± 0.6	3.8 ± 0.2
11	98.5 ± 2.4	12.8 ± 12.2	3.1 ± 12.6
12	100.0	93.9 ± 8.9	52.3 ± 2.4
13	24.4 ± 20.7	11.2 ± 19.8	10.8 ± 14.5
14	100.0	34.7 ± 14.9	17.3 ± 11.7
15	60.0 ± 9.5	0.0	14.2 ± 8.1
16	43.8 ± 3.3	12.3 ± 10.0	14.9 ± 0.1
17	90.0 ± 2.1	9.9 ± 2.1	12.4 ± 0.4
18	92.0 ± 7.1	37.7 ± 14.9	2.9 ± 2.1
19	0.0	4.3 ± 6.2	0.0
20	0.0	0.0	0.0
21	99.2 ± 3.8	21.1 ± 7.5	3.3 ± 4.8
22	87.6 ± 5.2	27.2 ± 23.1	14.2 ± 15.8
23	4.9 ± 1.1	0.0	5.5 ± 1.9
24	97.4 ± 5.4	70.1 ± 8.6	22.1 ± 16.9
25	100.0	64.7 ± 11.3	6.8 ± 12.9
26	78.4 ± 5.0	15.6 ± 10.8	12.3 ± 13.8
27	100.0	28.9 ± 15.0	19.4 ± 19.6
Nifurtimox	99.9 ± 0.2	100.0	20.8 ± 2.8

Other diterpenes, such as geranylgeraniol from *Pterodon pubescens* (Fabaceae), 6*β*-hydroxy-18-acetoxycassan-13,15-diene from *Myrospermum frutescens* (Fabaceae), cyclocoulterone and komaroviquinone from *Dracocephalum komarovi* (Lamiaceae), and 5-epi-icetexone from *Salvia gilliessi* (Lamiaceae), have previously demonstrated potent activity on epimastigote forms of *T. cruzi* [22–26]. Abietane diterpenoids taxodione, ferruginol, 6-hydroxysalvinolone, and uncinatone from the roots of *Clerodendrum eriophyllum* (Verbenaceae) have shown significant activity against promastigote forms of the trypanosomatid *Leishmania donovani*, with IC_{50} values of 0.1, 4.0, 3.2, and 0.2 µg/mL, respectively [27]. 5,6-Didehydro-7-hydroxy-taxodone from the aerial parts of *Plectranthus barbatus* showed selective activity against *P. falciparum* (IC_{50} 9.2 µM,) and *T. brucei* (IC_{50} 1.9 µM) [19].

▶ **Table 2** Activity on *T. cruzi* epimastigotes of abietanes **3, 4, 5**, and **12** at lower and intermediate concentrations (5, 2.5, 1.25, 0.63, 0.31, and 0.16 µg/mL). Data are expressed as percentage of growth inhibition ± standard deviation. All assays were carried out in quadruplicate and were repeated three times (n = 3). Data are expressed as % growth inhibition ± standard deviation relative to untreated controls.

Compound	% growth inhibition [mean ± SD]					
	5 µg/mL	2.5 µg/mL	1.25 µg/mL	0.63 µg/mL	0.31 µg/mL	0.16 µg/mL
3	83.0 ± 3.9	72.8 ± 3.3	59.2 ± 3.5	49.0 ± 0.7	19.4 ± 1.0	5.0 ± 3.2
4	99.7 ± 0.3	89.0 ± 0.7	71.5 ± 4.3	23.3 ± 6.4	1.2 ± 1.1	0.2 ± 0.5
5	98.0 ± 1.9	94.8 ± 4.9	61.6 ± 10.2	23.8 ± 3.4	0.7 ± 2.4	0.7 ± 0.3
12	64.1 ± 2.0	53.8 ± 6.6	35.2 ± 9.9	13.7 ± 0.3	0.8 ± 6.3	3.2 ± 0.8

Some compounds have been shown to have variable effects against the different forms of *T. cruzi* (epimastigotes, trypomastigotes, or amastigotes) [28]. Amastigotes are generally more sensitive than epimastigotes. Geranylgeraniol had an IC_{50} of 12.5 µg/mL against epimastigotes of *T. cruzi*, while its IC_{50} against amastigote forms was 2.0 µg/mL [25]. Moreover, 18-hydroxycassan-13,15-diene and 6*β*,-18-dihydroxycassan-13,15-diene, diterpenes showed IC_{50} values of 48.6 and 56.0 µg/mL, respectively, against epimastigotes and 17.4 and 16.6 µg/mL, respectively, against amastigotes [22]. This differential susceptibility could be attributed to differences in the environment of the parasites since epimastigotes and trypomastigotes are free-forms exposed to the external environment, while the amastigote forms live inside a host cell. On the other hand, one cannot exclude that the differences between the assays employed, especially the contact time with the compound, can influence the results of activity between amastigotes and epimastigotes.

Royleanone derivatives are tricyclic abietane diterpenes containing a hydroquinone, with an amphipathic nature, allowing them to cross or damage the cytoplasmic membrane. This gives them some antibacterial activity which can increase or decrease when the lipophilicity and/or the ability to form hydrogen bonds is modified [18]. This ability to alter the membranes may be the reason that these products have shown a high activity as compared to the other diterpenes studied.

The activity of compound **12** could be related to the presence of a C-2 *p*-substituted aromatic ester with a hydroxyl group, which increased its lipophilicity and membrane interactions [17]. This also applies to compounds **3**–**5**, with two aromatic esters (**3**), or an aromatic ester with a methoxy (**4**) or a chloride (**5**) substituent, respectively [18]. These two diterpenoids (**4** and **5**) have previously demonstrated interesting activity against *Mycobacterium tuberculosis*, showing a better activity than **6**, in which the aromatic ring is substituted with a nitro group [17].

Material and Methods

Natural and semisynthetic diterpenoids

Ten natural diterpenoids were isolated as previously described. Seven abietanes, 7*α*-acetoxy-6*β*-hydroxyroyleanone (**1**), 6,7-dehydroroyleanone (**9**), sugiol (**10**), coleon U (**11**), parvifloron D (**12**), (13S, 15S)-6*β*,7*α*,12*α*,19-tetrahydroxy-13*β*,16-cyclo-8-abietene-11,14-dione (**13**), *ent*-7α-acetoxy-15-beyeren-18-oic acid (**15**) [17, 29, 30], one halimane, (11*R*)-acetoxyhalima-5,13(E)-dien-15-oic acid (**16**) [31], two labdanes, 1,6-di-*O*-acetyl-9-deoxyforskolin (**19**), and a mixture (1 : 1) of 1,6-di-*O*-acetylforskolin and 1,6-di-*O*-acetyl-9-deoxyforskolin (**20**) [32] were obtained from *Plectranthus* spp.

▶ **Table 3** Cytotoxic effects on LC5 and NCTC 929 cells of abietanes **3, 4, 5,** and **12** and nifurtimox. All assays were carried out in quadruplicate and were repeated three times (n = 3). Data are expressed as % growth inhibition ± standard deviation relative to untreated controls.

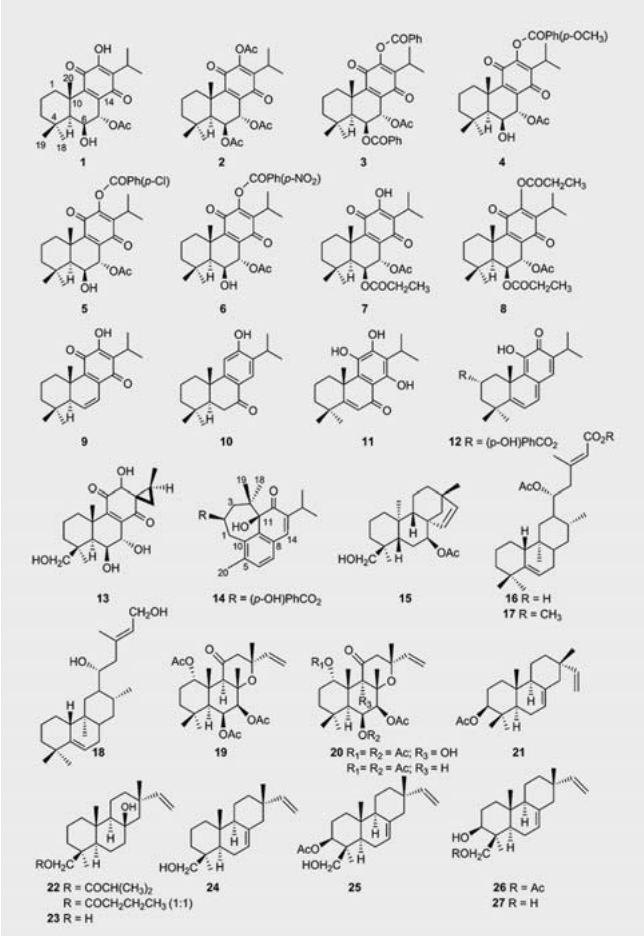
Compound	Concentration (µg/mL)	Concentration (µM)	% cytotoxicity [mean ± SD]	
			LC5	NCTC 929
3	100	166.7	94.6 ± 2.0	98.2 ± 0.6
	10	16.7	0.0	96.7 ± 0.4
	1	1.7	0.0	2.9 ± 0.1
4	100	190.5	95.7 ± 3.2	97.7 ± 0.4
	10	19.0	70.6 ± 3.7	94.3 ± 1.8
	1	1.9	0.0	0.0
5	100	189.0	87.2 ± 2.5	93.9 ± 1.6
	10	18.9	86.4 ± 3.1	82.0 ± 14.7
	1	1.9	7.3 ± 7.0	0.0
12	100	241.0	83.0 ± 0.7	85.7 ± 3.9
	10	24.1	17.6 ± 8.5	95.9 ± 2.7
	1	2.4	0.0	42.6 ± 4.3
Nifurtimox	100	348.1	4.7 ± 4.6	71.1 ± 1.5
	10	34.8	9.9 ± 9.2	25.5 ± 5.4
	1	3.5	6.7 ± 2.2	0.0

Seven pimaranes, 3*β*-acetoxy-7,15-isopimaradiene (**21**), a mixture (1 : 1) of 3*β*-isobutyryloxy- and 3*β*-butyryloxy-8*β*-hydroxy-15-isopimarene (**22**), 15-isopimarene-8*β*,19-diol (**23**), 7,15-isopimaradien-19-ol (**24**), 3β-acetoxy-7,15-isopimaradien-19-ol (**25**), 19-acetoxy-7,15-isopimaradien-3*β*-ol (**26**), and 7,15-isopimaradien-3*β*,19-diol (**27**) [20] were isolated from *Aeollanthus rydingianus*.

Semisynthetic abietane derivatives **2** to **8** were prepared from 7*α*-acetoxy-6*β*-hydroxyroyleanone (**1**) [17, 18], while microstegiol derivative (**14**) was synthesized from parvifloron D (**12**) [30]. Halimane methyl ester **17** and diol **18** were prepared from (11R)-acetoxyhalima-5,13(E)-dien-15-oic acid (**16**) via esterification and hydrolysis procedures [33] (▶ **Fig. 1**).

▶ **Table 4** Activity on *T. cruzi* amastigotes of abietanes **3, 4**, and **5** at concentrations of 5, 2.5, 1.25, 0.63, 0.31, and 0.16 µg/mL. All assays were carried out in triplicate and were repeated three times (n = 3). Data are expressed as % growth inhibition ± standard deviation relative to untreated controls.

Compound	% growth inhibition [mean ± SD]					
	5 µg/mL	2.5 µg/mL	1.25 µg/mL	0.63 µg/mL	0.31 µg/mL	0.16 µg/mL
3	100.0	100.0	81.4 ± 2.4	22.3 ± 17.3	0.0	0.0
4	100.0	100.0	100.0	100.0	58.5 ± 5.7	0.0
5	100.0	100.0	88.9 ± 1.3	36.6 ± 12.9	9.4 ± 2.2	6.0 ± 1.4
Nifurtimox	100.0	100.0	100.0	100.0	100.0	96.4 ± 0.8



▶ **Fig. 1** Chemical structures of compounds **1**–**27**.

Parasites

Trypanosoma cruzi Y strain and clone CL-B5 transfected with *Escherichia coli* β-galactosidase gene (*lacZ*) were used to test the antiparasitic effects of the test compounds on epimastigote and amastigote forms, respectively. *T. cruzi* Y strain epimastigotes were grown axenically at 28 °C in liver infusion tryptose (LIT) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco). CL-B5 epimastigotes were grown at 28 °C in LIT with 10% FCS and harvested during the exponential growth phase. Tissue culture derived trypomastigotes were obtained after infection of nonconfluent fibroblasts with epimastigotes in Minimal Essential Medium (MEM) without phenol red (Sigma).

Cell cultures

Human cell line LC5, derived from the lung tissue of a human embryo were grown in RPMI medium (Sigma) supplemented with 10% FCS at 37 °C. NCTC 929 cells were grown in MEM (Sigma) supplemented with 10% heat-inactivated FCS, penicillin G (100 U/mL) and streptomycin (100 µg/mL). Cell cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. For the experiments, cells in the pre-confluence phase were harvested with trypsin.

Epimastigote susceptibility assay

The activity on epimastigote forms was evaluated on cultures in LIT medium supplemented with 10% heat-inactivated (FCS). Parasites in logarithmic growth phase at an approximate density of 8–10×10^6 epimastigotes/mL were distributed in 96-well flat-bottom plates, each well receiving 90 µL of culture. Compounds were tested at several concentrations (100, 10 and 1 µg/mL) for 72 h. Nifurtimox (Bayer) was used as the positive control and parasite viability was analyzed by a modified MTT colorimetric assay method [34]. All assays were carried out in quadruplicate and were repeated three times. When compounds showed activity, intermediate and lower doses were assayed (5, 2.5, 1.25, 0.63, 0.31 and 0.16 µg/mL) and IC_{50} values (concentration that inhibits 50% of the growth of the parasites) were determined from nonlinear regression analysis (GraphPad Prism, version 7).

Amastigote susceptibility assay

The activity was evaluated with chlorophenol red-β-D-galactopyranoside (CPRG; Roche) dissolved in 0.9% Triton X-100 (pH 7.4) by a modified colorimetric method [35] described previously by Buckner et al [36]. NCTC 929 fibroblasts were established in 48-well tissue culture plates at a previously determined optimal concentration of 1×10^4 cells/well. NCTC 929-derived trypomastigotes were added to the monolayers at a parasite : cell ratio of 6 : 1 and incubated for 24 h at 33 °C with 5% CO_2. The infected cells were then washed twice with PBS to remove the extracellular trypomastigotes. Compounds were added in triplicate at concentrations of 5, 2.5, 1.25, 0.63, 0.31, and 0.16 µg/mL to give a final volume of 450 µL/well. The plates were incubated for 7 days at 33 °C. At this time, 50 µL of CPRG solution (final concentration

400 µM) in 0.3% Triton X-100 was added. Following 3 h of incubation at 37 °C, the colorimetric reaction was quantified by measuring at an optical density (OD) of 595 nm. The results were expressed as percentage of anti-amastigote activity (%AA) relative to control wells, as follows: %AA = 100-[(*At*-*Ab*)/(*Ac*-*Ab*)]×100, where *At* is the absorbance of treated wells, *Ac* the absorbance of control wells (not treated), and *Ab* the absorbance of blank wells (culture medium and vehicle only). Background controls (NCTC 929 cells only) were subtracted from all values. IC_{50} values (concentration that inhibits 50% of the growth of the parasites) were determined from nonlinear regression analysis (GraphPad Prism, version 7). All assays were carried out in triplicate and were repeated three times.

Cytotoxicity assay

LC5 cell line and NCTC clone 929 were used for these assays. Cells grown in RPMI or MEM supplemented with 10% FCS at 37 °C under humidified atmospheric conditions of 5% CO_2/95% air were seeded in 96-well flat-bottom microplates with 100 µL medium per well (initial densities of 10^4 cells per well) and incubated under the same conditions. After 24 h, the medium was removed and added fresh medium containing the compounds. Cells were exposed for 48 h to several concentrations of the test compounds (100, 10, and 1 µg/mL). The toxicity of the reference drug (nifurtimox) was also evaluated. Cell viability was analyzed by the MTT colorimetric assay method [37]. All assays were carried out in quadruplicate and were repeated three times.

Supporting information

Detailed data on the activities of compounds **3, 4, 5**, (and **12**) on *T. cruzi* epimastigotes and amastigotes are available as Supporting Information

Acknowledgements

This work has been supported by grants CTQ2012-38219-C03-01 and CTQ2015-64049-C3-1-R, MINECO, Spain. The authors are grateful for funding from Fundação para a Ciência e Tecnologia (FCT) under the project UID/MULTI/00612/2013. We want to thank Drs. Alicia Gomez-Barrio and Cristina Fonseca-Berzal (Department of Parasitology, Universidad Complutense de Madrid) for their kind assistance in conducting trials with amastigotes.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

[1] Rassi jr. A, Rassi A, Marin-Neto A. Chagas disease. Lancet 2010; 375: 1388–1402

[2] González-Coloma A, Reina M, Sáenz C, Lacret R, Ruiz-Mesia L, Arán VJ, Sanz J, Martínez-Díaz RA. Antileishmanial, antitrypanosomal and cytotoxic screening of ethnopharmacologically selected Peruvian plants. Parasitol Res 2012; 110: 1381–1392

[3] Alasbahi RH, Melzig MF. *Plectranthus barbatus*: a review of phytochemistry ethnobotanical uses and pharmacology – Part 1. Planta Med 2010; 76: 653–661

[4] Lukhoba CW, Simmonds MSJ, Paton AJ. *Plectranthus*: A review of ethnobotanical uses. J Ethnopharmacol 2006; 103: 1–24

[5] Al-Musayeib NM, Mothana RA, Matheeussen A, Cos P, Maes L. *In vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region. BMC Complement Altern Med 2012; 12: 49–55

[6] Tempone AG, Sartorelli P, Teixeira D, Prado FO, Calixto IA, Lorenzi H, Melhem MS. Brazilian flora extracts as source of novel antileishmanial and antifungal compounds. Mem Inst Oswaldo Cruz 2008; 103: 443–449

[7] Van Zyl RL, Khan F, Edwards TJ, Drewes SE. Antiplasmodial activities of some abietane diterpenes from the leaves of five *Plectranthus* species. S Afr J Sci 2008; 104: 62–64

[8] Batista O, Duarte A, Nascimento J, Simões MF, de La Torre MC. Structure and antimicrobial activity of diterpenes from roots of *Plectranthus hereroensis*. J Nat Prod 1994; 57: 858–861

[9] Batista O, Simões MF, Duarte A, Valdeira ML, de La Torre MC, Rodriguez B. Antimicrobial abietane from the roots of *Plectranthus hereroensis*. Phytochemistry 1995; 38: 167–169

[10] Dellar JE, Cole MD, Waterman PG. Antimicrobial abietane diterpenoids from *Plectranthus elegans*. Phytochemistry 1996; 41: 735–738

[11] Gaspar-Marques C, Rijo P, Simões MF, Duarte MA, Rodriguez B. Abietanes from *Plectranthus grandidentatus* and *P. hereroensis* against methicillin- and vancomycin-resistant bacteria. Phytomedicine 2006; 13: 267–271

[12] Ebrahimi SN, Zimmermann S, Zaugg J, Smiesko M, Brun R, Hamburger M. Abietane diterpenoids from *Salvia sahendica* – Antiprotozoal activity and determination of their absolute configurations. Planta Med 2013; 79: 150–156

[13] Pirttimaa M, Nasereddin A, Kopelyanskiy D, Kaiser M, Yli-Kauhaluoma J, Oksman-Caldentey KM, Brun R, Jaffe CL, Moreira VM, Alakurtti S. Abietane-type diterpenoid amides with highly potent and selective activity against *Leishmania donovani* and *Trypanosoma cruzi*. J Nat Prod 2016; 79: 362–368

[14] Cerqueira F, Cordeiro-Da-Silva A, Gaspar-Marques C, Simões F, Pinto MMM, Nascimento MSJ. Effect of abietane diterpenes from *Plectranthus grandidentatus* on T- and B-lymphocyte proliferation. Bioorg Med Chem 2004; 12: 217–223

[15] Burmistrova O, Simões MF, Rijo P, Quintana J, Bermejo J, Estévez F. Antiproliferative activity of abietane diterpenoids against human tumor cells. J Nat Prod 2013; 76: 1413–1423

[16] Burmistrova O, Perdomo J, Simões MF, Rijo P, Quintana J, Estévez F. The Abietane diterpenoid parvifloron D is a potent apototic inducer in human leukemia cells. Phytomedicine 2015; 22: 1009–1016

[17] Rijo P, Simões MF, Francisco AP, Rojas R, Gilman RH, Vaisberg AJ, Rodríguez B, Moiteiro C. Antimycobacterial metabolites from *Plectranthus*: royleanone derivatives against *Mycobacterium tuberculosis* strains. Chem Biodivers 2010; 7: 922–932

[18] Rijo P, Duarte A, Francisco AP, Semedo-Lemsaddek T, Simões MF. *In vitro* antimicrobial activity of royleanone derivatives against gram-positive bacterial pathogens. Phytother Res 2014; 28: 76–81

[19] Mothana RA, Al-Said MS, Al-Musayeib NM, El Gamal AA, Al-Massarani SM, Al-Rehaily AJ, Abdulkader M, Maes L. *In vitro* antiprotozoal activity of abietane diterpenoids isolated from *Plectranthus barbatus* Andr. Int J Mol Sci 2014; 15: 8360–8371

[20] Rijo P, Simões MF, Duarte A, Rodríguez B. Isopimarane diterpenoids from *Aeollanthus rydingianus* and their antimicrobial activity. Phytochemistry 2009; 70: 1161–1165

[21] Hanson JR. Diterpenoids of terrestrial origin. Nat Prod Rep 2011; 28: 1755–1772

[22] Mendoza DT, Ureña-González LD, Ortega-Barría E, Capson TL, Rios LC. Five new cassane diterpenes from *Myrospermum frutescens* with activity against *Trypanosoma cruzi*. J Nat Prod 2003; 66: 928–932

[23] Uchiyama N, Kiuchi F, Ito M, Honda G, Takeda Y, Khodzhimatov OK, Ashurmetov OA. New icetexane and 20 norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi*. J Nat Prod 2003; 66: 128–131

[24] Sanchez AM, Jimenez-Ortiz V, Sartor T, Tonn CE, García EE, Nieto M, Burgos MH, Sosa MA. A novel icetexane diterpene, 5-epi-icetexone from *Salvia gilliessi* is active against *Trypanosoma cruzi*. Acta Trop 2006; 98: 118–124

[25] Menna-Barreto RF, Laranja GA, Silva MC, Coelho MG, Paes MC, Oliveira MM, de Castro SL. Anti-*Trypanosoma cruzi* activity of *Pterodon pubescens* seed oil: geranylgeraniol as the major bioactive component. Parasitol Res 2008; 103: 111–117

[26] Lozano E, Strauss M, Spina R, Cifuente D, Tonn C, Rivarola HW, Sosa MA. The *in vivo* trypanocidal effect of the diterpene 5-epi-icetexone obtained from *Salvia gilliesii*. Parasitol Int 2016; 65: 23–26

[27] Machumi F, Samoylenko V, Yenesew A, Derese S, Midiwo JO, Wiggers FT, Jacob MR, Tekwani BL, Khan SI, Walker LA, Muhammad I. Antimicrobial and antiparasitic abietane diterpenoids from the roots of *Clerodendrum eriophyllum*. Nat Prod Commun 2010; 5: 853–858

[28] Izumi E, Ueda-Nakamura T, Dias Filho BP, Veiga Júnior VF, Nakamura CV. Natural products and Chagas' disease: a review of plant compounds studied for activity against *Trypanosoma cruzi*. Nat Prod Rep 2011; 28: 809–823

[29] Simões MF, Rijo P, Duarte A, Barbosa D, Matias D, Delgado J, Cirilo N, Rodríguez B. Two new diterpenoids from *Plectranthus* species. Phytochem Lett 2010; 3: 221–225

[30] Simões MF, Rijo P, Duarte A, Matias D, Rodríguez B. An easy and stereoselective rearrangement of an abietane diterpenoid into a bioactive microstegiol derivative. Phytochem Lett 2010; 3: 234–237

[31] Rijo P, Gaspar-Marques C, Simões MF, Jimeno ML, Rodríguez B. Further diterpenoids from *Plectranthus ornatus* and *P. grandidentatus*. Biochem Syst Ecol 2007; 35: 215–221

[32] Rijo P, Simões MF, Rodríguez B. Structural and spectral assignment of three forskolin-like diterpenoids isolated from *Plectranthus ornatus*. Magn Reson Chem 2005; 43: 595–598

[33] Rijo P, Rodríguez B, Duarte A, Simões MF. Antimicrobial properties of *Plectranthus ornatus* extracts, 11-acetoxyhalima-5,13-dien-15-oic acid metabolite and its derivatives. Nat Prod J 2011; 1: 57–64

[34] Martínez-Díaz RA, Ibáñez-Escribano A, Burillo J, de las Heras L, del Prado G, Agulló-Ortuño MT, Julio LF, González-Coloma A. Trypanocidal, trichomonacidal and cytotoxic components of cultivated *Artemisia absinthium* Linnaeus (Asteraceae) essential oil. Mem Inst Oswaldo Cruz 2015; 110: 639–699

[35] Fonseca-Berzal C, Merchán Arenas DR, Romero Bohórquez AR, Escario JA, Kouznetsov VV, Gómez-Barrio A. Selective activity of 2,4-diaryl-1,2,3,4- tetrahydroquinolines on *Trypanosoma cruzi* epimastigotes and amastigotes expressing β-galactosidase. Bioorg Med Chem Lett 2013; 23: 4851–4856

[36] Buckner FS, Verlinde CL, La Flamme AC, Van Voorhis WC. Efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing beta-galactosidase. Antimicrob Agents Chemother 1996; 40: 2592–2597

[37] Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63