Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 659–662

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Leishmanicidal Activity of Some Aliphatic Diamines and Amino-Alcohols

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Received 3 September 2001; revised 12 November 2001; accepted 5 December 2001

Abstract—A number of aliphatic diamines and amino-alcohols and several of their alkyl, acyl and carbamoyl derivatives, have been synthesised and evaluated in vitro on cultures of *Leishmania* spp. In general, diamine derivatives resulted to be more potent than their amino-alcohol or amino-ether analogues. Two diamine derivatives (**8b** and **9d**) and one amino-alcohol (**6a**) showed a fair inhibition of parasite growth, at concentrations below 10 μg/mL, with potencies close to that of the reference drug, amphotericin B. Some SAR considerations have been deduced.

Leishmaniasis, is a parasitic disease endemic to the American, African and Asian tropical countries.[1] It affects some 12 million people around the world and 350 million are at risk of becoming infected, of whom some 1.7 million will be infected each year.[2] The most common varieties of leishmaniasis are the cutaneous and mucocutaneous ones, they are not directly lethal, but provoke multiple granulomatose or diffuse, autoinoculable and even metastatic skin ulcers, resembling leprosy.[3] Drugs currently in use as the Antimony derivative glucantime, the bis-amidines, pentamidine and stilbamidine, and the glycomacrolide amphotericin B, display high liver and heart toxicities, develop clinical resistance after a few weeks of treatment[4] and it has been estimated that they contribute to increase co-infections leishmaniasis—AIDS.[5] For these reasons, it is necessary to discover new agents, either natural or synthetic, more potent and selective, for treating this increasing parasitosis.

A great number of natural and synthetic compounds have been tested in the past few years in anti-leishmanial assays. Their structures are very diverse. They often contain nitrogen heterocycles as: quinolines,[6] acridines,[7] phenothiazines,[8] pyrimidines[9] and purines,[10] but other types of compounds such as aniline derivatives,[11] flavonoids,[12] quinones,[13] certain amino acids, amides and esters,[14] in addition to alkyl-lysophospholipids[15] and Pt (II) and (IV) complexes,[16] have also been reported in the literature. In spite of the great number of compounds synthesised, no better substances than those already known have been introduced into the clinic. In this paper, we describe the synthesis of some lipidic amino-alcohols and diamines and their in vitro evaluation on cutaneous, mucocutaneous and visceral strains of *Leishmania*. These type of compounds, bearing a covalently bonded, non-hydrolysable, fatty chain attached to the ethanolamine or ethylenediamine fragments, were considered able to interact with membrane lipids, also to be transported into the cytoplasm and possibly, to interfere with the lipid or polyamine transport or metabolism of the parasite.[17,18]

Chemistry

The synthesis of the compounds to be tested is summarised in Scheme 1. Details of the reactions, experimental conditions and characterisation of the products, will be reported in a complete publication. The Boc-aminoacid **1**, prepared from diethyl 2-acetamidomalonate as reported by Gibbons et al.,[19] was transformed into the Boc-aminoalcohol **2a**, by treatment with ethyl chloroformate followed by sodium borohydride reduction of the intermediate mixed anhydride. Compound **2a** was benzylated to give the Boc-aminoether

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00837-X

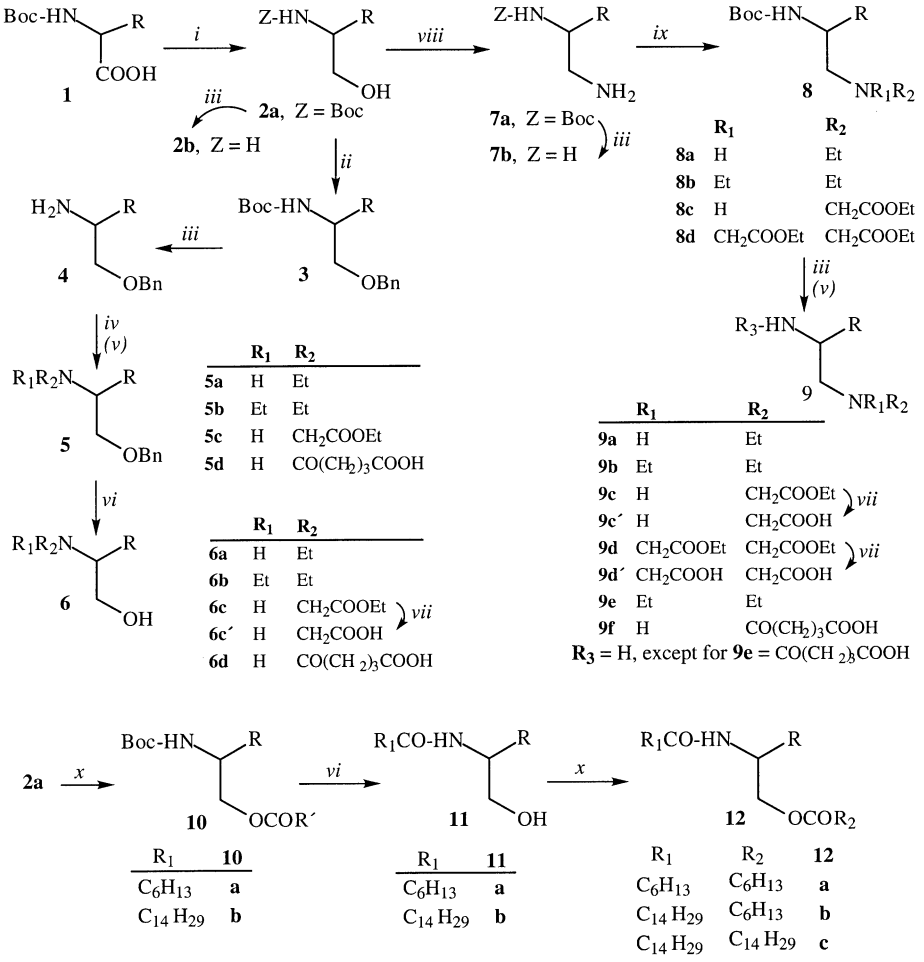
3, which was Boc-deprotected to give the aminoether **4**. The benzylic aminoether **4**, treated with 1.2 or 2.2 equiv of ethyl bromide gave the mono alkylated or dialkylated derivatives **5a** and **5b**, respectively, while treatment with ethyl bromoacetate led to the glycine ester **5c**. The acylation of **4** with glutaric anhydride provided the amidoacid **5d**. The hydrogenolytic debenzylation of compounds **5a–d** gave the corresponding free alcohol derivatives **6a–d**, and the hydrolysis of **5c** with 10% KOH/MeOH, yielded the glycine derivative **6c′**.

Compound **2a,** through mesylation, followed by substitution with sodium azide and reduction with $NaBH_4$/MeOH, in the presence of Pd–C,[20] was converted into the Boc-diamine **8**, which on alkylation/acylation with the above mentioned reagents, followed (or not) by Boc-deprotection, yielded the diamines and diamine derivatives **8a–d** and **9a–f**, respectively. Compounds **9c′** and **9d′** were obtained from **9c** and **9d** respectively, after treatment with 10% KOH/MeOH. Reaction of **9b** with glutaric anhydride gave **9e**.

The Boc-aminoalcohol **2a** was treated with heptanoyl chloride or palmitoyl chloride to give the esters **10a** and **10b**, respectively. The attempted Boc-deprotection of these compounds with TMSCl/PhOH in $HCCl_3$,[21] followed by chromatographic purification on silica gel, yielded the intramolecularly transamidated compounds **11a** and **11b**, which were further treated with the appropriated acyl chloride to obtain the amidoesters **12a–c**.

Biological Assays

The isolation, cultivation and maintenance of *Leishmania* promastigotes and the technique used have been previously described.[22] Briefly, promastigote inhibition studies were performed on *L. amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903) and *L. donovani* (MHOM/BR/74/PP75) grown at 25 °C in Schneider's drosophila medium containing 20% foetal bovine serum. Compounds were dissolved in 40 μL of dimethyl sulphoxide (DMSO, final concentration of this solvent 0.4%), then diluted in the medium and placed in microtitre plates in triplicate, with a final compound concentration of 100 μg/mL. The activity of compounds was evaluated after 48 h by optical observation of a drop of each culture using a microscope and comparison with control cell cultures and with those treated with the reference drug amphotericin B. Other con-



Scheme 1. The synthesis of lipidic diamines, amino-alcohols and their derivatives. $R = (CH_2)_{13}CH_3$; (i) (a) EtOOCCl/*N*-methylmorpholine/THF; (b) $NaBH_4$/MeOH; (ii) BnCl/NaH/DMF; (iii) HCl/THF/argon; (iv) EtBr (1.1 or 2.2 equiv) or $EtOOCCH_2Br$ (1.1 equiv)/Et_3N/DMF; (v) glutaric anhydride/EtOAc; (vi) H_2/Pd–C/AcOH; (vii) 10% KOH/MeOH; (viii) (a) $MsCl/Et_3N/CH_2Cl_2$; (b) NaN_3/DMF; (c) Pd-C/$CHCl_3$, $NaBH_4$/MeOH; (ix) EtBr (1.1 or 2.2 equiv) or $EtOOCCH_2Br$ (1.1 or 2.2 equiv)/Et_3N/DMF; (x) *n*-$C_6H_{13}COCl$ or *n*-$C_{15}H_{31}COCl/Et_2O$.

Table 1. In vitro activity of lipidic aminoalcohol derivatives on the growth of *Leishmania* promastigotes

Compd	*L. amazonensis* (PH8) (μg/mL)				*L. brasiliensis* (2903) (μg/mL)				*L. donovani* (PP75) (μg/mL)			
	100	50	25	10	100	50	25	10	100	50	25	10
1	+ +				+ +				+ +			
2a	+ +				+ +				+ +			
2b	+ +				+ +				+ +			
3	+				+				+			
4	+				+				+			
5a	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+
5b	+ +				+ +				+ +			
5c	+ +				+ +				+ +			
5d	+ +				+ +				+ +			
6a	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +
6b	+ + +	+ + +	+	+	+ + +	+ + +	+ +	+ +	+ + +	+ + +	+	+
6c	+ + +	+ +	+	+	+ + +	+ +	+	+	+ + +	+ +	+	+
6c′	+ + +	+	+		+ + +	+	+		+ + +	+	+	
6d	+ + +	+ +	+	+	+ + +	+ +	+	+	+ + +	+ +	+	+
11a	+ +				+				+ +			
11b	+				+				+			
12a	+ +				+ +				+ +			
12b	+				+				+			
12c	+				+				+			
Amphotericin B	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

+ + +: Total lysis of parasites; + +: 80–90% lysis; +: <70%; blank: no lysis.

centrations (50, 25 and 10 μg/mL) were tested after the total lysis of promastigotes was confirmed at 100 μg/mL. At least two independent experiments performed in triplicate with different stock solutions of samples were carried out.

Results and Discussion

The results of the evaluation of amino-alcohols and their derivatives are presented in Table 1 and those corresponding to the diamines and their derivatives are included in Table 2.

As can be seen, the amino-alcohol **6a** and the diamino derivatives **8b**, **9b** and **9d** provoked the total lysis of promastigotes of the three mentioned strains of parasites, at concentrations under 25 μg/mL and more than the 80% of lysis at 10 μg/mL for all of them, except for **9b** with value under 70%, after a relatively short period (48 h) of incubation. Compounds of group **6** were more potent than their corresponding benzylated analogues (**5**), revealing the convenience of the presence of a free hydroxyl group for the activity. Small size alkyl substitution seems better than medium size (**6a**/**6c**) or longer substitutions (**11a**/**11b**, **12a**/**12b** and **12c**). Finally, a free carboxylic acid at the end of the chain was less active than an ester function (**6c′**/**6c**).

With respect to the diamines, most of them produced total lysis of parasites at 50 μg/mL. In general Boc protected derivatives were more potent than those unprotected (**8a**–**8c**/**9a**–**9c**), except for the diester derivative **9d**. Certain selectivity of compound **8a** respecting *L.*

Table 2. In vitro activity of lipidic diamines and derivatives on *Leishmania* promastigotes

Compd	*L. amazonensis* (PH8) (μg/mL)				*L. brasiliensis* (2903) (μg/mL)				*L. donovani* (PP75) (μg/mL)			
	100	50	25	10	100	50	25	10	100	50	25	10
7a	+ +				+ +				+ +			
7b	+ +				+ +				+ +			
8a	+ + +	+ + +	+	+	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+	+
8b	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +
8c	+ + +	+ + +	+	+	+ + +	+ +	+	+	+ + +	+ +	+	+
8d	+ +				+ +				+ +			
9a	+ + +	+ +	+	+	+ + +	+ +	+	+	+ + +	+ +	+	+
9b	+ + +	+ + +	+ + +	+	+ + +	+ + +	+ + +	+	+ + +	+ + +	+ + +	+
9c	+ + +	+	+	+	+ + +	+	+	+	+ + +	+	+	+
9c′	+ +	+			+ +	+			+ + +	+		
9d	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +
9d′	+ + +	+ +	+		+ + +	+ +	+		+ + +	+ +	+	
9e	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+
9f	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+	+ + +	+ + +	+ +	+
Amphotericin B	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

+ + +: Total lysis of parasites; + +: 80–90% lysis; +: <70%; blank: no lysis.

brasiliensis (2903) strain could be noticed: >80% lysis versus <70% lysis for the other two strains, at 10 μg/mL. As it was appreciated for amino-alcohols and diesters, large groups at both amine ends are not recommended for the activity (**9b**/**9e**) and similarly the free acids are less potent than their corresponding esters (**9c′**/**9c** and **9d′**/**9d**). However the most potent monosubstituted compound **9f** is nearly as potent as the disubstituted **9d**, perhaps due to the presence of the amide group or to the longer separation between both funtional groups. Disubstitued amines are more potent than those monosubstituted (**8b**/**8a**, **9b**/**9a**, **9d**/**9c**), excepting the Boc diester **8d**.

We have found, therefore, a new leishmanicidal molecular prototype, less toxic (data not shown) than the reference drug Amphotericin B and displaying as the main structural features a fatty 1,2-diamine with the 1-NH_2 group disubstituted by small alkyl or alkoxycarbonylmethyl groups and with the NH_2 group at position 2 preferably free or protected.

Acknowledgements

This work was supported by JCyL (Grant No SA25/00B). This collaborative work was performed under the auspices of the CYTED Program (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo), SubProgram X.

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