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Synthesis and antikinetoplastid activity of a series of N,N′-substituted diamines

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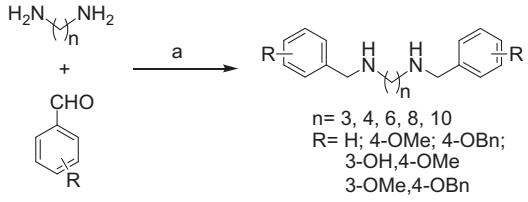
ABSTRACT

A series of 25 N,N′-substituted diamines were prepared by controlled reductive amination of free aliphatic diamines with different substituted benzaldehydes. The library was screened in vitro for antiparasitic activity on the causative agents of human African trypanosomiasis, Chagas' disease and visceral leishmaniasis. The most potent compounds were derived from a subset of diamines that contained a 4-OBn substitution, having a 50% parasite growth inhibition in the submicromolar (against *Trypanosoma cruzi*) or nanomolar (against *Trypanosoma brucei* and *Leishmania donovani*) range. We conclude that members of this series of N,N′-substituted diamines provide new lead structures that have potential to treat trypanosomal and leishmanial infections.

Neglected diseases are prevalent throughout the tropics and subtropics, representing a major public health problem in regions of the world least able to deal with the associated economic burden. In some cases, cost effective therapies are available, for example lymphatic filariasis can be treated at a cost as low a $2 per patient per episode,[1] but for most infections this is not the case. In these situations the current regimes are failing being beset with a series of problems ranging from toxicity and lack of efficacy through to drug resistance and cost.[2] This is exemplified by kinetoplastid infections such as Chagas disease, leishmaniasis and human African trypanosomiasis (HAT)[3] where front-line treatments are based on heavy metal-based compounds or on agents that are expensive. Against this backdrop there is urgent requirement for new orally administered agents targeting such infections that are cheap and present limited/no side effects.

Polyamine derivatives have been extensively studied as anticancer agents during the 90s,[4] with the use of such scaffolds being evaluated to address whether polyamine metabolism was a potential target for antiparasitic drugs.[5] Despite the considerable number of reports where different sets of substituted polyamine analogs have been prepared,[6] few systematic studies involving the synthesis of substituted diamines have been performed. In a limited number of cases the antiparasitic activity of such derivatives has been explored, like the pioneering work of Bitonti et al. where the bis(benzyl)polyamine analog, MDL 27,695 was reported.[7] More recently, libraries of N,N-substituted diamines based on putrescine and 1,3-propanediamine prepared by solid phase parallel synthesis[8] and a series of long chains N-monoalkylated diamines ($n = 2–6$).[9,10] The former agents are reported to show submicromolar activities against *P. falciparum* and *L. donovani*[8] while the latter exhibit leishmanicidal[8] and trypanocidal[10] activities. The examples mentioned lack the adequate target validation, but based on their structure it has been suggested that they interfere with polyamine metabolism.[11] Therefore, some of precedents described before have validated diamines derivatives as an attractive scaffold for the development of new antiparasitic drugs, and were chosen as our starting template to prepare new sets of analogs.

The backbone of the substituted *N,N′*-diamine derivatives generated here is shown in Scheme 1. We prepared a series of *N,N′*-diamine derivatives that contained (1) different length carbon chains between the two amino groups and/or (2) substitutions on the amine group; the type of side chain incorporated onto the amine was guided by



Scheme 1. Synthesis of N,N'-derivatives. Reagents: (a) (1) DCM/MeOH (3:1) reflux; (2) $NaBH_4$ DCM/MeOH (1:1).

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previous reports where the most of the biologically activity analogs contained, but not exclusively,[12] aromatic rings.[13] Those rings were introduced in the form of benzyl groups for synthetic reasons and also because they will give the compounds more flexibility compared to aniline analogs and will preserve the basicity of the amine, which is necessary for imparting specific physiological function to polyamines.

The logical approach to synthesize the analogs requires a reductive amination between a diamine and an aldehyde. Initially, to prepare the compounds the well established methodology using sodium triacetoxyborohydride was employed.[14] That methodology has been successfully used to produce reductive amination of aldehydes in situ, without the intermediary step of imine formation. Putrescine (1,4-diaminobutane) was used to test the reaction using a 1:2.2 ratio between diamine and benzaldehyde. Under those conditions a mixture of products was obtained which was composed by *N*-Bn, *N*,*N*′-dibenzyl and the *N*,*N*,*N*′-tribenzyl 1,4-diaminobutane. It was clear that after the *N*,*N*′- product was formed, a third molecule of aldehyde reacted with the product instead of reacting with the remaining free amine. Based on those results, we had to move to the stepwise procedure that allows for a more controllable diimine formation step. Using a procedure reported by Phanstiel and co-workers[15] putrescine was allowed to react with benzaldehyde in DCM/MeOH (3:1) overnight, with an aliquot evaporated and analyzed by NMR revealing a complete diimine formation. The solvent was evaporated and the intermediate redissolved in DCM/MeOH (1:1) and reduced with sodium borohydride overnight. The reaction provided exclusively the expected *N*,*N*′-dibenzyl-1,4-butilendiamine in 73% yield. The reaction conditions did not require further optimization based on the similar selectivity and yield obtained when using other diamines and benzaldehydes. Having found the adequate conditions,[16] a collection of 25 products was prepared. The library has two sources of diversity; the carbon chain length and the substituent on the aromatic ring. We selected five linear diamines covering different chain lengths (Scheme 1), with '*n*' varying from *n* = 3 to *n* = 10 and five benzaldehydes with different degrees of substitution; being R = H, R = 4-OMe, R = 4-OBn, R = 3-OH,4-OMe and R = 3-OMe,4-OBn. Isovanillin (R = 3-OH,4-OMe) was chosen over vanillin (R = 3-OMe,4-OH) because different reports[17] have shown vanillin to be less reactive on reductive aminations and both share a similar arrangement of substituents, being, in that sense, equivalent.

The synthesis of 25 compounds through this synthetic approach provided the expected products in good yields (Table 1). The reactivity of some aldehydes resulted in the reduction of the carbonyl to the corresponding benzyl alcohol, resulting in difficulty in purification of these analogs.

The average yield of purified compounds with different carbon chain lengths, and ordered by the substituent on the benzyl ring, were: 76% for R = H, 62% for R = 4-MeO, 78% for R = 4-BnO, 82% for R = 3-OH,4-MeO and 80% for R = 3-OMe,4-BnO; giving a global average yield of 76% (Table 1).

It is well established that kinetoplastid parasites share many biochemical and several life cycle features. In terms of their polyamine biosynthetic pathways, these pathogens show both differences and similarities.[11] The variations make it extremely difficult to find compounds that have potential to target all parasite species while the similarities are sufficient, with some qualitative and quantitative variations,[11] to indicate that compounds designed to block specific components could produce an inhibitory effect against the different kinetoplastid parasites. Based on this assumption, we decided to assay all compounds against the etiological agents of those parasitic diseases. Using well characterized growth inhibition assays,[18] all diamine compounds were tested for antiparasitic activity against *Trypanosma cruzi* epimastigotes (Chagas' disease), *Trypanosoma brucei* bloodstream form (HAT) and *Leishmania donovani* promastigotes (visceral leishmaniasis) and for mammalian cytotoxicity using

Table 1
Structure and yields of N,N′-substituted diamines prepared

Compound	*N*	R	Yield[a] (%)
1	3	H	79
2	4	H	73
3	6	H	46
4	8	H	94
5	10	H	89
6	3	4-OMe	78
7	4	4-OMe	77
8	6	4-OMe	30
9	8	4-OMe	59
10	10	4-OMe	66
11	3	4-OBn	67
12	4	4-OBn	77
13	6	4-OBn	86
14	8	4-OBn	99
15	10	4-OBn	60
16	3	3-OH, 4-OMe	96
17	4	3-OH, 4-OMe	80
18	6	3-OH, 4-OMe	82
19	8	3-OH, 4-OMe	78
20	10	3-OH, 4-OMe	72
21	3	3-OMe, 4-OBn	50
22	4	3-OMe, 4-OBn	84
23	6	3-OMe, 4-OBn	81
24	8	3-OMe, 4-OBn	88
25	10	3-OMe, 4-OBn	89

[a] After purification.

African green monkey kidney epithelial (Vero) cells. For each agent, dose response curves were generated from which the concentration of drug that inhibited cell growth by 50% (IC_{50}) determined (Table 2).

Based on this data it is clear that some scaffolds represent new potent antiparasitic structures, showing activity across all three species tested with few showing signs of cytotoxicity toward the mammalian line. In general, each of the five substituted diamines sub-sets displayed a lower trypanocidal activity against *T. cruzi* than towards the other parasites. For *T. cruzi*, 11 compounds exhibited growth inhibitory properties below 10 μM with 8 having an effect at sub-micromolar levels. In contrast, most scaffolds affected *T. brucei* (21 out of 25) or *L. donovani* (17 out of 25) growth at concentrations below the 10 μM cutoff with several having an effect at the nanomolar range. This difference in the compound susceptibilities may reflect differences in the parasites polyamine biosynthetic systems: *T. cruzi* lacks a functional ornithine decarboxylase, therefore is auxotrophic for putrescine, while *T. brucei* and *L. donovani* have capacity for de novo polyamine biosynthesis.[5] For one diamine sub-set (**16–20**) addition of a hydroxyl group at the 3-position on the benzyl side chain generally resulted in several derivatives exhibiting no activity against any of the parasites screened. Additionally those scaffolds that did have an affect generally showed lower growth inhibitory properties than the equivalent compounds in other sub-sets: compare the IC_{50} value of **19** against *T. brucei* and *L. donovani* with the IC_{50} values obtained with **4**, **9**, **14**, and **24**.

This anomaly may be explained when considering that the phenol group increases the compound's polarity therefore altering that scaffolds ability to cross biological membranes. Incorporation of a 4-benzyloxy group onto the benzyl group results in a noticeable enhancement of the growth inhibitory activity. In fact, compounds **11–15** with only a 4-benzyloxy substituent on the phenyl ring show an average IC_{50} of around 1 μM toward *T. cruzi* and *L. donovani* and below 0.40 μM toward *T. brucei*. For this group of compounds, the antiparasitic activity is generally dependent on the carbon chain length with longer chains resulting in lower trypanocidal/