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Ivermectin-derived leishmanicidal compounds

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ABSTRACT

In the present study a family of macrocyclic and acyclic analogues as well as seco-analogues of avermectins were prepared from commercial Ivermectin (IVM) and their antileishmanial activity assayed against axenic promastigote and intracellular amastigote forms of *Leishmania amazonensis*. Contrarily to the filaricidal activity, the leishmanicidal potentiality of avermectin analogues does not appear to depend on the integrity of the non-conjugated $\Delta^{3,4}$-hexahydrobenzofuran moiety. Conjugated $\Delta^{2,3}$-IVM or its corresponding conjugated secoester show higher anti-leishmania activity than the parent compound. Surprisingly, the diglycosylated northern sub-unit exhibits the same anti-amastigote potentiality as the southern hexahydrobenzofuran. As expected for compounds derived from the widely used Ivermectin antibiotic, little toxicity has been noticed for most of the novel analogues prepared.

1. Introduction

Leishmaniases constitute a group of endemic tropical diseases provoked by protozoan parasites of the genus Leishmania, transmitted by the phlebotomine sandfly. About 12 million people are infected worldwide, particularly in the developing countries and 350 million people are at risk.[1] Depending upon involved parasite species, leishmaniasis occurs either as cutaneous/muco-cutaneous (CL) or the most severe visceral form (VL, or Kala-azar), that is often fatal when untreated. No vaccine yet exists and conventional chemotherapy with pentavalent antimonials, pentamidine or amphotericin-B exhibit limitations such as parenteral administration, long course of treatment, toxic side effects, high treatment cost and/or drug resistance. Despite recent advances in the disease knowledge and promising drug discovery programs,[2] therapy of leishmaniasis still represents a major health problem,[3] with increasing drug resistance constituting a major concern for the future.[4]

The use of known pharmaceutical drugs (old drugs) for new therapeutic applications is an interesting cost-effective strategy with several obvious advantages such as low development cost due to known pharmaco-toxicological profile and established industrial processes.[5] Aiming at developing new orally effective drugs against leishmaniasis, we focused our efforts on Ivermectin **1**, a well known antiparasitic semi-synthetic analogue of the avermectin family of macrolides, widely used for its potent anti-helminthic properties against livestock parasitic diseases and human filariasis.[6]

Apart from two early studies demonstrating the in vitro and in vivo activities of Ivermectin against *Leishmania donovani*, a causative agent of VL in the Old World,[7] and the in vitro inhibitory concentration of 100 μg/ml against the promastigote insect form of *Leishmania major*, a causative agent of CL also in the Old World,[8] nothing is reportedly known on the leishmanicidal properties of the avermectin family of compounds. We thus proposed to investigate the effect of Ivermectin and several of its analogues or seco-analogues on *Leishmania amazonensis*, a causative agent of CL in South America. Besides its oral activity, another decisive argument for undertaking such a study starting from commercial Ivermectin is the existence of improved efficient industrial fermentation/hydrogenation processes as well as its recent availability as a generic drug that makes these molecules cost-effective starting materials for the development of more efficient or differently profiled therapeutic drugs.

Therefore, several semi-synthetic Ivermectin analogues were tested against *L. amazonensis* promastigotes and intracellular

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amastigotes and the main results of this comparative study are presented here.

2. Results and discussion

2.1. Chemistry

The structures of all the Ivermectin analogues tested in the present study are depicted in Figure 1. For clarity, these compounds have been gathered in three sub-families of derivatives: those where the integrity of the Ivermectin C1–C25 carbon skeleton of the natural avermectins is preserved (**1**–**6**), those corresponding to the northern sub-unit after selective oxidative scission of the C10–C11 double bond (**7**–**11**) and finally those corresponding to the C1–C9 southern counter part (**12** and **13**).

A number of these Ivermectin-derived analogues were prepared according to methodologies already described. IVM–monosaccharide **2** and IVM–aglycone **3** were obtained upon hydrolysis conditions reported by Mrozik et al.[9] The physical data were identical to those already reported.[10] According to a methodology already applied by us to open avermectin macrolides,[11] refluxing Ivermectin in anhydrous ethanol in the presence of $Ti(OEt)_4$ afforded IVM ethyl secoester **4** (68% yield).

Selective ozonolysis of C10–C11 double bond of Ivermectin secoester **4** has been carried out as previously described, affording the northern alcohol **7** and the corresponding 5-*O*TBS C1–C10 southern alcohol which was subsequently deprotected under acidic conditions to afford the desired southern diol **12** in only a 12% yield probably due to the lability of the final compound. No attempt has been made to optimize this step. Spirodiol **10** and the isomeric 1,4-pentanediol derivatives **11a** and **11b** were subsequently prepared by ozonolysis of subunit **7** in MeOH at −78 °C followed by $NaBH_4$ reduction and chromatographic separation. Respective configurations of the newly created stereogenic center at C-14 in **11a** and **11b** have not been assigned.

The presence of the intact non-conjugated $\Delta^{3,4}$-double bond has been claimed to be important for the antiparasitic activity of avermectins.[12] Therefore, for bioassay comparisons, conjugated $\Delta^{2,3}$-Ivermectin **5**, and the corresponding unprecedented conjugated secoester **6** were synthesized (Scheme 1). According to known procedure,[13] $\Delta^{2,3}$-(4*S*)-Ivermectin **5** was prepared upon treatment of commercial Ivermectin **1** with substoichiometric amount of DBU until total consumption of **1**, monitored by the disappearance of the 174 ppm ^{13}C NMR signal in crude reaction mixture aliquots. The resulting conjugated $\Delta^{2,3}$-Ivermectin ethyl secoester **6** was obtained by refluxing **5** in anhydrous ethanol in the presence of $Ti(OEt)_4$ for five days under inert atmosphere. The reaction was slower than in the case of **1**, probably due to the lower reactivity of the conjugated carboxyl group. Interestingly, this process provides one single (4*S*) epimer in more than 98% of diastereomeric excess , a result quite different from the approximately 20:80 ratio of (4*S*)/(4*R*) isomers obtained when inverting the conjugation—transesterification sequence.

The novel conjugated $\Delta^{2,3}$-(4*S*) southern alcohol **13** was subsequently prepared by selective ozonolysis of conjugated secoester **6** followed by $NaBH_4$ reduction as above. During the whole sequence, no protection of the 5-OH group is required, probably as a result of the greater stability of the hexahydrobenzofuran unit resulting from the conjugation of the double bond. The route used here to

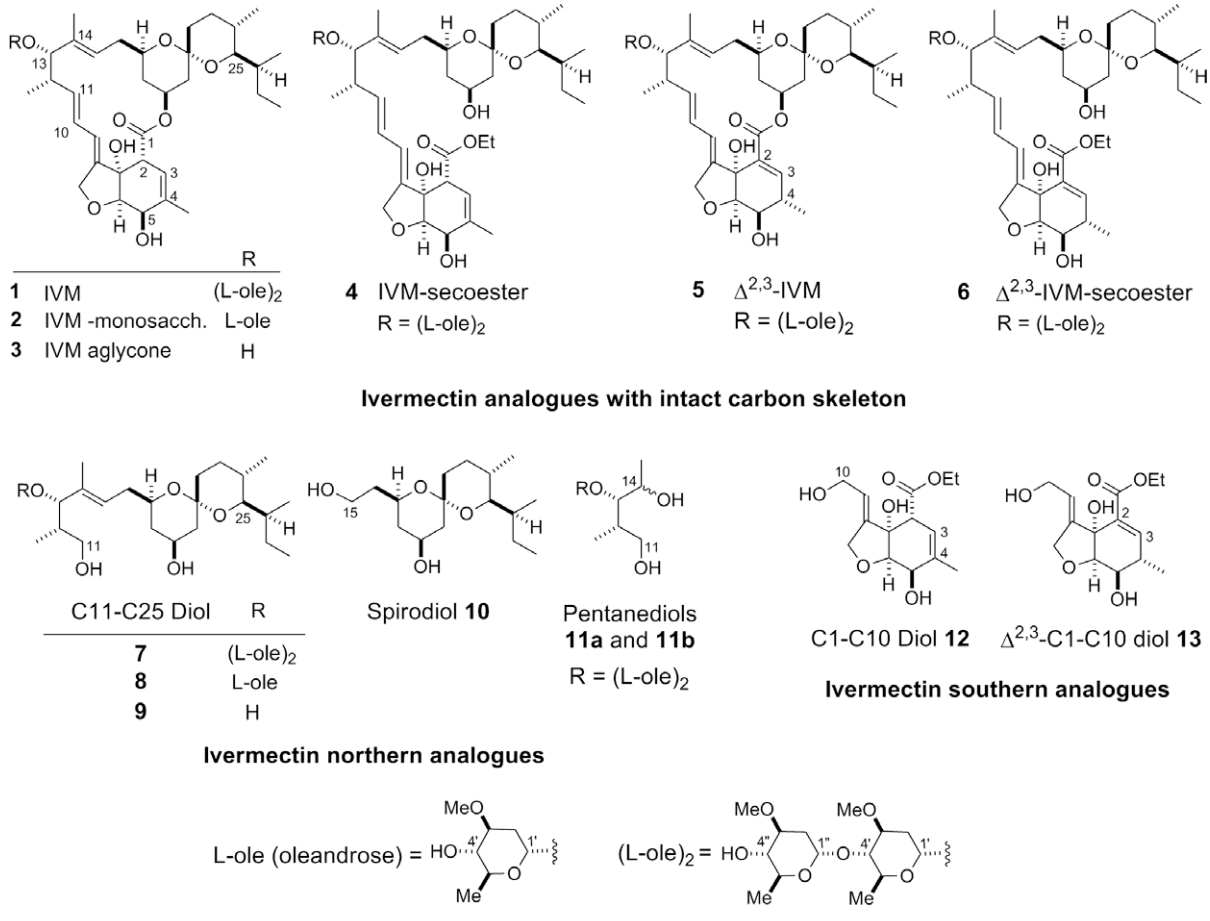
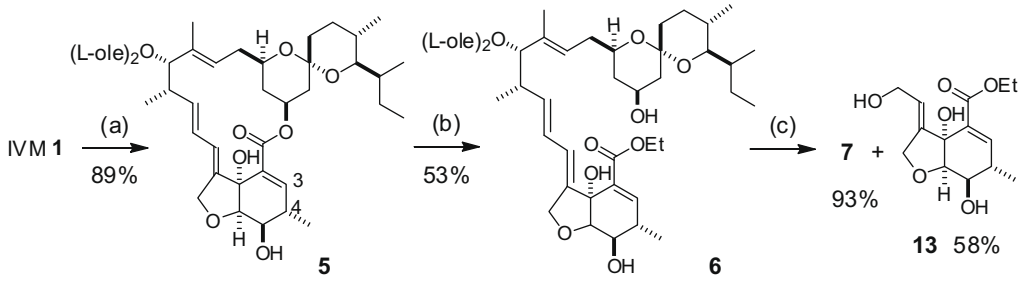


Figure 1. Tested analogues and seco-analogues derived from IVM.



Scheme 1. Preparation of conjugated $\Delta^{2,3}$-Ivermectin analogues: (a) DBU (0.4 equiv), THF, 85 °C, 15 h; (b) ethanol (10 equiv), $Ti(OEt)_4$ (1.5 equiv), 90 °C, 5 days; (c) (i) O_3, 3:1 CH_2Cl_2/EtOH, −78 °C; (ii) $NaBH_4$ (4.0 equiv), MeOH, 0 °C, 40 min.

obtain **13** represents a flexible alternative to the one previously described by Hanessian et al.[14]

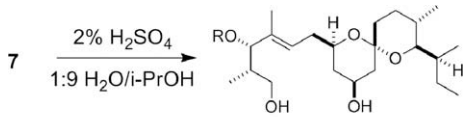
Finally, the northern C11-C25 monosaccharide **8** as well as the corresponding aglycone **9** was prepared by controlled hydrolysis of northern disaccharide **7** using slightly modified conditions in comparison with those used for intact Ivermectin (Scheme 2).

2.2. Biological activities

The antileishmanial activities of the compounds were determined in vitro against both the insect promastigote and the intra-macrophage amastigote forms of *L. amazonensis* in three independent experiments. Pentostam and Amphotericin B were used as reference drugs for anti-promastigote and anti-amastigote activities, respectively. Two controls were used because Pentostam is ineffective against promastigotes. The mean IC_{50} (the concentration required to induce half of the maximum inhibition) values are summarized in Table 1. High IC_{50} on macrophages reflects undesirable cytotoxicity to mammalian cells, and was determined by the release of the cytoplasmic enzyme lactate dehydrogenase (LDH).[15]

As can be seen in Table 1, compounds **1**–**7** possessing intact Ivermectin backbone, particularly the conjugated $\Delta^{2,3}$-conjugated secoester **6**, were very active against axenic cultures of promastigotes, indicating a direct leishmanicidal action. Noteworthy, compound **6** was more potent than the control drug Amphotericin B. The activity of Ivermectin **1** against *L. amazonensis* is in agreement with the antileishmanial activity previously described against *L. donovani* and *L. major* promastigotes.[7,8] Compounds **8**–**13** did not show significant anti-promastigote activity (IC_{50} > 60 μM).

We also assessed the activity of the compounds against the amastigote parasite forms that would require transmembrane transport to reach the macrophage phagolysosome where amastigotes grow. Whereas compounds **2**–**3**, **8**–**10** and **12** had intermediate activity, IVM **1** and compounds **4**–**7** as well as **13** were shown to be more capable at inhibiting parasite growth than the control drug Pentostam. It is worth noting that both northern sub-units **9** and **10** were active against intracellular amastigotes (IC_{50} = 30.5 μM and 54.5 μM, respectively) but not against axenic promastigotes (IC_{50} > 90 μM).



Conditions		R	yield
r.t., 24 h	**8**	L-ole	90%
50 °C, 24 h	**9**	H	40%

Scheme 2. Controlled hydrolysis of the northern disaccharide **7**.

Table 1
Antileishmanial and cytotoxic activities of IVM analogues compared with reference drugs

Compound	IC_{50}(μM)[a]			
	Promastigotes	Amastigotes	Macrophages	Selectivity index[b]
IVM analogues with intact carbon skeleton				
1 (IVM)	7.0 ± 1.5	5.0 ± 1.3	72.4 ± 1.5	14.5
2	7.7 ± 1.7	29.7 ± 1.2	>90	>3.0
3	8.3 ± 1.6	32.0 ± 1.6	>90	>2.8
4	10.7 ± 1.6	6.5 ± 1.2	42.9 ± 1.6	0.8
5	13.8 ± 1.5	3.6 ± 1.3	65.5 ± 2.1	18.2
6	2.8 ± 1.7	4.6 ± 1.2	27.1 ± 1.6	5.8
Northern-derived IVM analogues				
7	17.1 ± 1.5	13.2 ± 1.2	55.0 ± 1.4	4.2
8	65.7 ± 1.2	41.5 ± 1.3	32.1 ± 1.6	0.8
9	>90	30.5 ± 1.4	>90	>3.0
10	>90	54.5 ± 1.4	>90	1.7
11a	>90	>90	86.2 ± 1.1	<1.0
11b	>90	>90	>90	—
Southern-derived IVM analogues				
12	>90	38.6 ± 1.2	>90	2.3
13	>90	13.8 ± 1.2	>90	6.5
Reference drugs				
Pentostam	—	17.9 ± 1.2	>90	>5.0
Amphotericin B	4.4 ± 1.3	—	—	—

[a] Values are means and standard deviations of triplicate samples.
[b] The selectivity index was calculated as macrophage value/Amastigote value.

With respect to the cytotoxicity against the macrophage host cells, secoester **4** and **6**, together with the northern analogues **7** and **8**, revealed more toxic than the parent IVM **1**, whilst most of the other Ivermectin-derived analogues exhibited lower toxicity ($IC_{50} \geqslant 60$ μM).

3. Discussion–conclusion

For commercial Ivermectin **1**, the results obtained are consistent with previous observations reported in the literature (see above).

Dealing with anti-promastigote activities (Fig. 2), it clearly appears that the integrity of the Ivermectin skeleton is required. The skeleton can be either under the macrolactone form as in IVM **1** itself, monosaccharide **2**, aglycone **3** or $\Delta^{2,3}$ -conjugated macrolide **5**, or under the corresponding secoester form as in **4** or **6**. The later $\Delta^{2,3}$-conjugated secoester appeared to be the most active anti-promastigote analogue prepared from **1**, showing an in vitro activity of the same order as that of the very potent control drug Amphotericin B. Interestingly, contrarily to the anti-amastigote case (see below), neither the northern nor southern dissected analogues demonstrate important activities. Particularly striking is the fact that conjugation of the C3–C4 double bond resulted in an increase of the anti-promastigote activity, a tendency rather in

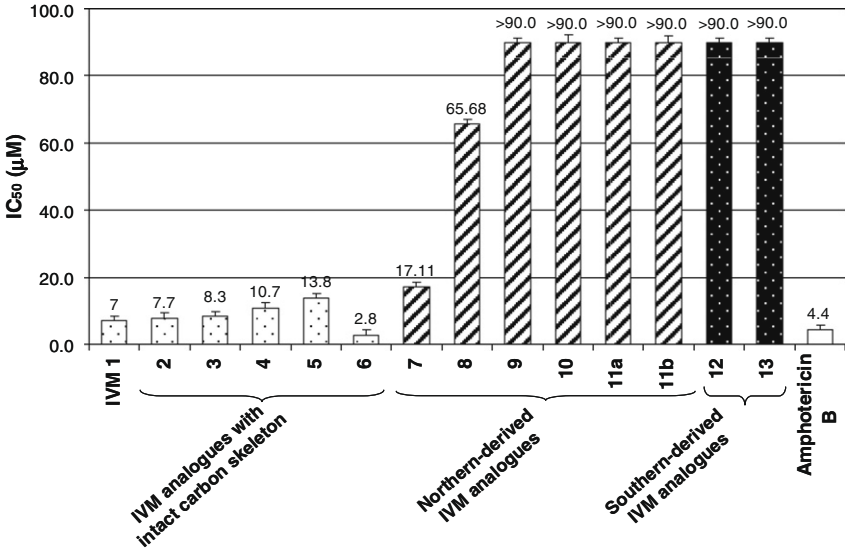


Figure 2. Comparative anti-promastigote activity of the different IVM analogues.

contradiction with that observed for the antihelminthic activity of avermectins for which the native double bond has been demonstrated to be necessary.[12]

Concerning the anti-amastigote activity of the IVM analogues on infected macrophages, the results obtained show some specific differences in comparison with the results observed above for the promastigotes, as illustrated in Figure 3. Here again, IVM analogues with non-fragmented skeleton show greater activity. $\Delta^{2,3}$-Conjugated macrolide **5** or its corresponding $\Delta^{2,3}$-secoester **6** are almost equally active and significantly more efficient than standard Pentostam. However, for the anti-amastigote efficiency, contrarily to the anti-prosmastigote case, the presence of the polar disaccharide side chain is required (compare **1** with **2** and **3** in both cases). Furthermore, the diglycosylated northern analogue **7** as well as the southern moiety **12**, and particularly the conjugated $\Delta^{2,3}$-southern analogue **13**, exhibit relevant anti-amastigote activities, which was not the case for promastigotes. These differences may account for different mechanisms of action, but also for an increased stability of the conjugated $\Delta^{2,3}$-analogues under the test conditions in comparison with the native $\Delta^{3,4}$-isomers.

The reason why **7**, **12** and **13** are active against the intracellular amastigotes but not the promastigotes that are more readily accessible to drug does not appear obvious and requires more thorough investigations.

In a first consideration, as illustrated by the differences observed in the activity of **1**, **2** and **3**, the necessity of the presence of the diglycosylated side chain in the amastigote case may reflect polarity or recognition requirements for the macrophage internalization of the substances. The importance of such glycosyl substituents is reminiscent of the results of a recent study on the anti-amastigote activity of the two flavonoids quercetin and quercetin 3-rhamnoside (quercitrin).[16] The latter glycoside is more active than the aglycone, probably due to a better ability for intra-macrophage uptake of the glycoside form. Furthermore, in the case of quercitrin, the known affinity of rhamnose for macrophage membranes may be, at least, partially responsible for the higher activity observed.[17]

The possibility that oleandrosyl residues, which are formal 2-deoxy analogues of rhamnose, present in compounds **1**, **4**–**6** and also in the northern moiety **7** favored the binding of these mole-

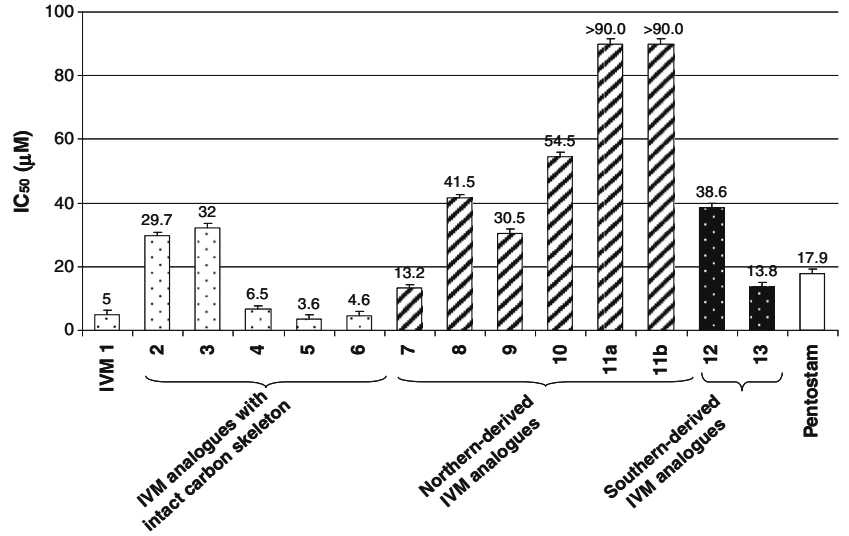


Figure 3. Comparative anti-amastigote activity of the different IVM analogues.

cules to the macrophage membrane through lectin-related receptors,[18] should also be considered. Such recognition would explain the increased activity of these glycosylated molecules against the intracellular parasites.

Taking into account both anti-promastigote and anti-amastigote activities observed during the present study, compounds **1–7** revealed the most promising. However, considering the cytotoxic profile to macrophages culture, only $\Delta^{3,4}$-macrolides **1–3** as well as $\Delta^{2,3}$-conjugated macrolide **5** deserve further attention due their higher selectivity against the parasite.

Oral drugs are highly needed for both cutaneous and visceral leishmaniasis due to the obvious drawbacks of intramuscular and intravenous conventional chemotherapy. Miltefosine, the only oral drug currently licensed for treating antimony-resistant visceral leishmaniasis in India also poses limitations due to its teratogenicity and a narrow therapeutic window.[4] Due to the oral effectiveness of Ivermectin in other infections, and the absence of acid-sensitive groups in the in vitro-active **7**, **12** and **13** analogues, it is expected that they also serve for oral administration. Although the majority of drug targets is conserved amongst the various Leishmania species as apparently is the Ivermectin target in *L. donovani* and *L. major*,[7,8] the activity of those analogues against *L. donovani* remains to be confirmed.

In conclusion, the present study confirms and complements preliminary studies on the antileishmanial properties of Ivermectin, a well-known semi-synthetic macrolide widely used since more than 20 years by the oral route for its antihelmintic properties. Our attempts to prepare analogues with improved antiparasitic activity were successful, leading to some molecules more active against *L. amazonensis* than the parental compound. More interestingly, the present preliminary SAR observations clearly establish a different profile than for antihelminthic activities for which the non-conjugated southern hexahydrobenzofuran is claimed to be an essential structural feature for optimal activity. Furthermore, different specific structural requirements are demonstrated for anti-promastigote or anti-amastigote activity, a result that, in the perspective of a future pharmacological application, may serve to design more optimized molecules against the targeted parasite.

4. Experimental

4.1. Biological assays

4.1.1. Obtention of *L. amazonensis*-GFP

L. amazonensis (Josefa strain) transfected with the green fluorescence reporter protein (GFP), was periodically isolated as amastigotes from experimentally infected mice and maintained as promastigotes in DMEM culture medium (Sigma Aldrich) supplemented with 10% heat inactivated foetal calf serum (HIFCS) at 27 °C as previously described.[15] Transfected promastigotes were periodically selected in 150 μg/mL of geneticin antibiotic.

4.1.2. Anti-promastigote activity

Promastigotes of GFP-transfected *L. amazonensis* were cultured at 5×10^5 cells/ml in 0.2 mL of DMEM culture medium containing 5% HIFCS, 0.5% DMSO plus the drugs at 0, 10, 30 and 90 μM for 72 h at 27 °C in 96-well culture plates. Amphotericin B was used as a reference drug. At the end of culture time, the fluorescence intensity of the cultures was measured using a plate-reader fluorometer (Bio-Tek) at 435 nm excitation and 538 nm emission.[15]

4.1.3. Anti-amastigote activity

Mouse peritoneal macrophages were plated at 2×10^6 cells/well of 24-well culture plates for adherence and then infected with 10^7 GFP-transfected fluorescent promastigotes for 4 h at 37 °C. Cell monolayers were washed to remove free parasites and cultured for a further 72 h with varying concentrations of the test compounds (0, 10, 30 and 90 μM) in 0.5% DMSO. Controls were 0.5% DMSO alone or Pentostam (Welcome). The fluorescence intensity of the infected cell monolayers was measured as for the antipromastigote activity. Maximum and minimum inhibitory activities were fluorescence units of uninfected macrophages and infected cells without drugs, respectively.

4.1.4. Cytotoxicity to macrophages

Mouse peritoneal macrophages were plated at 2×10^6 cells/well in 24-well culture plates and incubated for 48 h at 37 °C in 1 mL of DMEM culture medium containing 5% HIFCS and different concentrations of the test compounds (0, 10, 30 and 90 μM) in 0.5% DMSO. The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) into the culture medium was measured using an assay kit (Doles Reagentes, Brazil).[15] Maximum and minimum release values were cells cultured with 2% Triton X-100 or 0.5% DMSO, respectively.

4.1.5. Data analysis

The data were analyzed by one-way analysis of variance followed by a Tukey posttest.

4.2. Chemistry

4.2.1. General

Optical rotations were determined on a JASCO instrument, model DIP-370. Mass spectra were obtained by electron-spray on a Micromass ZQ 4000 spectrometer by direct introduction. High-resolution, high mass-accuracy measurements were performed either on a Micromass Autospec mass spectrometer with EBE configuration using 70 eV electron ionisation (EI) at the Instituto de Química, Campinas, Brazil or on a Micromass ZAB-SpecTOF spectrometer at the Centre Régional de Mesures Physiques de l'Ouest, CRMPO, Rennes, France, using electron spray ionisation (ESI) and methanol as solvent (4 kV acceleration, 60 °C source temperature). Infrared spectra were obtained in potassium bromide on a Nicolet-Nexus 670 model instrument (wavelengths are given in wavenumbers). ^{1}H NMR spectra were recorded on a Bruker DRX 300 or Bruker DRX 400 instruments. The chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants (J) are given in Hertz (Hz). ^{13}C NMR spectra were recorded on a Bruker AC 200 at 50.32 MHz or on a Bruker DRX 300 instrument at 75.47 MHz. The chemical shifts are expressed in parts per million (ppm), and are referenced to residual chloroform (δ = 77.0 ppm).

Commercial Ivermectin {$[\alpha]_D$ = +34.8 (*c*, 1.00, $CHCl_3$)} used throughout this work was a mixture of 22,23-dihydroavermectin B1a and B1b, in a ratio greater than 97:3 (HPLC analysis) and is therefore described throughout this paper as the B1a constituent. This material was kept under high vacuum for several hours before use. Most of the reagents (Aldrich) were used without treatment. Titanium(IV) ethylate [$Ti(OEt)_4$], was prepared before use by refluxing [$Ti(O\textit{i}\text{-}Pr)_4$] in anhydrous ethanol, followed by distillation under reduced pressure (150–170 °C, 650–1300 Pa), and stored under nitrogen. Ozone was obtained from a BMT 802 ozone generator (BMT Messtechnik GMBH, Berlin, Germany) and the enriched ozone stream was controlled using a flow rotameter. All the solvents were distilled before use.

For clarity, avermectins/Ivermectin carbon and proton numbering has been used for all compounds throughout the paper.

4.2.2. Southern diol (12)

A solution of 5-*O*-(*tert*-butyldimethylsilyl) southern alcohol (800 mg, 2.0 mmol) resulting from ozonolysis of 5-*O*-(*tert*-butyldimethylsilyl) IVM as previously described,[11] was stirred in MeOH (6.0 mL) under nitrogen at room temperature for 90 min in the presence of PTSA (1% w/v). After total substrate consumption, the mixture was diluted by AcOEt (50 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give, after purification by flash chromatography on silica gel (hexanes/AcOEt mixtures from 1:0 to 0:1), 71 mg (12%) of southern alcohol **12** as colourless oil.

^{1}H NMR (200 MHz; $CDCl_3$): δ = 5.67 (dddd, 1H, J = 2.2, 6.1 Hz, H-9), 5.41 (dt, 1H, J = 1.8, 4.3 Hz, H-3), 4.65 (br d, 1H, J = 14.0 Hz, H_A-8a), 4.47 (br d, 1H, J = 14.0 Hz, H_B-8a), 4.41 (br d, 1H, J = 4.9 Hz, H-5), 4.20 (q, 2H, J = 7.1 Hz, $COOCH_2CH_3$), 4.19 (d, 1H, J = 4.9 Hz, H-6), 4.11 (br d, 2H, J = 6.1 Hz, H_2-10), 3.41 (app. dt, 1H, J = 2.0, 4.3 Hz, H-2), 2.91 (br s, 3H, HO-5. HO-7 and HO-10), 1.84 (br s, 3H, Me-4), 1.29 (t, 3H, J = 7.1 Hz, $COOCH_2CH_3$) ppm. ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 173.0 (C-1), 145.0 (C-8), 138.9 (C-4), 120.3 (C-9), 117.3 (C-3), 82.7 (C-6), 78.2 (C-7), 68.4 (C-8a), 67.9 (C-5), 61.5 ($COOCH_2CH_3$), 60.0 (C-10), 47.4 (C-2), 19.2 (Me-4), 14.1 ($COOCH_2CH_3$) ppm. HRMS (ESI, MeOH): m/z calcd for $C_{14}H_{20}O_6$ [M^+] 284.1260; found 284.1263.

4.2.3. $\Delta^{2,3}$-(4*S*)-Ivermectin (5)

Ivermectin **1** (5.36 g, 6.12 mmol) and DBU (0.37 mL, 2.47 mmol, 0.4 equiv) were dissolved in 30 mL anhydrous THF and the solution stirred at 85 °C for 15 h before cooling to room temperature. The reaction mixture was diluted with diethyl ether (150 mL) and washed with aqueous 0.5 N HCl (2 × 100 mL). The aqueous layer was washed with diethyl ether (2 × 100 mL), and the combined organic extracts were washed with brine (2 × 100 mL), dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give, after purification by silica gel flash chromatography (hexanes/AcOEt mixtures from 4:1 to 1:4), 4.78 g (89%) of $\Delta^{2,3}$-(4*S*)-Ivermectin **5** as a pale-yellow foam.

$[\alpha]_D$ = +206.0 (c, 0.73, $CHCl_3$). IR (KBr): ν = 3500, 3000–2850, 1699, 1456, 1383, 1200–1000, 983 cm^{-1}. ^{1}H NMR (400 MHz; $CDCl_3$): δ = 6.18 (dt, 1H, J = 2.4, 10.8 Hz, H-9), 6.15 (d, 1H, J = 1.6 Hz, H-3), 5.78 (dd, 1H, J = 10.0, 14.8 Hz, H-11), 5.69 (dd, 1H, J = 10.8, 14.8 Hz, H-10), 5.41 (d, 1H, J = 3.6 Hz, H-1″), 5.39–5.29 (m, 1H, H-19), 4.94 (br d, 1H, J = 10.8 Hz, H-15), 4.80 (s, 1H, HO-7), 4.76 (d, 1H, J = 3.2 Hz, H-1′), 4.59 (dd, 1H, J = 2.4, 14.0 Hz, H_A-8a), 4.51 (dd, 1H, J = 2.4, 14.0 Hz, H_B-8a), 4.05 (d, 1H, J = 2.0 Hz, H-6), 3.93 (br s, 1H, H-13), 3.88–3.55 (m, 5H, H-5, H-3′, H-5′, H-3″ and H-5″), 3.53–3.43 (m, 1H, H-17), 3.47 (s, 3H, OMe), 3.44 (s, 3H, OMe), 3.25–3.20 (app d, 1H, H-25), 3.25 (t, 1H, J = 9.2 Hz, H-4′ or H-4″), 3.17 (t, 1H, J = 9.2 Hz, H-4″ or H-4′), 2.61 (br s, 1H, HO-4″), 2.56–2.43 (m, 2H, H-4 and H-12), 2.36–2.18 (m, 4H, H_2-16, H_{eq}-2′ and H_{eq}-2″), 1.95 (dd, 1H, J = 4.3, 12.4 Hz, H_{eq}-20), 1.87 (br d, 1H, J = 12.0 Hz, H_{eq}-18), 1.70 (br s, 1H, HO-5), 1.68 (br d, 1H), 1.60–1.35 (m, 10H, including H_2-22, H_2-23 and H_2-27), 1.46 (br s, 3H, Me-14), 1.30–1.20 (m, 9H, Me-5′, Me-5″ and Me-4), 1.16 (d, 3H, J = 6.8 Hz, Me-12), 0.93 (t, 3H, J = 7.6 Hz, Me-27), 0.84 (d, 3H, J = 6.4 Hz, Me-24), 0.79 (d, 3H, J = 5.2 Hz, Me-26), 0.71 (q, 1H, J = 12.0 Hz, H_{ax}-18) ppm. ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 168.8 (C-1), 138.9 (C-8), 138.6 (C-3), 138.0 (C-11), 134.7 (C-14), 129.7 (C-2), 125.4 (C-10), 122.7 (C-9), 118.0 (C-15), 98.4 (C-1″), 97.3 (C-21), 94.7 (C-1′), 82.9 (C-6), 81.8 (C-13), 80.3 (C-4′), 79.2 (C-3′), 78.4 (C-5), 78.1 (C-3″), 76.6 (C-25), 75.9 (C-4″), 72.0 (C-8a), 68.9 (C-19), 68.1 (C-7), 67.8 (C-5″), 67.1 (2C, C-5′ and C-17), 56.5 (OMe), 56.3 (OMe), 40.3 (C-20), 39.6 (C-12), 36.8 (C-18), 35.7 (C-22), 35.3 (C-26), 34.4 (C-2′), 34.3 (C-16), 34.1 (C-2″), 33.1 (C-4), 31.1 (C-24), 27.9 (C-23), 27.2 (C-27), 20.1 (Me-12), 18.3 (Me-5′), 17.6 (Me-5″), 17.3 (Me-24), 16.8 (Me-4), 15.1 (Me-14), 12.3 (Me-26), 11.9 (Me-27) ppm. ES MS: m/z (%) = 898 (100) [M^++Na], 569 (11), 307 (22). HRMS (ESI, MeOH): m/z calcd for $C_{48}H_{74}O_{14}$ [M^+] 874.5079; found 874.5063.

4.2.4. $\Delta^{2,3}$-(4*S*)-Ivermectin ethyl secoester 6

An oven-dried 25-mL flask was charged under nitrogen with $\Delta^{2,3}$-(4*S*)-Ivermectin **5** (2.47 g, 2.82 mmol), ethanol (1.65 mL, 29.0 mmol; 10.0 equiv) and $Ti(OEt)_4$ (0.89 mL, 4.25 mmol, 1.5 equiv). The reaction mixture was stirred under reflux for five days. After cooling to room temperature, the mixture was diluted with diethyl ether (200 mL) and washed with aqueous 0.5 N HCl (3 × 100 mL). The aqueous layer was washed with diethyl ether (100 mL), and the combined organic fractions were washed with brine (2 × 150 mL), dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give, after purification by silica gel flash chromatography (hexanes/AcOEt mixtures from 4:1 to 1:9), 0.62 g (25%) of recovered **5** and 1.37 g (53%) of $\Delta^{2,3}$-(4*S*)-Ivermectin ethyl secoester **6** as a pale-yellow foam.

$[\alpha]_D$ = +58.3 (c, 1.06, $CHCl_3$). IR (KBr): ν = 3473, 3000–2850, 1694, 1456, 1382, 1150–1000, 986 cm^{-1}. ^{1}H NMR (400 MHz; $CDCl_3$): δ = 6.63 (d, 1H, J = 1.8 Hz, H-3), 6.33 (app dt, 1H, J = 2.4, 10.0 Hz, H-9), 5.97–5.84 (m, 2H, H-10 e H-11), 5.44 (br t, 1H, J = 6.8 Hz, H-15), 5.28 (d, 1H, J = 3.2 Hz, H-1″), 4.72 (d, 1H, J = 2.8 Hz, H-1′), 4.66 (dd, 1H, J = 2.4, 14.0 Hz, H_A-8a), 4.54 (dd, 1H, J = 2.4, 14.0 Hz, H_B-8a), 4.22 (m, 2H, $COOCH_2CH_3$), 4.07 (m, 1H, H-19), 4.02 (d, 1H, J = 2.4 Hz, H-6), 3.78–3.45 (m, 5H, H-17, H-3′, H-5′, H-3″ and H-5″), 3.63 (d, 1H, J = 8.4 Hz, H-13), 3.55 (dd, 1H, J = 2.4, 9.6 Hz, H-5), 3.42 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.17–3.08 (m, 3H, H-4′, H-4″ and H-25), 2.63 (ddddd, 1H, J = 1.8, 7.1, 9.6 Hz, H-4), 2.45 (m, 1H, H-12), 2.33–2.23 (m, 3H, H_2-16 and H_{eq}-2″), 2.09 (app ddd, 1H, J = 1.0, 4.8, 12.0 Hz, H_{eq}-2′), 2.00–1.87 (m, 3H, including H_{eq}-20 and H_{eq}-18), 1.64 (br d, 1H, J = 10.1 Hz), 1.54 (br s, 3H, Me-14), 1.53–1.45 (m, 6H, H_2-22, H_2-23, H_2-27), 1.34–1.22 (m, 9H, including Me-5′ and Me-5″), 1.32 (t, 3H, J = 7.2 Hz, $COOCH_2CH_3$), 1.12 (d, 3H, J = 6.0 Hz, Me-12), 1.11 (app q, 1H, J = 11.6 Hz, H_{ax}-18), 0.92–0.86 (m, 6H, Me-4 e Me-27), 0.80 (d, 3H, J = 6.8 Hz, Me-24), 0.78 (d, 3H, J = 6.0 Hz, Me-26) ppm. ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 167.6 (C-1), 144.5 (C-8), 140.4 (2C, C-3 e C-11), 133.8 (C-14), 128.7 (C-2), 127.2 (C-10), 125.3 (C-15), 123.0 (C-9), 99.0 (C-1″), 97.2 (C-21), 93.3 (C-1′), 86.6 (C-13), 83.8 (C-6), 81.8 (C-4′), 79.3 (C-3′), 78.3 (C-3″), 77.9 (C-5), 77.2 (C-25), 76.2 (C-4″), 71.9 (C-7), 68.2 (2C, C-8a e C-5″), 67.6 (C-17), 66.7 (C-5′), 64.7 (C-19), 61.1 ($COOCH_2CH_3$), 56.3 (OMe), 56.1 (OMe), 45.0 (C-20), 40.6 (C-18), 38.3 (C-12), 35.8 (C-22), 35.4 (C-26), 34.8 (C-2′), 34.5 (C-2″), 34.1 (C-16), 33.1 (C-4), 31.3 (C-24), 28.1 (C-23), 27.4 (C-27), 18.0 (Me-12), 17.6 (Me-5″), 17.3 (Me-5′), 16.7 (2C, Me-4 and Me-24), 14.0 ($COOCH_2CH_3$), 12.4 (Me-26), 11.4 (Me-27) ppm. ES MS: m/z (%) = 944 (100) [M^++Na], 926 (6), 403 (7), 291 (12). HRMS (ESI, MeOH); m/z calcd for $C_{50}H_{80}O_{15}$ [M^+] 920.5497; found 920.5466.

4.2.5. $\Delta^{2,3}$-(4*S*) Southern diol (13)

A trace amount of Sudan Red 7B was added to a magnetically stirred solution of $\Delta^{2,3}$-(4*S*)-Ivermectin ethyl secoester **6** (1.28 g, 1.39 mmol) in 38.5 mL of 3:1 CH_2Cl_2/EtOH mixture. The mixture was cooled to −78 °C under nitrogen and then ozone was bubbled until TLC monitoring showed complete consumption of the starting material (TLC monitoring was done after a rapid nitrogen purge, independently of the solution colour fading). The reaction mixture was then purged with nitrogen, added with $NaBH_4$ (211 mg, 5.58 mmol, 4.0 equiv) in 9.6 mL of methanol, allowed to warm to 0 °C and maintained at this temperature for 40 min. The stirred solution was diluted with water at 0 °C and aqueous 1.0 N HCl was added dropwise down to pH 4.0. The phases were separated and the aqueous layer was extracted with chloroform (2 × 50 mL), saturated with NaCl and re-extracted with 9:1

$CHCl_3$/MeOH mixture (2 × 50 mL). The combined organic extracts were washed with brine (2 × 100 mL), dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (4:1 hexanes/AcOEt) to give, in order of elution, 874 mg (93%) of known northern alcohol **7** as a white powder and 230 mg (58%) of $\Delta^{2,3}$-(4*S*) southern diol **13** as a colourless oil.

IR (KBr): ν = 3407, 3000–2850, 1695, 1456, 1372, 1200–1000 cm^{-1}. ^1H NMR (400 MHz; $CDCl_3$): δ = 6.63 (d, 1H, *J* = 2.0 Hz, H-3), 5.94 (dddd, 1H, *J* = 2.5, 5.8, 6.8 Hz, H-9), 4.95 (br s, 1H, HO-7), 4.60 (app. ddt, 1H, *J* = 1.3, 2.5, 13.7 Hz, H_A-8a), 4.43 (app. ddt, 1H, *J* = 1.3, 2.5, 13.7 Hz, H_B-8a), 4.24 (q, 2H, *J* = 7.2 Hz, $COOCH_2CH_3$), 4.12 (ddt, 1H, *J* = 1.3, 6.8, 13.5 Hz, H_A-10), 4.09 (ddt, 1H, *J* = 1.3, 5.8, 13.5 Hz, H_B-10), 4.03 (d, 1H, *J* = 2.4 Hz, H-6), 3.56 (dd, 1H, *J* = 2.4, 9.4 Hz, H-5), 2.59 (ddddd, 1H, *J* = 2.0, 7.3, 9.4 Hz, H-4), 2.40 (br s, 2H, HO-5 and HO-10), 1.33 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$), 1.26 (d, 3H, *J* = 7.3 Hz, Me-4) ppm. ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 167.5 (C-1), 144.4 (C-3), 143.6 (C-8), 128.9 (C-2), 122.8 (C-9), 83.4 (C-6), 78.3 (C-7), 72.3 (C-5), 67.9 (C-8a), 61.3 ($COOCH_2CH_3$), 60.3 (C-10), 33.4 (C-4), 16.8 (Me-4), 14.1 ($COOCH_2CH_3$) ppm. HRMS (ESI, MeOH): *m/z* calcd for $C_{14}H_{20}O_6$ [M^+] 284.1260; found 284.1258.

4.2.6. Northern monosaccharide (8)

Northern alcohol **7** (400 mg, 0.595 mmol) was added to a solution of 2% H_2SO_4 in 1:9 H_2O/*i*-PrOH mixture (40 mL) and stirred at rt for 24 h. Then chloroform (50 mL) was added, and the solution transferred into a separatory funnel, washed with aqueous $NaHCO_3$ solution (3 × 30 mL). The aqueous layer was re-extracted with chloroform (2 × 30 mL), and the combined organic extracts were washed with brine (50 mL), dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2/AcOEt mixtures from 1:0 to 1:1) to give 314 mg (90%) of northern alcohol monosaccharide **8** as a white powder.

$[\alpha]_D$ = −16.7 (*c*, 0.73 , AcOEt). IR (KBr): ν = 3406, 3000–2850, 1458, 1383, 1150–1000, 987 cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$): δ = 5.52 (br t, 1H, *J* = 6.8 Hz, H-15), 4.82 (d, 1H, *J* = 3.0 Hz, H-1′), 4.08 (m, 1H, H-19), 3.80 (d, 1H, *J* = 9.8 Hz, H-13), 3.75–3.30 (m, 5H, H_2-11, H-17, H-3′, H-5′), 3.40 (s, 3H, OMe), 3.17–3.07 (m, 2H, H-4′, H25), 2.57 (br s, 1H, HO-11), 2.53 (br s, 1H, HO-4′), 2.28 (br t, 2H, *J* = 6.8 Hz, H_2-16), 2.14 (dd, 1H, *J* = 4.9, 13.2 Hz, H_{eq}-2′), 2.02–1.87 (m, 3H, including H_{eq}-18 and H_{eq}-20), 1.63 (br d, 1H, *J* = 7.6 Hz), 1.55 (br s, 3H, Me-14), 1.58–1.43 (m, 6H, H_2-22, H_2-23, H_2-27), 1.37–1.22 (m, 4H), 1.29 (br d, 3H, *J* = 6.0 Hz. Me-5′), 1.13 (q, 1H, *J* = 11.7 Hz, H_{ax}-18), 0.88 (t, 3H, *J* = 7.2 Hz, Me-27), 0.84–0.73 (m, 9H, Me-12, Me-24, Me-26) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 132.8 (C-14), 128.1 (C-15), 97.2 (C-21), 92.8 (C-1′), 85.5 (C-13), 78.5 (C-3′), 77.3 (C-25), 76.1 (C-4′), 68.0 (C-5′), 67.6 (C-17), 66.6 (C-11), 64.8 (C-19), 56.6 (OMe-3′), 45.0 (C-20), 40.6 (C-18), 36.8 (C-12), 35.8 (C-22), 35.4 (C-26), 34.1 (C2′), 34.0 (C-16), 31.3 (C-24), 28.1 (C-23), 27.5 (C-27), 18.0 (Me-5′), 17.4 (Me-24), 14.1 (Me-12), 12.5 (Me-26), 11.4 (Me-27), 10.9 (Me-14) ppm. HRMS (ESI, MeOH); *m/z* calcd for $C_{29}H_{50}O_7$ [M^+–18] 510.3557; found 510.3560.

4.2.7. Northern aglycone (9)

Northern alcohol **7** (400 mg, 0.595 mmol) was added to a solution of 2% H_2SO_4 in 1:9 H_2O/*i*-PrOH mixture (40 mL) and stirred at 50 °C for 24 h. Subsequent chloroform extraction as above furnished a crude product which gave 90 mg (40%) of northern alcohol aglycone **9** as a white powder after chromatography (CH_2Cl_2/AcOEt mixtures from 1:0 to 2:3).

$[\alpha]_D$ = +59.4 (*c*, 0.96, AcOEt). IR (KBr): ν = 3390, 3000–2850, 1460, 1383, 1150–1000, 984 cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$): δ = 5.33 (br t, 1H, *J* = 6.8 Hz, H-15), 4.00 (m, 1H, H-19), 3.80 (d, 1H, *J* = 8.9 Hz, H-13), 3.70-3.15 (m, 5H, including H_2-11, H-17), 3.07 (br d, 1H, H25), 2.20 (m, 2H, H_2-16), 2.00-1.75 (m, 3H, including H_{eq}-18 and H_{eq}-20), 1.56 (br s, 3H, Me-14), 1.65–1.35 (m, 6H, H_2-22, H_2-23, H_2-27), 1.35–1.15 (m, 4H), 1.02 (q, 1H, *J* = 11.6 Hz, H_{ax}-18), 0.82 (t, 3H, *J* = 7.2 Hz, Me-27), 0.78–0.60 (m, 9H, Me-12, Me-24, Me-26) ppm. ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 137.8 (C-14), 124.2 (C-15), 97.3 (C-21), 84.6 (C-13), 77.2 (C-25), 68.0 (C-11), 67.6 (C-17), 64.8 (C-19), 44.8 (C-20), 40.2 (C-18), 37.0 (C-12), 35.7 (C-22), 35.4 (C-26), 33.9 (C-16), 31.2 (C-24), 28.0 (C-23), 27.3 (C-27), 17.4 (Me-24), 13.8 (Me-12), 12.4 (Me-26), 11.7 (Me-27), 11.2 (Me-14) ppm. HRMS (ESI, MeOH); *m/z* calcd for $C_{22}H_{40}O_5$ [M^+] 384.2876; found 384.2876.

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References and notes

1. World Health Organization. http://www.who.int/leishmaniasis/en/ (2008); http://www.who.int/tdr/diseases/leish/default.htm (2008).
2. Croft, S. L.; Coombs, G. H. *Trends Parasitol.* **2003**, *19*, 502.
3. Desjeux, P. *Comp. Immun. Microbiol. Infect. Dis.* **2004**, *27*, 305.
4. Croft, S. L.; Sundar, S.; Fairlamb, A. H. *Clin. Microbiol. Rev.* **2006**, *19*, 111.
5. For an example: Christensen, D. *Sci. News* **2003**, *162*, 296.
6. Omura, S. *Int. J. Antimicrob. Agent* **2008**, *31*, 91.
7. Rifaat, L. K.; Mohammad, M. A.; Jawdat, S. Z. *Jpn. J. Med. Sci. Biol.* **1989**, *42*, 51.
8. Rasheid, K. A.; Morsy, T. A. *J. Egypt Soc. Parasitol.* **1998**, *28*, 207.
9. Mrozik, H.; Eskola, P.; Arison, B. H.; Albers-Schönberg, G.; Fisher, M. H. *J. Org. Chem.* **1982**, *47*, 489.
10. Chabala, J. C.; Mrozik, H.; Tolman, R. L.; Eskola, P.; Lusi, A.; Peterson, L. H.; Woods, M. F.; Fisher, M. H. *J. Med. Chem.* **1980**, *23*, 1134.
11. Santos, A. R.; Ferreira, M. L. G.; Kaiser, C. R.; Férézou, J.-P. *Eur. J. Org. Chem.* **2005**, 3348.
12. For example, against mites, conjugated avermectin B1a exhibited only one-fifth of the natural product activity: Pivnichny, J. V.; Arison, B. H.; Preiser, F. A.; Shim, J.-S. K.; Mrozik, H. *J. Agric. Food Chem.* **1988**, *36*, 826.
13. Hanessian, H.; Dubé, D.; Hodges, P. J. *J. Am. Chem. Soc.* **1987**, *109*, 7063.
14. Hanessian, H.; Ugolini, A.; Hodges, P. J.; Dubé, D. *Tetrahedron Lett.* **1985**, *27*, 2699.
15. Boeck, P.; Falcão, C. B.; Leal, P. V.; Cechinel-Filho, V.; Torres-Santos, E. C.; Yunes, R. A.; Rossi-Bergmann, B. *Bioorg. Med. Chem.* **2006**, *16*, 1538.
16. Muzitano, M. F.; Tinoco, L. W.; Guette, C.; Kaiser, C. R.; Rossi-Bergmann, B.; Costa, S. S. *Phytochemistry* **2006**, *67*, 2071.
17. Bozue, J. A.; Parthasarathy, N.; Phillips, L. R.; Cote, C. K.; Fellows, P. F.; Mendelson, I.; Shafferman, A.; Friedlander, A. M. *Microb. Pathog.* **2005**, *38*, 1.
18. Monteiro, G. C. T. S.; Hirata, R.; Andrade, A. F. B.; Mattos-Guaraldi, A. L.; Nagao, P. E. *Int. J. Mol. Med.* **2004**, *13*, 175.