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Leishmanicidal and trypanocidal activities of 2-aminocyclohexanol and 1,2-cyclohexanediamine derivatives

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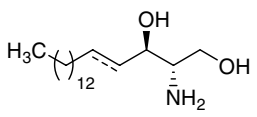
Abstract—A number of aminoalcohols, diamines and other related cycloanalogues of sphingosine have been synthesized and assayed in vitro against three *Leishmania* spp. and *Trypanosoma cruzi*. Most of the compounds were potent parasiticides, with IC_{50} values in the μM or lower range and potencies higher than those of pentamidine and benznidazol, the common therapeutic agents against these parasitoses.

Leishmaniasis and trypanosomiasis are widespread parasitic diseases, vectored by insects and affecting the poorest people of tropical and subtropical regions principally.[1] Both types of infections are considered within the most relevant group of neglected tropical diseases[2] and targeted by the WHO and other health organizations for prevention, control and eradication.[3] Leishmaniasis, produced by several *Leishmania* spp., occurs in three cutaneous (CL), muco-cutaneous (MCL) and visceral (VL) forms. CL and MCL forms produce drastic deforming lesions of skin and tissues, while VL usually leads to death if untreated. American trypanosomiasis, commonly known as Chagas disease, is provoked by *Trypanosoma cruzi* and, after a long asymptomatic and untreated period, usually leads to fatal results. African trypanosomiasis, known as the sleeping sickness, practically incapacitates the infected patient for any activity and also leads to death. Currently, great efforts are addressed to vaccine development, vector elimination with selective pesticide applications but, up to now only a few old and inadequate drugs are in clinical use.

Drugs in current use against leishmaniasis such as antimonial derivatives (glucantime), bis-amidines (pentamidine) and polyene macrolides (amphotericin B) display high liver, kidney or heart toxicities, develop clinical resistance easily[4] and could be contributing to increase the incidence of leishmaniasis-AIDS co-infections.[5] Nifurtimox and benznidazol, the two drugs in use for the treatment of trypanosomiasis (sleeping sickness and Chagas disease), are toxic,[6] have serious undesirable effects[7] and do not guarantee a complete cure. Consequently, it could be stated that the therapeutic control of these diseases is worsening. Due to these reasons, there exists a real necessity of implementing new methods for disease control; particularly of discovering alternative new drugs, being safer, more effective and inexpensive enough to allow their worldwide use against these parasitic infections.

Sphingosine is a natural unsaturated C-18 linear aminodiol, which mainly integrates sphingolipids, ceramides and other lipidic metabolites.



Sphingosine and **dihydrosphingosine**

Keywords: Synthesis; Aminoalcohols; Diamines; Sphingosine; SAR; In vitro assays; *Leishmania*; *Trypanosoma*.
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Sphingosine-1-phosphate has been recognised as a multifunctional physiologic mediator, with roles on regulating cardiogenesis, vascular system formation, oocyte survival and immune cell trafficking. Several other physiologic roles remain to be elucidated.[8] It has also been proposed as intracellular second messenger[9] and, among other functions, is able to balance and control the levels of sphingolipid metabolites and, consequently, to regulate signalling pathways, determining whether the cell survives or dies. Sphingosine, its metabolites and their derivatives have been considered as target molecules for the design of potential candidates in the development of new drugs.[10] In fact, several reports describe antimicrobial, antiparasitic and other pharmacological properties for linear or substituted aminoalcohols, aminodiols and diamines.[11]

Our group was interested in this type of lipidic molecules, particularly in linear 2-aminoalkanols related to dihydrosphingosine, 1,2-diamines and their intermediates and derivatives, whose synthesis and leishmanicidal activity were previously reported.[12] Aiming to obtain new potential antiparasitics, we designed several series of compounds with the structural basis of sphingosine and dihydrosphingosine and incorporating the amino and hydroxyl groups on a cyclic skeleton (Fig. 1).

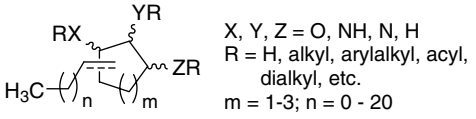
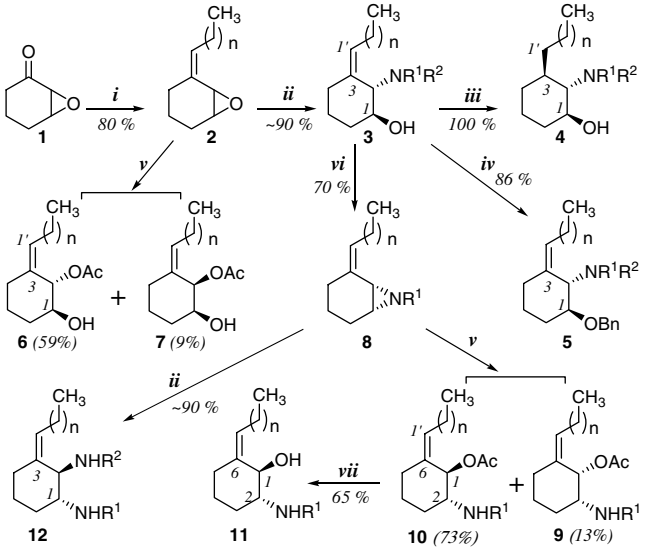


Figure 1. Global structure of potential antiparasitic sphingosine cycloanalogues.



Scheme 1. The synthesis of compounds of type **2**–**12**. Reagents and conditions: [R^1, R^2: H(**a**), *n*-Bu(**b**), *n*Hex(**c**), Bn(**d**), NH_2Et(**e**), Et_2(**f**); see Table 1]. (i) $PPh_3(CH_2)_nCH_3$, BuLi, THF, −70 °C; (ii) primary or secondary amine, CH_3CN, $LiClO_4$, Δ; (iii) H_2/Pd–C, MeOH; (iv) BnBr, NaH, TBAI, toluene, Δ; (v) AcOH, EtOAc; (vi) DIAD, PPh_3, MTBE; (vii) *p*-TsOH, MeOH, Δ.

In this paper, we report on the synthesis of several representative cyclic analogues of sphingosine and the results of their in vitro evaluation against promastigotes of cutaneous, mucocutaneous and visceral *Leishmania* spp. and against epimastigotes of *T. cruzi*.

Compounds tested in this study were, principally, racemic *trans* and *cis* 3(6)-alkylidene(alkyl)-2-amino-cyclohexanols and derivatives, along with cyclohexanediamine and cyclohexanediol analogues, as well as the cyclohexane-fused aziridine intermediates. Their synthesis, starting from cyclohexenone oxide (**1**), is summarized in Scheme 1. The epoxyolefins of type **2**, with alkylidene side chains of different sizes, were obtained through Wittig olefination of **1**, according to the procedure described by Koreeda.[13] In that conditions the *Z* configuration of the olefin was the only detected. Side-chain sizes varied from C_5 to C_{22} (n = 3–20, Scheme 1). Nevertheless, on the basis of previous bioactivity results found with linear open-chain compounds,[10] not all the combinations of *n* values, R^1 and R^2 represented in Scheme 1 were synthesized, and only a selected number of those compounds expected to display potent antiparasitic activity (Table 1) were actually assayed.

Epoxides of type **2** were treated with alkyl and dialkylamines in presence of $LiClO_4$ to obtain the series of alkyl(dialkyl)aminocyclohexanols, **3**.[14] The following simple chemical descriptions refer only to the actually assayed compounds, which belong, principally, to the series with a C_{14} side-chain (n = 12), attached to the cyclohexane ring.

Under catalytic hydrogenation (H_2/Pd–C) the olefinic *N*-*n*-hexylamino derivative **3c** was transformed into the saturated analogue **4c**, while under similar conditions the *N*-benzyl derivative **3d** led to the saturated and debenzylated aminoalcohol **4a**. The diethylaminoether **5f** was prepared through benzylation of the diethylaminoalcohol **3f**. The mixture of *trans*/*cis* acetoxycyclohexanols **6**/**7** was obtained by ring opening of epoxide **2** with acetic acid in EtOAc. Through intramolecular dehydration under the Mitsunobu conditions,[15] the (alkyl)aminocyclohexanols **3b** (*N*-*n*-Bu), **3c** (*N*-*n*-Hex) and **3d** (*N*-Bn) provided the aziridines **8b**, **8c** and **8d**, respectively. Treatment of **8b** with acetic acid in EtOAc led to the *cis*/*trans* mixture of acetoxycyclohexylamines **9b**/**10b**. The major reaction product, the *trans* isomer **10b**, was saponified in acid medium to obtain the free aminoalcohol **11b**. Finally, cyclohexanediamines **12bc** and **12dc** were, respectively, obtained by treatment of aziridines **8b** and **8d** with *n*-hexylamine.

All the compounds shown in Scheme 1 and Table 1 were obtained as racemic mixtures. Their structures, including the relative configurations depicted in Scheme 1, as well as their main conformations, were established through the complete analysis of their 1D and 2D NMR, NOE difference, ROESY, MS and IR spectra and conformational calculations. In representative cases the unambiguous assignment of all significant ^1H and ^{13}C NMR signals was performed. The complete descrip-

Table 1. Antiparasitic activities for some selected compounds of type **3**–**6** and **8**–**12**

Compound	R^1	R^2	*Leishmania* spp.			Chagas *T. cruzi*
			L. amazonensis	*L. braziliensis*	*L. donovani*	
3*	H	*n*-Hex	1.2	1.2	1.2	**0.15**
3c	H	*n*-Hex	1.0	1.0	1.0	**0.27**
3e	H	$-(CH_2)_2NH_2$	1.1	1.1	1.1	**0.14**
3f	Et	Et	3.0	2.7	2.5	7.4
4a	H	H	**0.26**	**0.29**	**0.29**	**0.19**
4c	H	*n*-Hex	**0.5**	1.5	1.5	**0.12**
5f	Et	Et	*157*	*148*	*157*	*106*
6	—	—	11.6	13.3	13.3	8.8
8c	*n*-Hex	—	2.4	2.4	1.8	51
8d	Bn	—	16.2	16.2	19.9	31
9b	*n*-Bu	—	9.1	8.8	8.6	8.6
10b	*n*-Bu	—	7.3	7.3	9.6	1.2
11b	*n*-Bu	—	**0.11**	**0.11**	**0.11**	**0.24**
12bc	*n*-Bu	*n*-Hex	14.0	14.0	17.4	116
12dc	Bn	*n*-Hex	15.5	10.8	13.0	98
	Pentamidine		29.4	29.4	29.4	—
	Amphotericin B		0.2	0.2	0.2	—
	Benznidazol		—	—	—	7.4

IC_{50} values (μM); $n = 12$; *($n = 8$); *L. amazonensis* (pH 8), *L. braziliensis* (M2903), *L. donovani* (PP75), *T. cruzi* (Tulahuen strain).

tion of the chemical procedures summarized in Scheme 1 and the characterisation of the compounds described here will be reported soon. As representative examples, spectral data for several of those most potent antiparasitic compounds found in this research (**3e**, **4a**, **4c** and **11b**) are included in the references and notes section.[16]

The parasiticidal effects of compounds included in Table 1 were evaluated, in vitro, against cultured promastigotes of *L. amazonensis* (IFLA/BR/67/pH8), *L. braziliensis* (MHOM/BR/75/M2903) and *L. donovani* (MHOM/BR/74/pp75), according to a previously reported procedure.[17] Pentamidine and amphotericin B were used as reference drugs.[18] Similarly, compounds in Table 1 were tested in vitro against cultured epimastigotes of *T. cruzi* (Tulahuen).[19] Benznidazol was used as the reference drug in this assay.[20]

It can be observed that, in general, all types of compounds assayed resulted highly potent against both parasite types. The only exception is represented by the benzyl ether **5f**, whose main structural difference relates to the alkylation degree of the amino and hydroxyl groups, which result unable to form hydrogen bonds, in this case. Most compounds displayed IC_{50} values in the μM range against *Leishmania*; several of them, **11b**, **4a** and **4c**, resulted fairly more potent than the most commonly used clinical agent pentamidine. Very interestingly, compound **11b** resulted some 250 times more potent than pentamidine and even slightly more potent than amphotericin B.

Due to the small number of compounds evaluated and the diversity of types involved, it seems not reasonable the attempt of analysing SAR aspects, but several observations and comparisons on the results can be made. Globally considered, the antileishmanial potency of the different structural types can be ordered as follows: **11** ∼ **4** > **3** > **8** ∼ **9/10** ∼ **6** ∼ **12** ≫ **5**, and it can be stated that aminocyclohexanol derivatives are, in general, more potent than diamine, aziridine and diol derivatives. From another point of view, it can be deduced that compounds having a greater number of labile H atoms (NH, OH), and simultaneously the lower lipophilicity (log *P*), result to be the most potent leishmanicides. The presence (**3** and **11**) or absence (**4**) of the exocyclic olefin seems to be not too relevant for the activity. Additionally, it should be noted that aminoalcohol **11b** is one order of magnitude more potent than type **3**, which have exchanged positions of functions and side-chain on the ring. These facts denote that the molecular regiochemistry could influence the activity significantly. On the other hand, it should be noted that the most typically bioactive (reactive) aziridine derivatives (**8**) result less potent than aminoalcohols and similar as bis-monosubstituted diamines. Nevertheless, it could be considered that aziridines **8c** and **8d** do not contain any labile (NH or OH) hydrogen atom, as it is the case of the almost inactive benzyl ether **5f**.

Relating to the activities against *T. cruzi*, the assayed compounds behaved similarly as against *Leishmania*. Six compounds displayed IC_{50} values below the μM level, corresponding to a trypanocidal potency 60 times higher than that of benznidazol, in the case of compound **4c**.

Eight compounds included in Table 1 had been previously submitted to cytotoxicity assays on neoplastic cells with not enough interesting results. The LC_{50} values found for compounds **3***, **3c**, **3f**, **4c** and **11b**, assayed on A-549 lung carcinoma cells, were 14.2, 23.4, >25.7, 10.9 and 25.2 μM, respectively; thus revealing a certain degree of selectivity for parasites in comparison with human neoplastic cells.

In summary, in spite of the fact that only a few types and a small number of cyclic analogues of sphingosine have

been evaluated, we can conclude that these compounds constitute a new family of highly potent antiparasitics, that must be explored further for optimising structure, performing in vivo assays and establishing of their mechanism of action. Indeed, assays against other parasitic and microbial pathogens are currently in course, while chemical structure optimisation, dealing with ring and side-chain sizes, regiochemical and stereochemical aspects, as well as the synthetic work, are also in progress.

Acknowledgments

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16. (a) Compound **3e** [(*Z*)-*trans*-2-(2-aminoethylamino)-3-tetradecylidenecyclohexanol]: Mp: 104–105 °C. IR (KBr, ν_{max}): 3381, 2919, 2850, 1578, 1470, 1090, 992 cm^{-1}. HRMS: 465.4791 $[M+H]^+$; calcd 465.4778. ^1H NMR (δ ppm, $CDCl_3$): 3.93 (m, H-1); 3.59 (br s, H-2); 1.80 (m, H-4_{ax}); 2.20 (m, H-4_{eq}); 2.12 (m, H-5_{ax}); 2.55 (m, H-5_{eq}); 1.65 (m, H-6_{ax}); 1.95 (m, H-6_{eq}); 5.51 (t, J = 7.0 Hz, H-1′); 2.05 (m, H_2-2′); 2.98 (m, H_2-1″); 2.81 (m, H_2-2″); 4.50 (br s, NH+OH); 1.25 [br s, –$(CH_2)_9$–]; 0.86 (t, J = 7.0 Hz, –CH_3). ^{13}C NMR (δ ppm, $CDCl_3$): 70.7 (C-1); 59.2 (C-2); 135.4 (C-3); 31.9 (C-4); 22.1 (C-5); 27.2 (C-6); 130.1 (C-1′); 28.2 (C-2′); 48.5 (C-1″); 41.2 (C-2″); 30.4 (CH_2); 29.7 $(CH_2)_n$; 29.3 (CH_2); 22.6 (CH_2); 14.2 (CH_3); (b) Compound **4a** [(1,2-*trans*-1,3-*cis*)-2-amino-3-tetra-decylcyclohexanol]: Oil. IR (KBr, ν_{max}): 3331, 3279, 2930, 2852, 1596, 1460, 1056, 843, 719 cm^{-1}. ^1H NMR (δ ppm, $CDCl_3$): 3.44 (m, H-1); 2.48 (t, J = 8.0 Hz, H-2); 1.47 (m, H-3); 0.95 (m, H-4_{ax}); 1.83 (br s, H-4_{eq}); 1.50 (m, H-5); 1.00 (m, H-6_{ax}); 2.05 (m, H-6_{eq}); 1.27 [br s, (–CH_2–)$_9$]; 0.79 (t, J = 6.6 Hz, –CH_3); 3.90 (br s, NH+OH). ^{13}C NMR (δ ppm, $CDCl_3$): 79.6 (C-1); 61.5 (C-2); 43.2 (C-3); 30.7 (C-4); 23.4 (C-5); 33.4 (C-6); 31.8 (CH_2); 30.0 (CH_2); 29.9 (CH_2); 29.6 $(CH_2)_n$; 22.4 (CH_2); 14.0 (CH_3); (c) Compound **4c** [*trans*-2-hexylamino-3-tetradecyl-cyclohexanol]: Oil. *IR* (KBr, ν_{max}): 3300, 2920, 2853, 1460 cm^{-1}. ^1H NMR (δ ppm, $CDCl_3$): 3.40 (m, H-1); 2.10 (t, J = 10.5 Hz, H-2); 1.48 (m, H-3); 0.91 (m, H-4_{ax}); 1.83 (m, H-4_{eq}); 1.71 (m, H_2-5); 1.25 (m, H-6_{ax}); 2.05 (m, H-6_{eq}); 2.70 (m, H_2-1″); 1.54 (m, H_2-2″+H_2-3″); 1.25 [br s, –$(CH_2)_{11}$–]; 0.87 (t, J = 6.8 Hz, 2× CH_3). ^{13}C NMR (δ ppm, $CDCl_3$): 70.7 (C-1); 68.2 (C-2); 39.3 (C-3); 29.9 (C-4); 23.2 (C-5); 32.2 (C-6); 45.9 (C-1″); 33.1 (C-2″); 31.8 (CH_2); 31.6 (CH_2); 30.7 (CH_2); 29.3 (CH_2); 26.8 (CH_2); 26.4 $(CH_2)_n$; 22.5 (CH_2); 14.1 $(CH_3)_2$; (d) Compound **11b** [(*Z*)-*trans*-2-butylamino-6-tetradecylidenecyclohexanol]: Oil. IR (KBr, ν_{max}): 3377, 2924, 2854, 1460, 1120, 1022 cm^{-1}. HRMS: 366.3731 $[M+H]^+$; calcd 366.3730. ^1H NMR (δ ppm, $CDCl_3$): 4.33 (d, J = 5.5 Hz, H-1); 2.71 (m, H-2); 1.80 (m, H-3_{ax}); 2.05 (m, H-3_{eq}); 1.50 (m, H_2-4); 1.95 (m, H-5_{ax}); 2.31 (m, H-5_{eq}); 5.33 (t, J = 7,3 Hz, H-1′); 2.16 (m, H_2-2′); 2.60 (m, H_2-1″); 1.40 (m, H_2-2″); 1.25 (br s, –$(CH_2)_{10}$–); 0.90 (t, J = 6.9 Hz, CH_3″); 0.87 (t, J = 6,6 Hz, CH_3′). ^{13}C NMR (δ ppm, $CDCl_3$): 71.0 (C-1); 60.4 (C-2); 26.7 (C-3); 23.4 (C-4); 32.3 (C-5); 136.4 (C-6); 128.2 (C-1′); 27.2 (C-2′); 46.8 (C-1″); 33.3 (C-2″); 31.9 (CH_2); 30.6 (CH_2); 29.7 (CH_2); 29.4 (CH_2); 22.7 (CH_2); 20.4 (CH_2); 14.1 (CH_3′); 14.0 (CH_3″).
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18. *Leishmania* spp. promastigotes were grown at 27 °C in Schneider's drosophila medium containing 10% of foetal bovine serum. Compounds were dissolved in DMSO (final concentration of solvent <0.1%) and liquid medium and placed in microcells. Promastigote cultures in the logarithmic phase were transferred at the level of 10^5 parasites per cell. The activity of the compounds was evaluated after incubation during 72 h and compared with either control and treated cultures. Assays were done in triplicate.
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