Antileishmanial and Antitrypanosomal Activity of Triterpene Derivatives from Latex of Two *Euphorbia* Species

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The *in vitro* activity on *Leishmania infantum* promastigotes and *Trypanosoma cruzi* epimastigotes of 25 semisynthetic terpenoid derivatives has been evaluated. These compounds were obtained through chemical modifications of the major components of *Euphorbia resinifera* (*α*-euphol and *α*-euphorbol) and *Euphorbia officinarum* (obtusifoliol and 31-norlanosterol). Leishmaniasis and Chagas´ disease are major worldwide health problems. The drugs of choice for their treatment are still problematic in both cases, and therefore there is an urgent need to discover new drugs with high activity and low side effects. Natural products have become a key source of new drugs in the last years. The genus *Euphorbia* has been the subject of abundant phytochemical and pharmacological research because of its potential medical applications, but the antiparasitic effects of derivatives from plants of this genus are still unknown. Our results showed that 76% and 64% of the test compounds had antiparasitic effects on *L. infantum* and *T. cruzi*, respectively. The different activities on both parasites, especially their moderate effects on mammalian cells, indicate an interesting selective toxicity.

Key words: Triterpenes, Antileishmanial, Antitrypanosomal

Introduction

Today there is a great and urgent need to discover new antiparasitic compounds with different chemical structures and mechanisms of action because of the increase in the incidence of some parasitic diseases and the appearance of re-emerging parasitosis. In addition, drugs for the treatment of some parasitic diseases are scarce and sometimes non existent. Leishmaniasis and Chagas´ disease are two major parasitic diseases that cause significant morbidity and mortality, and their treatment is still problematic.

Natural products have become a key source of new drugs in the last years (Fournet and Muñoz, 2002; Newman and Cragg, 2007). The *Euphorbia* genus includes a wide and diverse group of plants which are characterized by the presence of an irritant latex rich in euphol and euphorbol triterpenes (Singla and Pathak, 1990). This genus has been the subject of abundant phytochemical and pharmacological research because of its potential medical applications. Extracts of numerous *Euphorbia* species have been found to demonstrate a number of interesting biological activities against pathologies, such as swelling and warts, or anticancer properties (Fatope *et al*., 1996). Furthermore, *Euphorbia officinarum* is used in traditional medicine to treat skin and ophthalmologic diseases (Daoubi *et al*., 2007). A number of biologically active natural compounds have been isolated from this genus: euphol and euphorbol triterpenes have been reported as anti-inflammatory and antiviral compounds which affect *Xenopus* cell division and human breast cancer cell viability (Yasukawa *et al*., 2000; Akihisa *et al*., 2002; Wang *et al*., 2003). Other phorbol and ingenol derivatives have a potent antiproliferative activity in tumour cells (Irie *et al*., 2002; Kedei *et al*., 2004). Also, some derivatives from *Euphorbia*

cauducifolia have proven molluscicidal activity (Baloch *et al*., 2009), and several diterpenes from *Euphorbia kansui* have shown insecticidal (Shi *et al*., 2008a) and antinematodal activity (Shi *et al*., 2008b). However, there are hardly any studies on the effects of *Euphorbia* derivatives on parasites and protozoa (Duarte *et al*., 2008), and therefore their potential antiparasitic and antiprotozoal activities remain unknown.

Trypanosoma cruzi is the aethiologic agent of Chagas´ disease, a public health problem in many Latin American countries, including Europe due to the immigration from several countries of South America (Florián-Sanz *et al*., 2005; Muñoz *et al*., 2007; Gascon *et al*., 2010). The treatment of Chagas´ disease is a challenge, since the only available drugs, such as nifurtimox and benznidazole, possess severe side effects. Moreover, their lack of efficiency has involved problems in their production and distribution (Muelas-Serrano *et al*., 2000; Sra *et al*., 2004). Leishmaniasis is a protozoan parasitic disease endemic in 88 countries, which causes considerable morbidity and mortality. The drugs most commonly used to treat leishmaniasis are pentavalent antimonials which require high dose regimens with extended treatments with compounds such as amphotericin B, paromomycin or pentamidine. However, all these drugs are far from being satisfactory due to unacceptable side effects at effective doses. Therefore, the search for new compounds to improve the actual treatments of Chagas´ disease and leishmaniasis is urgently needed (Dejeux, 2001; Croft and Coombs, 2003; Croft *et al*., 2005; Davis and Kedzierski, 2005; Murray *et al*., 2005).

In this work, the *in vitro* activities on *Leishmania infantum* promastigotes and *Trypanosoma cruzi* epimastigotes of 25 semisynthetic terpenoid derivatives obtained by means of chemical modifications of the major compounds of *E. resinifera* and *E. officinarum* have been evaluated. Additionally, the selective cytotoxicity of these compounds on mammalian CHO cells has been tested.

Material and Methods

Plant material and chemical procedures

Latex from *E. resinifera* and *E. officinarum* were collected in the area of Dennat and Agadir (Morocco), and the procedures for extraction, isolation, and synthesis for obtaining the triterpene derivatives were performed as previously described (Mazoir *et al*., 2008). The list of tested compounds is shown in Table I. Obtusifoliol and 31-norlanosterol were obtained from *E. officinarum*; α-euphol and α-euphorbol were obtained from *E. resinifera*. The chemical structures of these compounds and their derivatives are shown in Figs. 1 and 2.

Antileishmanial screening

Leishmanicidal activity was assayed on promastigote forms of *L. infantum*, strain PB75, cultured at 28 °C in RPMI medium supplemented with 10% foetal calf serum. Parasites in the logarithmic growth phase were distributed in 96-well flat-bottom plates. The compounds to be tested were dissolved in DMSO (< 0.2% in order to avoid the toxicity of the solvent on the protozoa) and the resulting solution added to the cultures at several concentrations (at first 100, 10, and 1 μg/ml; after, when some activity was detected, intermediate doses were evaluated) for 72 h. Amphotericin B was used as reference drug, and parasite viability was analysed by a modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay (González-Coloma *et al*., 2002). The activity was calculated as percentage of growth inhibition.

Trypanocidal activity

This activity was assayed on epimastigote forms of *T. cruzi*, strain Y, cultured in LIT medium. Parasites in the logarithmic growth phase (from an initial culture with $2 \cdot 10^6$ epimastigotes/ml) were distributed in 96-well flat-bottom plates. Each well was treated with increasing concentrations of the compounds (as in leishmanicidal assays) for 96 h. Nifurtimox was used as reference drug, and parasite viability was analysed by a modified MTT colorimetric assay (Muelas-Serrano *et al*., 2000). All assays were carried out in triplicate, and the activity was calculated as percentage of growth inhibition.

Cytotoxicity assays

Mammalian Chinese hamster ovary cells (CHO) were used for these assays. The non-selective cytotoxicity of the test compounds was evaluated as described previously (Mazoir *et al*., 2008).

Table I. Compounds tested.

Compound	Chemical name
E. officinarum	
1	3β-Tosyloxy-4α,14α-dimethyl-5α-ergosta-8-en-24-one ($C_{36}H_{54}O_4S$)
2	31-Norlanostenol ($C_{29}H_{50}O$)
3	3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene ($C_{36}H_{56}O_3S$)
4	3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{36}H_{52}O_5S$)
5	3β-Acetoxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione ($C_{31}H_{48}O_4$)
6	4α,14α-Dimethyl-5α-ergosta-8-ene-3,24-dione ($C_{30}H_{48}O_2$)
7	4α,14α-Dimethyl-5α-ergosta-8,24-dien-3-one ($C_{30}H_{48}O$)
8	4α,14α-Dimethyl-5α-cholest-8-ene-3,7,11-trione-7-thiosemicarbazone ($C_{30}H_{43}O_2N_3S$)
9	4α,14α-Dimethyl-5α-cholest-8-ene-3,7,11-trione-7-thiadiazoline ($C_{34}H_{51}O_4N_3S$)
10	4α,14α-Dimethyl-5α-cholesta-7,9-dien-3-one thiosemicarbazone ($C_{30}H_{49}N_3S$)
11	3β-Acetoxy-norlup-20-one ($C_{31}H_{50}O_3$)
12	3β-Hydroxy-norlup-20-one ($C_{29}H_{48}O_2$)
13	4α,14α-Dimethyl-5α-cholest-8-ene-3,7,11-trione-3-thiadiazoline ($C_{34}H_{51}O_4N_3S$)
E. resinifera	
14	3β-Acetoxy-elemo-lanosta-8-en-24-one ($C_{31}H_{50}O_3$)
15	3β-Acetoxy-25,26,27-trisnor(5α,13α,14β,17α)lanosta-8-en-24-al ($C_{29}H_{46}O_3$)
16	Elemo-lanosta-8-en-3,24-dione ($C_{31}H_{48}O_2$)
17	3-Oxo-25,26,27-trisnor(5α,13α,14β,17α)lanosta-8-en-24-al ($C_{27}H_{42}O_2$)
18	3β-Hydroxy-25,26,27-trisnor(5α,13α,14β,17α)lanosta-8-en-24-al ($C_{27}H_{44}O_2$)
19	24-Methylen-elemo-lanosta-8-en-24-epoxy-3β-ol ($C_{31}H_{52}O_2$)
20	3β-Tosyloxy-24-methylen-elemo-lanosta-8,24-dien-7,11-dione ($C_{38}H_{54}O_3S$)
21	3β-Acetoxy-24-methylen-elemo-lanosta-8,24-dien-7,11-dione ($C_{33}H_{50}O_4$)
22	3β-Tosyloxy-24-methylen-elemo-lanosta-8,24-dien-11-one ($C_{38}H_{53}O_4S$)
23	3β-Acetoxy-24-methylen-elemo-lanosta-8,24-dien-7,11-dione-11-thiosemicarbazone ($C_{34}H_{33}O_3N_3S$)
24	3β-Hydroxy-elemo-lanosta-8-en-24-one ($C_{31}H_{50}O_2$)
25	24-Methylen-elemo-lanosta-2,8,24-trien-7,11-dione ($C_{31}H_{48}O_2$)

In all cases, once active concentrations are known, intermediate doses were tested and ED_{50} values (the effective dose to give 50% cell viability) were determined from linear regression analysis (% cell viability on log dose).

Results and Discussion

The leishmanicidal and trypanocidal activities of the compounds (Figs. 1 and 2) as well as the cytotoxicity on CHO cells are shown in Table II. Our results showed that 72% and 36% of the test compounds had important antiparasitic effects ($ED_{50} < 10\,\mu$g/ml) on *L. infantum* and *T. cruzi*, respectively. In contrast, mammalian CHO cells were affected ($ED_{50} < 50\,\mu$g/ml) by a lower number of compounds (44%), with some of them (28%) being selective parasite toxicants.

The strongest leishmanicidal compounds were **9** and **22** ($ED_{50} < 3\,\mu$g/ml) followed by **19**, **21** ($ED_{50} < 4\,\mu$g/ml) > **5**, **20**, **24**, **25** ($ED_{50} < 6\,\mu$g/ml) > **6**, **12** ($ED_{50} < 8\,\mu$g/ml) > **7**, **8**, **15**, **16**, **17**, **18**, **23** ($ED_{50} < 10\,\mu$g/ml), and **10** ($ED_{50} < 15\,\mu$g/ml). Overall *T. cruzi* was less sensitive to these compounds, with **21** and **22** ($ED_{50} < 5\,\mu$g/ml) being the most active, followed by **6**, **8**, **20** ($ED_{50} < 7\,\mu$g/ml) > **9**, **15**, **23**, and **25** ($ED_{50} < 10\,\mu$g/ml). Therefore **5**, **7**, **10**, **12**, **16**, **17**, **18**, **19**, and **24** were selective leishmanicidal terpenes (11 – 2 times more potent). Compound **23** was the most cytotoxic to CHO cells. The rest of cytotoxic compounds had moderate effects. Therefore, all strong antiparasitic compounds had low to moderate cytotoxicity.

Comparing the antiparasitic activity of the products and their toxicity on mammalian CHO cells (considering it as a control for nonspecific cytotoxicity), we can see that **5**, **7**, **10**, and **12** showed significant selective leishmanicidal activity with a low or moderate nonspecific cytotoxicity. In addition, **8**, **20**, and **22** showed significant activity against both parasites (*L. infantum* and *T. cruzi*) without nonspecific cytotoxicity associated. Among them, **5** and **10** stood out as strong selective leishmanicidal products without associated toxicity, **20** and **22** as strong leishmanicidal and trypanocidal products without associated toxicity.

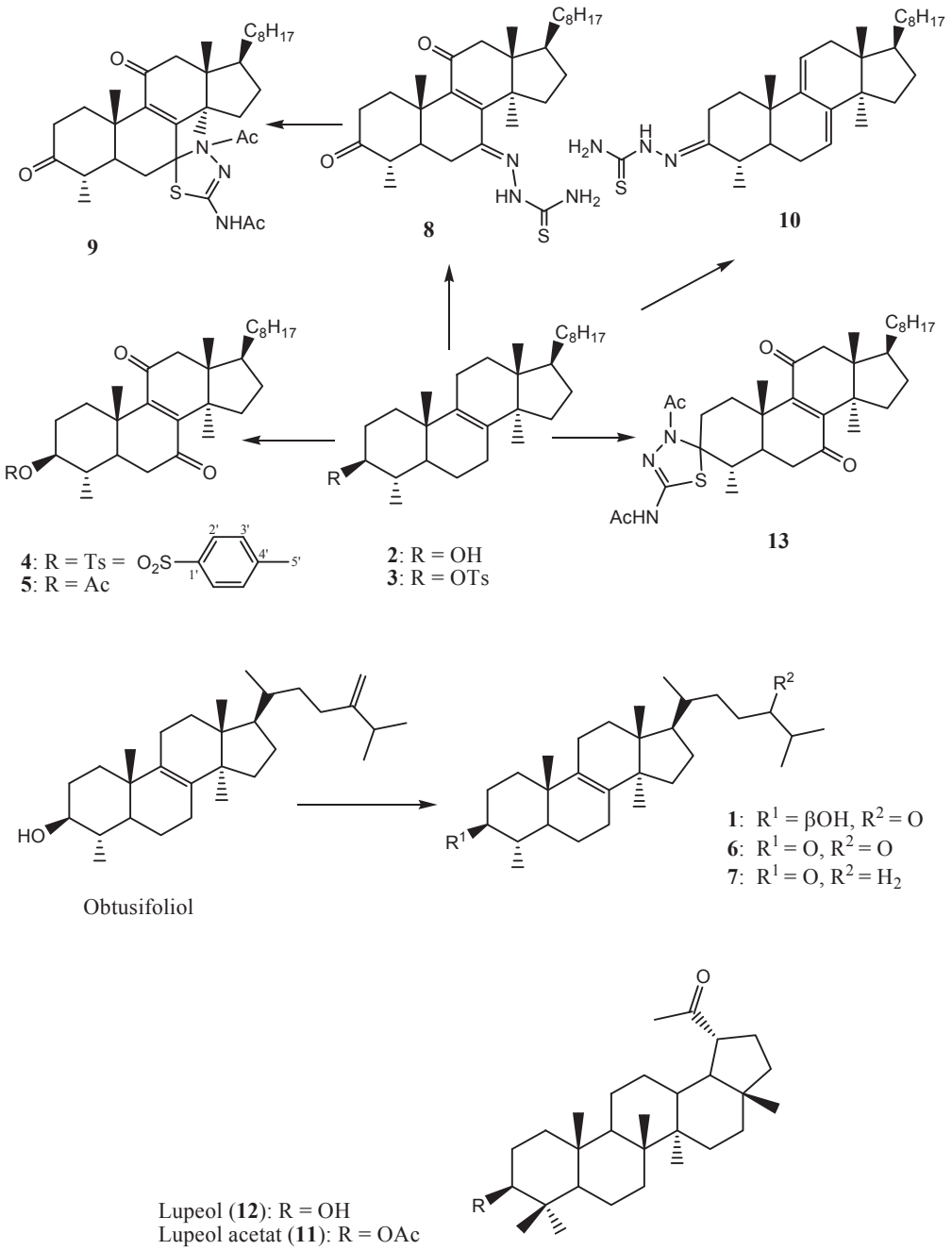

Fig. 1. Chemical structures of the friedelane derivatives from *Euphorbia officinarum* tested.

Overall, among the tetracyclic triterpenes, the most active compounds were highly oxygenated with ketone/OH substituents at C-3 and C-7 and/or C-11 and/or the presence of a substituent at C-24 (epoxy or ketone group) in the lateral chain. The presence of a hydroxy group at C-3 determined the activity of the lupeol derivative **12**.

A number of terpenes with antileishmanial and antitrypanosomal activity have been reported but their modes of action are not clear. Different au-

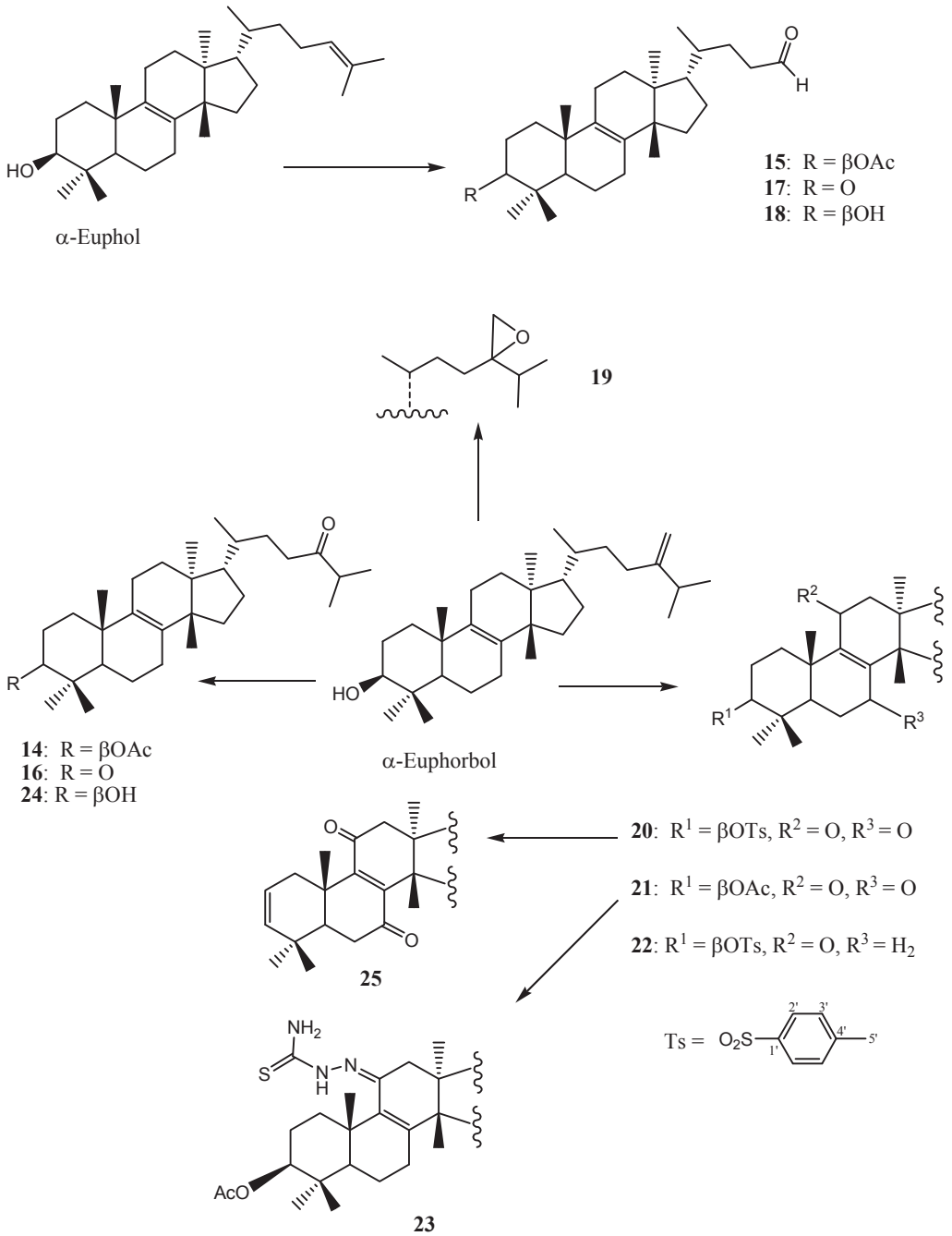

Fig. 2. Chemical structures of the friedelane derivatives from *Euphorbia resinifera* tested.

thors suggested that they can be related with the inhibition of the synthesis of proteins and nucleic acids or with the inhibition of a membrane-associated calcium-dependent ATPase pump (Goijman *et al.*, 1984; Mishina *et al.*, 2007). *T. cruzi* and *Leishmania* parasites have a strict requirement for specific endogenous sterols (ergosterol and analogues) for survival and growth (Urbina *et al.*,

Table II. Leishmanicidal, trypanocidal, and cytotoxic effects of the tested compounds on mammalian CHO cells. Data are expressed as ED_{50} (μg/ml)[a] and 95% confidence limits (lower – upper).

Compound	*L. infantum*	*T. cruzi*	CHO cells
1	66.11 (51.06–85.61)	> 100	> 100
2	37.77 (34.21–41.70)	72.42 (64.48–81.34)	> 100
3	> 100	> 100	> 100
4	> 100	> 100	> 100
5	5.14 (4.67–5.65)	25.81 (23.73–28.07)	> 100
6	7.74 (4.70–12.73)	6.64 (5.56–7.93)	22.35 (21.15–23.63)
7	9.14 (6.58–12.71)	27.81 (25.85–29.92)	82.39 (80.45–84.38)
8	$10 > ED_{50} > 1$	6.50 (4.12–10.26)	75.56 (71.18–80.22)
9	2.41 (2.07–2.80)	$10 > ED_{50} > 1$	18.27 (15.23–21.91)
10	12.64 (11.1–14.41)	62.12 (37.50–102.91)	> 100
11	≈ 100	> 100	> 100
12	6.25 (5.21–7.50)	21.32 (15.38–29.55)	50.87 (44.52–58.13)
13	37.66 (26.9–52.73)	62.23 (59.0–65.66)	> 100
14	> 100	> 100	≈ 100
15	$10 > ED_{50} > 1$	9.15 (7.21–11.60)	23.40 (19.08–28.72)
16	$10 > ED_{50} > 1$	29.95 (15.63–57.39)	20.59 (13.12–32.32)
17	$10 > ED_{50} > 1$	23.87 (22.53–25.29)	28.30 (23.17–34.57)
18	8.18 (7.20–9.28)	28.46 (26.69–30.35)	35.86 (29.22–44.01)
19	4.42 (3.84–5.09)	46.57 (41.79–51.9)	28.24 (23.58–28.24)
20	5.16 (4.7–5.68)	6.98 (6.12–7.97)	> 100
21	3.73 (3.18–4.39)	5.33 (4.91–5.79)	32.91 (29.36–36.9)
22	2.13 (1.86–2.45)	$5 > ED_{50} > 1$	> 100
23	9.27 (6.30–13.65)	$10 > ED_{50} > 1$	5.25 (3.98–6.92)
24	5.84 (5.02–6.79)	34.15 (31.74–36.74)	$50 > ED_{50} > 25$
25	5.99 (4.91–7.32)	9.77 (8.17–11.68)	13.92 (10.69–18.14)
Amphotericin B	0.03		
Nifurtimox		2.22	

[a] ED_{50} (μg/ml), concentration needed to produce 50% epimastigote/promastigote mortality and to maintain 50% CHO cell viability.

2002). Therefore, our results suggest a possible sterol metabolism interference by the triterpenes tested.

In conclusion, the triterpenes tested showed high activity against both parasites, particularly *Leishmania*. However, the different activities on both parasites, and especially their moderate effects on mammalian cells, indicate an interesting selective toxicity. These preliminary positive results suggest further work with these compounds (especially **5**, **10**, **20**, and **22**) in order to discover new drugs with high activity and low side effects against diseases caused by protozoan parasites, such as leishmaniasis and Chagas´ disease.

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