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Diamine and aminoalcohol derivatives active against *Trypanosoma brucei*

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ABSTRACT

Twenty compounds selected as representative members of three series of long-chain 1,2-diamines, 2-amino-1-alkanols and 1-amino-2-alkanols structurally related to dihydrosphingosin, were synthesized and tested in vitro for their ability to inhibit the sleeping sickness parasites *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Eight compounds showed EC_{50} values in the submicromolar range, with selectivity indexes up to 39 related to the respective cytotoxicity values for Vero cells. The parasite phenotype detected after treatment with the most potent compounds showed irreversible cell morphology alterations of the flagellar pocket that lead to inhibition of cell growth and parasite death.

Trypanosoma brucei is the causal agent of Human African Trypanosomiasis (HAT), a vector-borne neglected disease transmitted by insects of *Glossina* genus, the tsetse fly. Also known as sleeping sickness, the disease is distributed among 36 sub-Saharan countries, with more than 7000 new cases reported in 2009 and more than 60 million people at risk.[1] The clinical manifestation of HAT depends on the sub-species involved. *T. b. rhodesiense* is responsible of the acute form of the disease, mainly distributed in Eastern and Southern Africa, whereas in West and Central Africa, *T. b. gambiense*, is responsible of chromic infections that may develop symptoms years later. Wild animals, as well as livestock, are reservoirs of the HAT for both species, being an obstacle to eradicate this disease. Evolution of infection begin with an early stage or haemolymphatic phase, where the parasite replicates in blood and body fluids, provoking lymphadenophathy and fever, followed by a second stage where the infectious cells cross the blood–brain barrier causing neurologic symptoms and if untreated, patients would fall into coma and die.[2] Drugs used in the present time exhibit shortage in healing and potent toxicity.[3] Vaccination is hampered by antigenic variation, a process developed by the parasite that changes the glycoprotein surface eluding the host immune response. Thus, it is evident the need of developing new drugs and therapeutic strategies to improve efficacy and reduce toxicity in treatments.

Success in the treatment of HAT is conditioned to an early diagnosis, since current pharmacological formulations that get through the blood–brain barrier have very high toxic side effects and complicated administration. The therapeutic arsenal to treat HAT is scarce, mainly based on a few old drugs, which were donated to the WHO by the manufacturers Sanofi-Aventis and Bayer. Pentamidine isethionate and suramin are used, respectively, against the first stage of *T. b. gambiense* and *T. b. rhodesiense* disease. The very toxic melarsoprol is used against the second stage HAT caused by *T. b. gambiense*. Eflornithine is safer than melarsoprol and was recommended as first line treatment for second stage HAT caused by *T. b. gambiense*, but recently clinical trials showed that a nifurtimox–eflornithine combination therapy presents advantages over the eflornithine monotherapy. Unfortunately, *T. b. rhodesiense* is resistant to eflornithine so actually the only choice against the second stage is melarsoprol. Due to the lack of really safe and efficacious drugs, the discovery and development of better and cheaper new compounds against HAT is needed.[2,4]

In an attempt to discover new types of therapeutic agents against *T. brucei*, along with several series of fused heterocyclic compounds, we prospectively evaluated a number of aliphatic 1,2-diamine, 1-amino-2-alkanol and 2-amino-1-alkanol derivatives (Fig. 1), structurally considered as simplified sphingosin pseudo-analogues. Preliminarily, a small library of 20 compounds representative of the three series were selected for evaluation on the basis of previous bioactivity results found for this type of compounds against *Mycobacterium tuberculosis*[5] and *Leishmania* spp. parasites[6] as well as, those found for a collection of cyclic analogues tested against *Leishmania* spp. and *Trypanosoma cruzi*.[7]

The preparation of the 1,2-diamine and 2-aminoalkan-1-ol derivatives being assayed was previously reported by us.[5,6,8,9]

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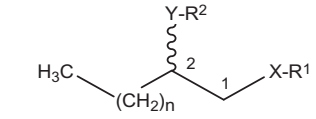
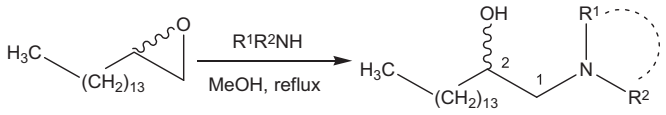


X, Y = O, NH, N, n = 9, 13, 15.
R^1,R^2 = H, Et, *n*-Bu, *n*-Pent, *n*-Hex, cyclohex, Bn, 4-Me-piperidin-1-yl, 4-Bu-piperidin-1-yl, morpholin-1-yl, Boc, HOOC-$(CH_2)_{2(3)}$CO-.

Figure 1. Global structure and variants of the evaluated compounds.



Scheme 1. The synthesis of 1-amino-2-alkanol derivatives.

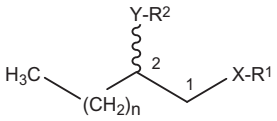
Compounds of the new series, with inverted positions for the amino and the hydroxyl functions to mimic positions 2 and 3 of dihydrosphingosin, were easily prepared by nucleophilic attack of 1,2-epoxyhexadecane with the appropriate alkyl-, dialkyl- or heterocyclic amine derivative (Scheme 1). Examples of preparation and characterisation by physical and spectral data for compounds of the new series are given in the references and notes section.[10,11]

After a prospective preliminary assay of parasite growth inhibition, at 1 and 100 μM concentrations of the compounds, that served to reveal the anti-trypanosomal potential of the three series, all the compounds were assayed in vitro for 72 h against the human-infective strain *T. b. rhodesiense* EATRO3, grown and tested as bloodstream form,[12] with various concentrations of the different test compounds, according to a reported procedure based on the Alamar Blue assay[13] and covering a range from 0.05 to 2.5 μM.[14] The 50% effective concentration (EC_{50}) value of each compound was determined and the results are shown in Table 1. Cells cultured without any compound, incubated with the calculated proportion of DMSO used as solvent of the samples served as control and pentamidine was used as reference drug showing an EC_{50} value of 3.7 nM against *T. b. rhodesiense*, a value significantly lower than that previously reported by Bodley and Shapiro.[15]

As it can be seen in Table 1 the three series of compounds tested contain representative elements displaying anti-trypanosomal effects with submicromolar EC_{50} values, though no one attained the potency of the reference drug. In the case of those 1,2-alkanediamine derivatives **1** to **5**, two compounds attained the submicromolar level for their EC_{50} values, the ethylamino/Boc (*t*-butoxycarbonyl) derivative **2** resulting as the most potent trypanocide within those compounds evaluated of this series. It can be observed, though being not enough sure for this short group, that the presence of the Boc substituent on N^2 (compounds **1** and **2**) seems comparatively useful for enhancing the activity, while the increase of size or polarity of the substituent on N^1 (compounds **3**, **4** and **5**) fairly lead to lowering the antiparasitic potency.

Five members of the series of 2-amino-1-alkanol derivatives (compounds **6**–**13**), also attained micromolar or submicromolar levels in their respective EC_{50} values. For this series the preferred substituent on N^2 was also the ethyl group and the comparison

Table 1
Activity of long-chain diamine and aminoalcohol derivatives against *Trypanosoma brucei rhodesiense* EATRO3



Compd[a]	X	Y	R^1	R^2	*n*	*T. brucei rhodesiense* EC_{50} (nM) ± SD
1	NH	NH	H	Boc	13	**961 ± 67**
2	NH	NH	Et	Boc	13	**704 ± 55**
3	NH	NH	*n*-Hex	Boc	13	>2500
4	NH	NH	HO_2CCH_2–	H	13	>2500
5	NH	NH	$HO_2C(CH_2)_2CO$–	Boc	13	>2500
6	O	NH	H	H	13	>2500
7	O	NH	H	Et	9	1087 ± 88
8	O	NH	H	Et	13	1139 ± 26
9	O	NH	H	Et	15	**584 ± 14**
10	O	NH	H	*n*-Bu	13	**817 ± 57**
11	O	NH	H	$HO_2C(CH_2)_3CO$–	13	>2500
12	O	N	H	Et_2	13	**722 ± 58**
13	O	NH	Bn	$HO_2C(CH_2)_2CO$–	13	>2500
14	NH	O	*n*-Hex	H	13	>2500
15	NH	O	Cyclohexyl	H	13	**790 ± 36**
16	NH	O	2-Propyl	H	13	**436 ± 4**
17	N	O	Et_2	H	13	2001 ± 95
18	NH	O	4-Me-piperazin-1-yl	H	13	**524 ± 12**
19	NH	O	4-Bu-piperazin-1-yl	H	13	1781 ± 194
20	NH	O	Morpholin-1-yl	H	13	>2500
Pentamidine						3.7 ± 0.4
Suramin						185.3 ± 8.5

EC_{50} values under 1 μM are bolded.
[a] All the compounds were obtained as racemic mixtures and evaluated per triplicate.