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Diamine derivatives with antiparasitic activities

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Abstract—There are a lack of effective chemotherapies for many parasitic diseases. Polyamine pathways have been reported as potential targets for the development of new chemotherapies against parasitic diseases. In the present study, different libraries of substituted diamines totalling 78 compounds have been synthesized on solid support and their activities against malaria and leishmania parasites have been determined. Most active compounds demonstrated submicromolar activities against both organisms and their structure–activity relationships are discussed.

Parasitic diseases are a worldwide problem that have a deep impact on public health in developing countries. Malaria, leishmaniasis and trypanosomiasis are caused by protozoan parasites that have been targeted for control and eradication by the World Health Organisation's Global Defense Against the Infectious Disease Threat.[1] In spite of the progress made during the last fifty years in the understanding of the biochemistry and the development of new agents against these diseases, the availability of adequate chemotherapies remains unsolved. The increasing resistance of malaria[2] and the lack of adequate chemotherapies against trypanosomiasis and leishmaniasis[3] promotes an ongoing drug discovery effort to identify mechanistically novel, nontoxic, cost-effective chemotherapies to treat these worldwide public health problems.

Polyamines are essential for cell proliferation and differentiation. It has been shown that interfering with their function or biosynthesis can block cellular growth.[4] Mechanisms of parasitic acquisition of polyamines are different from those of mammalian cells, and in addition, interference with this key pathway should have more severe consequences for the parasite than the host.[5] Blockade of parasitic biosynthesis of polyamines has been reported to be a valid chemotherapeutic approach and there are, consequently, an interesting number of known molecular targets that are under study.[6] Various polyamines derivatives with antiparasitic activities have been prepared or isolated from natural sources. For example, 1-lipidoyl substituted ethylenediamines have been prepared showing micromolar activities against *Leishmania* promastigotes.[7] 1,3,5-triazine-substituted polyamines[8] and lipophilic mono- and di-quaternary ammonium salts[9] have shown potent antimalarial activities. Polyamines derivatives have been synthesized or isolated from natural sources as specific inhibitors of trypanothione reductase, a molecular target for chemotherapies against leishmaniasis and trypanosomiasis.[10] With these reported precedents and the observation that polyamines derivatives show activities against more than one parasitic diseases, we decided to explore the polymer supported synthesis of diamines derivatives, as minimal structural prototypes of polymine compounds. Different strategies to prepare polyamines on solid support have been reported and used to generate bioactive compounds.[11] First, we examined appropriate polymer supports for our synthetic approach, having found that trityl resin has been successfully used for synthesis of polyamine derivatives on solid support.[12] Putrescine and 1,3-propanediamine were directly loaded on 2-chlorotrityl chloride resin by reaction with Hünig's base as a catalyst and DMF as solvent. The presence of a free amine group was confirmed using a Ninhydrin test. The loaded resins **2a–b** were allowed to react under conditions of reductive amination with a library of different aldehydes. First, Schiff's bases were formed by reaction of the loaded diamines and the aldehydes in trimethylorthoformate (TMOF):dichloromethane (DCM) for 16 h, at which

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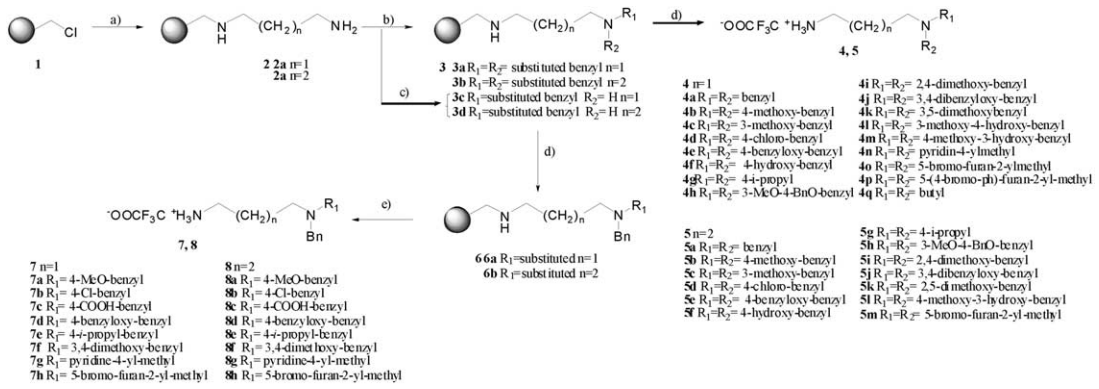

Figure 1. (a) Hünig's base, DMF, polyamine; (b) 1–5.0 equiv RCHO, DCM:TMOF, 2-$NaBH(AcO)_3$, AcOH, DMF; (c) 1–2.5 equiv RCHO, DCM:TMOF, 2-$NaBH(AcO)_3$, AcOH, DMF; (d) BnBr, DBU, DMF; (e) 10% TFA/DCM.

time the product was reduced using sodium triacetoxyborohydride and acetic acid in DMF.[13] After initial attempts we were able to optimize conditions to produce single or double reductive aminations. In fact, when 5 equivalents of aldehyde was used, two consecutive reductive aminations were effected giving the corresponding tertiary amine. On the other hand, when 2.5 equivalents of aldehyde were used, the secondary amine was obtained by reaction of only one aldehyde. As we expected the solid support functioned as a protecting group for the other amino group for all these reactions.[12] With these conditions optimized, a library of various aldehydes was allowed to react in parallel (individual vessels) with the loaded diamines **2a** and **2b** under double reductive amination conditions; obtaining the corresponding libraries of tertiary amines **3a** and **3b** respectively. Then, libraries **3a–b** were cleaved using 10% trifluoroacetic acid in dichloromethane (TFA/DCM) to provide the corresponding trifluoroacetate salts **4a–q** and **5a–m**. At the same time, single reductive amination reactions were done over loaded diamines **2a–b** and a selection of aldehydes providing libraries of secondary amines **3c** and **3d**, respectively. Those libraries were then *N*-alkylated by reaction with 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) and benzyl bromide in DMF[14] to build tertiary amines libraries **6a** and **6b**. Cleavage from the resin was then effected using 10% TFA/DCM providing the trifluoroacetate salts **7a–h** and **8a–h** (Fig. 1).[15]

Compounds were characterized and submitted for in vitro bioassay against two different strains of malaria parasite (*Plasmodium falciparum* D6 and chloroquine *resistant* W2) and Leishmania promastigotes (*Leishmania donovani*). The results showed that 23 of the compounds in libraries **4** and **5** were active against Leishmania. Activities ranged from 26.15 to 0.67 μM for the most active. On the other hand, only 9 compounds were active against the D6 clone with activities between 10.2 to 1.07 μM and 13 against W2 clone with a range from 9.34 to 1.68 μM (Tables 1–2). Considering the library compounds **7** and **8**, the same pattern of activities were observed over different diseases (Table 3). A careful examination over the antileishmanial activities showed a correlation with the 4-substituent's volume. In fact, increasing the size of the substituent **4a** R = H, **4b** R = OMe, **4g** R = *i*-Pr, **4e** R = OBn decreased EC_{50} 24.15 > 10.04 > 2.65 > 1.12 μM, showing clearly that the volume was proportional to the activity. Exactly the same tendency was observed for the library **5**. A general overview showed that the most active compounds against both diseases were **4e**, **4h** and **4j** for library **4**, and **5e**, **5h** and **5j** for library **5** (Tables 1–2). For compounds **8**, **8d** and **8e** had the best spectrum of activities (Table 3), while for **7**, likewise, **7d** and **7e** were the most versatile substituents for promoting broad-spectrum activity.

This entire group of compounds shares a common moiety, a 4-benzyloxy group. Based on these and the above observations, we considered introduction of diversity at the benzyloxy ring for improvement of antiparasitic activities.

To carry this out, two new scaffolds needed to be prepared by double reductive aminations of the terminal free amine with 4-hydroxy-benzaldehyde and with

Table 1. Antiparasitic activities[a] of compounds **4a–q**

Compd	LCMS (M + H)	Leishmania[b]	Malaria[b] D6 Clone	Malaria[b] W2 Clone
4a	255.23	24.17	NA	NA
4b	315.19	10.04	NA	9.34
4c	315.19	12.60	NA	NA
4d	323.11	8.69	NA	10.06
4e	467.17	1.12	4.99	6.03
4f	287.18	NA	NA	NA
4g	339.21	2.65	NA	9.50
4h	527.28	0.67	2.97	2.81
4i	375.13	20.47	NA	9.42
4j	679.02	1.64	2.90	3.03
4k	375.2	9.42	NA	NA
4l	347.14	NA	NA	NA
4m	347.20	NA	NA	NA
4n	257.24	NA	NA	NA
4o	390.86	19.76	NA	NA
4p	542.93	8.51	5.62	NA
4q	301.19[c]	4.66	6.33	NA
Amphotericin A		0.01		
Pentamidine		1.35		
Artemisin			0.020	0.020
Chloroquine			0.014	0.31

[a] Values are means of three experiments, (NA = not active).
[b] Activities are μM EC_{50} values determined by in vitro assay.
[c] (M + TFA).

Table 2. Antiparasitic activities[a] of compounds **5a–m**

Compd	LCMS (M + H)	Leishmania[b]	Malaria[b] D6 Clone	Malaria[b] W2 Clone
5a	269.14	26.15	NA	NA
5b	329.09	10.62	NA	6.78
5c	329.15	10.85	NA	6.10
5d	337.02	3.77	NA	NA
5e	481.12	1.85	3.20	4.88
5f	301.09	NA	NA	NA
5g	353.19	3.00	NA	7.29
5h	541.19	1.83	1.07	1.68
5i	389.08	NA	7.76	NA
5j	693.37	1.86	3.47	2.23
5k	389.08	5.37	NA	NA
5l	361.13	NA	NA	NA
5m	404.82	19.23	NA	NA

[a] Values are means of three experiments, (NA = not active).
[b] Activities are μM EC_{50} values determined by in vitro assay.

Table 3. Antiparasitic activities[a] of compounds **7a–h** and **8a–h**

Compd	LCMS (M + H)	Leishmania[b]	Malaria[b] D6 Clone	Malaria[b] W2 Clone
7a	285.2	4.52	NA	NA
7b	289.16	1.86	NA	7.70
7c	389.1	8.97	NA	NA
7d	361.17	1.37	4.43	3.79
7e	297.21	1.58	7.31	2.19
7f	315.12	3.97	NA	5.60
7g	255.23	NA	NA	NA
7h	323.04	20.58	NA	5.72
8a	299.15	1.45	NA	2.23
8b	303.11	1.61	NA	NA
8c	403.14	10.32	NA	NA
8d	375.13	0.37	5.73	7.78
8e	311.23	1.88	9.66	10.13
8f	329.15	8.36	NA	3.62
8g	270.21	NA	NA	NA
8h	337.01	21.05	NA	NA

[a] Values are means of three experiments, (NA = not active).
[b] Activities are μM EC_{50} values determined by in vitro assay.

vanillin. Then, the resulting 4 compounds could be alkylated on the free phenolic moiety with different alkyl halides to introduce the new diversity. Starting from loaded propylenediamine **2a** and putrescine **2b**, double reductive aminations with 4-hydroxy-benzaldehyde and vanillin were performed following the same procedure as before (Fig. 2). Thus, **9a** was a product of just two reductive aminations with 4-hydroxybenzaldehyde, while **9b** was the product of double reductive aminations with vanillin. Either **9c** or **9d** were the same except that $n = 1$ for **9a**, **9b**; but $n = 2$ for **9c**, **9d**.

Subsequent *O*-alkylation reactions were conducted in DMF using DBU as a base and adding different alkyl, benzyl or aryl halides, leading to libraries **10a–d**.

Compounds were cleaved from the polymeric support using 10% TFA/DCM generating the trifluoroacetate salts **11a–n** and **12a–n**. Tables 4 and 5 show the antiparasitic activities of compounds from these libraries. Clearly, our assumption regarding that the benzyl moiety attached to the aromatic ring(s) will increase activities against malaria was confirmed; only 4 compounds were inactive against the W2 clone, these same compounds being active against the D6 clone of *P. falciparum*. It is interesting to note that the small change from a 4-benzyloxy to a 4-(2-phenylethyl)oxy moiety was all that was required to stifle antimalarial efficacy against the W2 clone (i.e., **11a**, **11i**, **12a** and **12i**), but not the D6 clone.

All of the compounds from Tables 4 and 5 were active against leishmania promastigotes in vitro.

In this study, in vitro EC_{50} values ranged from 27.00 to 0.88 μM for *Leishmania donovani*. Likewise, for *Plasmodium falciparum*, EC_{50} values ranged from 8.68 to 0.68 μM for the D6 clone and 6.64 to 0.69 μM for the W2 clone. It is noteworthy that in both cases diamine chain length did not affect activity, for example, compounds with the same substitution pattern that only differed in diamine spacing ($n = 1$ or $n = 2$), had similar activities.

In conclusion, we have identified a series of novel diamines with the most active compounds being **4h**, **5h**, **7d**, **8d**, **11b** and **d** and **12b** and **d** against leishmania. Against the *P. falciparum* D6 clone and W2 clone the most active compounds with the best balance between W2:D6 activity were **4h**, **5h**, **7d**, **8d**, **11n** and **12m**. The introduction

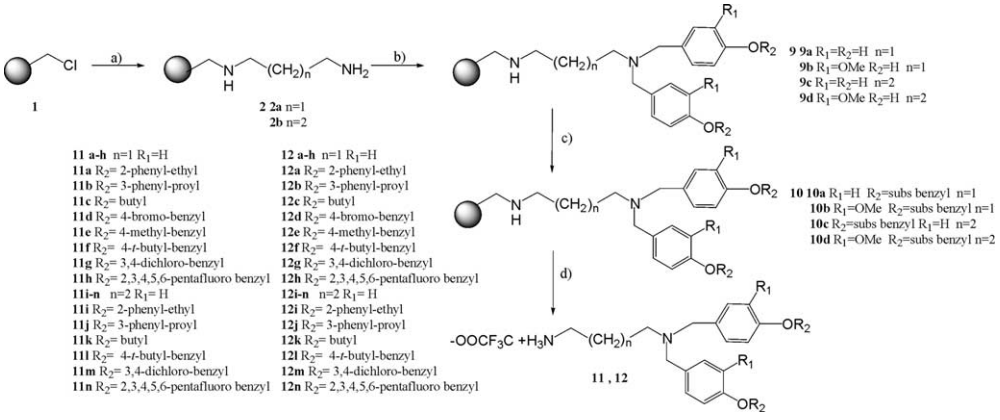

Figure 2. (a) Hünig's base, DMF, polyamine; (b) 1-Aldehyde, DCM:TMOF, 2-$NaBH(AcO)_3$, AcOH, DMF; (c) Substituted benzyl bromide, DBU, DMF; (d) 10% TFA/DCM.

Table 4. Antiparasitic activities[a] of compounds **11a–n**

Compd	LCMS (M + H)	Leishmania[b]	Malaria[b] D6 Clone	Malaria[b] W2 Clone
11a	391.95	11.10	6.94	NA
11b	523.07	0.94	1.88	5.34
11c	399.07	1.27	4.10	6.44
11d	624.89	0.91	1.14	2.44
11e	495.02	1.07	1.41	4.60
11f	579.17	1.37	2.02	4.04
11g	602.94	0.97	1.34	3.20
11h	647.02	3.81	2.24	3.16
11i	404.93	27.00	8.68	NA
11j	537.06	2.46	1.12	3.07
11k	413.05	2.47	1.86	4.18
11l	447.00	8.92	3.21	6.42
11m	616.94	3.82	1.50	2.59
11n	662.02	2.97	1.16	2.07

[a] Values are means of three experiments, (NA = not active).
[b] Activities are μM EC_{50} values determined by in vitro assay.

Table 5. Antiparatic activities[a] of compounds **12a–n**

Compd	LCMS (M + H)	Leishmania[b]	Malaria[b] D6 Clone	Malaria[b] W2 Clone
12a	451.04	14.17	4.25	NA
12b	583.15	1.00	2.01	3.44
12c	459.1	1.22	4.37	6.64
12d	684.92	0.88	1.23	2.25
12e	555.09	1.20	1.42	3.59
12f	639.2	2.13	2.52	3.85
12g	664.93	2.57	1.54	4.11
12h	706.99	1.95	2.68	3.29
12i	465.02	12.96	7.43	NA
12j	597.14	2.96	1.04	2.53
12k	473.09	3.92	4.09	2.90
12l	653.26	3.00	1.07	2.09
12m	676.97	3.66	1.16	0.69
12n	721.05	2.52	0.68	2.16

[a] Values are means of three experiments, (NA = not active).
[b] Activities are μM EC_{50} values determined by in vitro assay.

of a benzyloxy group into the aromatic ring has been observed to enhance antimalarial activity in vitro.

The fact that the compounds demonstrated activities against both diseases leads us to think that these compounds may eventually prove useful as broad-spectrum antiparasitic agents. When one examines the overall assay results, **4h**, **5h**, **7d**, **8d**, **11g** and **12d** appear to possess the best balance of μM EC_{50} values. Most of the well-balanced dual inhibitors were in fact the same as those considered most active, the discrepancies arising for series **11** and **12** compounds. In these instances, fortuitously, the 3-methoxy-4-benzyloxybenzyl moieties had not been included in the libraries. Thus, the compounds surfacing next were halogenated benzyl rings. In *P. falciparum*, our experience with other classes of antimalarials is that halogenation of aromatic rings improves activity presumably by an effect on aromatic ring metabolism.

We notice as an overall pattern emerging from this simple selection of substituents that a *para*-substituent such as a benzyloxy group imparted the greatest effect on potency. Additional flanking oxygenation (e.g., 3-methoxy and 4-benzyloxy) was also beneficial.

In the future we will explore activities against other parasites and will look for the specific molecular target of these compounds.

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15. **General experimental procedure:** The compounds were prepared on an Argonaut Quest® 210 synthesizer using 5 mL reaction vessels. To 2-chorotrityl resin (50 mg, 1.08

mmol/gm, preswelled with DCM for 15 min) was added 500 μL of anhydrous DMF followed by diisopropylethylamine (7 equiv). The mixture was stirred for 20 min, and a solution of substituted benzaldehyde (5 equiv) and DMAP (5 mg) in DMF (1000 μL) was added. The mixture was stirred for 24 h at which time all of the vessels were drained and the resin was capped with methanol for 30 min and drained again. The resins were then subjected to a wash sequence using DMF (3×3 mL), MeOH (3×3 mL), and DCM (3×3 mL). The reaction vessels (RVs) were finally washed with TMOF. The resin was suspended in TMOF–DCM (1:2, v/v, 700 μL) and diverse amines (10 equiv) were added. Schiff's base formation was allowed to continue for 16 h, and then a solution of sodium triacetoxyborohydride (10 equiv) in DMF (500 μL) and acetic acid (7 equiv) was added to all of the RVs. The reactions were allowed to proceed for another 18 h, at which time all of the RVs were drained and washed using DMF (3×3 mL), THF (3 mL), THF–H_2O (1:1, 3 mL), H_2O (3 mL), THF (3 mL), MeOH (3×3 mL), and DCM (2×3 mL). The resin in all the RVs was suspended in DMF (500 μL) and to each solution a different benzyl bromide (10 equiv) and DBU (4 equiv) was added and the mixtures were stirred for 24 h. A wash sequence as described above was carried out and a final washing of all the RVs was carried out with anhydrous diethyl ether (2×3 mL). The resin was dried overnight and the products were cleaved from resin using 10% TFA in DCM (4 mL) for 25 min, followed by filtration. The filtrates were evaporated in a Speedvac® to obtain the products whose purity was analyzed by HPLC using a C_{18} Symmetry column running a gradient from (0.1% TFA) H_2O–AcCN 90:10 to 10:90 for 30 min at a flow rate of 1 mL/min. The identity of the products was confirmed by LC-MS and NMR spectroscopy.

The purities of the compounds in series **4** and **5** were determined by analytical HPLC as 82% (±4%); **7** and **8**: 75% (±5%); **9** and **10**: 70% (±8%); and **11** and **12**: 68% (±6%).