SUPPLEMENTARY MATERIAL

Active sesquiterpene lactones against *Leishmania amazonensis* and *Leishmania braziliensis*

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Abstract

Seventeen sesquiterpene lactones (SLs) isolated from five species of the tribe Vernonieae were evaluated for their *in vitro* activity against promastigotes of *Leishmania amazonensis* and *L. braziliensis*. Additionally, a quantitative structure activity relationship has been made, since all these natural compounds were found to have potent to mild antileishmanial properties. The most active compounds against *L. braziliensis* were **16** and **17** (IC₅₀ values 1.45 and 1.34 µM respectively), followed by compound **15** with IC₅₀ value of 1.60 µM against *L. amazonensis*. The three glaucolide type SLs (**4–6**) were the least active against both parasites. The computational study allowed us to establish that lipophilicity and polarizability play an important role in the antiparasitic activity. This is the first report of the known germacradiendiolides **16** and **17** from *Elephantopus mollis*. The activity data of the compounds **1–17** assayed against *Leishmania* parasites is reported here for the first time.

Keywords: Vernonieae; sesquiterpene lactones; antileishmanial activity; *Leishmania amazonensis*; *Leishmania braziliensis*

Experimental

Plant material and isolation of compounds

All compounds tested in this study were isolated from five plant species belonging to the tribe Vernonieae. Vouchers are on deposit in the Herbarium of the Fundación Miguel Lillo, Tucumán, Argentina. Plant parts (stem, flowers and leaves) were air-dried at room temperature and afterwards powdered in preparation for analysis. Extraction of the plant material was done using solvents as ethyl acetate, chloroform and methanol. Different extracts obtained were processed using column chromatography and reverse phase HPLC following protocols previously reported (Borkosky, Alvarez Valdez et al. 1996; Borkosky, Bardon et al. 1997; Pollora et al. 2003). Pure compounds isolated were assessed by UV, IR, NMR mono- and bidimensional and MS analyses and identified by their spectroscopy features in comparison with previously reported literature data (Kurosawa et al. 1970; Lee et al. 1975; Borkosky, Alvarez Valdez et al. 1996; Borkosky, Bardon et al. 1997; Pollora et al. 2003). Compounds **1–4** and **6** came from a Bolivian collection of *Vernonanthura pinguis*; compound **5** was present on a Bolivian collection of *Eirmocephala megaphylla*; **7–10** came from an Argentine collection of *Vernonanthura nebularum*; the isogoyazensolides **11–13** and the goyazensolides **14** and **15**, from an Argentine collection of *Centratherum punctatum* spp. *punctatum*. Compounds **16** and **17** were obtained from the Argentine herb *Elephantopus mollis*.

Compounds assayed

- (1) 1-hidroxy-1,4-epoxy-8-methacryloyloxy-13-acetoxy-5E, 7(11) germacradien-6,12-olide. Molecular weight: 404.
- (2) 1-hidroxy-1,4-epoxy-8-seneciolyoxy-13-acetoxy-5E, 7(11) germacradien-6,12-olide. Molecular weight: 420.
- (3) 1-hidroxy-1,4-epoxy-8-tigloyloxy-13-acetoxy-5E, 7(11) germacradien-6,12-olide. Molecular weight: 420.
- (4) 13-acetyloxy-4,5-10,1- diepoxy-8-methacryloyloxy-germacra-7(11)-en-6,12-olide; common name: Vernonataloide. Molecular weight: 406.
- (5) 10,13-diacetyloxy-4,5- epoxy-8-methacryloyloxy-1-oxogermacra-7(11)-en-6,12-olide; common name: Glaucolide A. Molecular weight: 462.

- (6) 8, 10, 13-triacetyloxy-4,5- epoxy-1-oxogermacra-7(11)-en-6,12-olide; common name: Glaucolide B. Molecular weight: 438.
- (7) 2-Hidroxy-2,5-epoxy-8-methacryloxygermacra-3Z,11(13)-dien-6,12-olide. Molecular weight: 348.
- (8) 2-Methoxy-2,5-epoxy-8-methacryloxygermacra-3Z,11(13)-dien-6,12-olide. Molecular weight: 362.
- (9) 2-Methoxy-2,5-epoxy-8-angeloxygermacra-3Z,11(13)-dien-6,12-olide. Molecular weight: 376.
- (10) 2-Hydroxy-2,5-epoxy-8-angeloxygermacra-3Z,11(13)-dien-6,12-olide. Molecular weight: 362.
- (11) 1-Oxo-3,10-epoxy-5-hidroxy-8-metacryloyloxy-germacra-2,4(15),11(13)-trien 6,12-olide. Common name: Isolychnophorolide B. Molecular weight: 360.
- (12) 1-Oxo-3,10-epoxy-5-hidroxy-8-angeloyloxy-germacra-2,4(15),11(13)-trien-6,12-olide; common name: Isocentratherin. Molecular weight: 374.
- (13) 1-Oxo-3,10-epoxy-5-hidroxy-8-tigloyloxy-germacra-2,4(15),11(13)-trien-6,12-olide. Molecular weight: 374.
- (14) 1-Oxo-3,10-epoxy-8-epoxymethacryloyloxy-15-hydroxygermacra-2,4,11(13)-trien-6,12-olide. Molecular weight: 376.
- (15) 1-Oxo-3,10-epoxy-8-angeloyloxy-15-hydroxygermacra-2,4,11(13)-trien-6,12-olide; common name: Centratherin. Molecular weight: 374.
- (16) 8-(2-Methylpropenoyl)-1(10),4,11(13)-germacatriene-12,6;14,2-diolide. (2 α ,4E,6 α ,8 α)-form; common name: Isodeoxyelephantopin. Molecular weight: 344.
- (17) 8-(2-Methylpropenoyl)-1(10),4,11(13)-germacatriene-12,6;14,2-diolide. (2 β ,4E,6 α ,8 α)-form; common name: Deoxyelephantopin. Molecular weight: 344.

Parasites growth

Promastigotes form of *L. amazonensis* complex (clon 1: Lma, MHOM/BR/76/LTB-012) and *L. braziliensis* complex (strand M2904 C192 RJA) obtained from *in vitro* cultures of Instituto de Investigaciones Farmaco Bioquímicas (IIFB), were cultivated at 26 °C in Schneider's Insect Medium, pH 6.8, supplemented with 10% calf bovine serum inactivated at 56 °C for 30 min. Promastigotes were fixed with glutaraldehyde (5%, 180 μ L) and

counted in a Neubauer chamber.

Leishmanicidal assay

Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites/mL, were seeded on a 96-well flat bottom microtiter plate and different concentration of the test substances (0.75 – 100 $\mu\text{g/mL}$) dissolved in DMSO were added. The micro well plates were incubated for 72 h at 26 °C. After incubation, a solution of XTT (1mg/mL) in PBS, phosphate buffer saline (pH 7.0 at 37 °C) with PMS, phenazine methosulphate (Sigma-Aldrich, 0,06mg/mL), was added (50 $\mu\text{l/well}$), and incubated again for 4 h at 26 °C. DMSO (1%) and Amphotericin B Sigma-A2411 (0.5 $\mu\text{g/mL}$) were used as negative and positive controls during the evaluations (Salamanca Capusiri et al. 2008). Optical density of each well was obtained on a Synergy HT Microplate Reader (Biotec) at λ 450 nm.

Data analysis

The IC_{50} values (the concentration of a substance needed to reduce 50% parasites viability) were calculated using Microsoft Excel 2007 and expressed as mean value $\pm$ standard error (Table S1). All assays were carried out in triplicate.

Computational methods

Structures of compounds **1** – **17** were drawn in Avogadro 1.0.1 software and further minimized to their lowest energy conformation using the semi empirical PM3 method in MOPAC 2009 software. Different chemical descriptors such as heat of formation, dipole moment, and HOMO and LUMO energies were obtained as a result of the geometry optimization. In addition, the extended topochemical atom indices (ETA descriptors) were calculated by using the freely-available PaDel-Descriptor software. The ETA descriptors provide precise information regarding the molecular topology and their electronic environment. The logarithm of partition coefficient between n-octanol and water (LogP) using Chem3D Ultra 8.0 software was also calculated.

In order to build a QSAR model, the wide variety of molecular descriptors calculated for each molecule was collected (X) and it was assumed that biologic activity (y) is a function of some of the calculated molecular descriptors; $y = f(X)$. Statistical regression analysis of data was undertaken using the LINEST curve fitting routine provided by Microsoft Office Excel 2007 and different plots of the calculated descriptors vs. experimental IC_{50} values

were obtained. The coefficient of determination (R^2) was calculated in all cases and it has been used as a measure of the total variance of the response explained by the regression model. Descriptors that best fitted (higher R^2) were selected.

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Figure S1. Correlation between molecular descriptors and leishmanicidal activity expressed as IC₅₀ (μM) against *L. amazonensis* (Lam) and *L. braziliensis* (Lbr). (a) CLogP; (b) ALogP; (c) bpol descriptor. R² values are presented in Table S2.

Table S1. *In vitro* leishmanicidal activity of compounds **1–17**.

Compound	IC ₅₀ ^a - <i>L. amazonensis</i>		IC ₅₀ ^a - <i>L. braziliensis</i>	
	[µg/ml]	[µM]	[µg/ml]	[µM]
1	2.60 ± 0.10	6.44 ± 0.25	2.40 ± 0.05	5.94 ± 0.13
2	5.80 ± 0.15	13.8 ± 0.36	5.90 ± 0.30	14.1 ± 0.71
3	2.60 ± 0.15	6.19 ± 0.36	2.40 ± 0.60	5.71 ± 1.40
4	19.4 ± 1.00	47.8 ± 2.46	12.9 ± 2.30	31.8 ± 6.40
5	16.2 ± 0.14	35.1 ± 0.30	10.8 ± 0.46	23.4 ± 1.00
6	20.2 ± 0.20	46.1 ± 0.46	12.2 ± 0.80	27.9 ± 1.82
7	2.55 ± 0.07	7.33 ± 0.20	1.06 ± 0.09	3.05 ± 0.26
8	1.20 ± 0.05	3.31 ± 0.01	1.00 ± 0.10	2.76 ± 0.27
9	2.50 ± 0.20	6.65 ± 0.53	1.30 ± 0.10	3.46 ± 0.27
10	1.05 ± 0.05	2.90 ± 0.13	1.10 ± 0.10	3.04 ± 0.28
11	1.40 ± 0.01	3.89 ± 0.03	3.00 ± 0.15	8.33 ± 0.42
12	1.30 ± 0.01	3.48 ± 0.03	1.86 ± 0.60	4.97 ± 1.60
13	3.30 ± 0.18	8.82 ± 0.48	3.70 ± 0.05	9.89 ± 0.13
14	3.90 ± 0.35	10.4 ± 0.93	4.70 ± 0.20	12.5 ± 0.53
15	0.60 ± 0.10	1.60 ± 0.26	1.10 ± 0.01	2.94 ± 0.03
16	1.13 ± 0.05	3.28 ± 0.03	0.50 ± 0.10	1.45 ± 0.29
17	1.00 ± 0.01	2.90 ± 0.03	0.46 ± 0.05	1.34 ± 0.15
Amphotericin B^b	0.25 ± 0.05	0.27 ± 0.05	0.07 ± 0.01	0.08 ± 0.01

^a IC₅₀ values express as mean ± standard deviation of three determinations.^b Positive control.

Table S2. Values for the correlation between experimental activities and molecular parameters: CLogP, ALogP and bpol.

	<i>L. amazonensis clon 1</i>		<i>L. braziliensis</i>	
	R²	Equation^a	R²	Equation^a
CLogP	0.82	$y = 7.26X^2 - 32.6X + 36.2$	0.77	$y = 4.06X^2 - 19.1X + 23.7$
ALogP	0.81	$y = 8.98X^2 - 34.2X + 34.9$	0.77	$y = 4.04X^2 - 18.4X + 23.1$
bpol	0.83	$y = 0.50X^2 - 35.3X + 618$	0.80	$y = 0.35X^2 - 24.4X + 433$

^aBiological activity is expressed as function of calculated molecular descriptors; $y=f(X)$.