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Original article

Synthesis and antiprotozoal activities of dicationic bis(phenoxymethyl)benzenes, bis(phenoxymethyl)naphthalenes, and bis(benzyloxy)naphthalenes

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ABSTRACT

A series of 37 dicationically substituted bis(phenoxymethyl)benzene bis(phenoxymethyl)naphthalene, and bis(benzyloxy)naphthalene analogues of pentamidine was prepared and evaluated for antiprotozoal activities and cytotoxicity in in vitro. 1,3-Bis(4-amidinophenoxymethyl)benzene (**1**) was the most active against *Trypanosoma brucei rhodesiense* ($IC_{50} = 2.1$ nM). 1,3-Bis[4-(*N*-isopropylamidino) phenoxymethyl]benzene (**2**) was most active against *Plasmodium falciparum* ($IC_{50} = 3.6$ nM) and displayed a selectivity index more than 50 times greater than that of pentamidine. Several other compounds displayed lower antiplasmodial IC_{50} values and higher selectivity indices relative to pentamidine. 1,4-Bis(4-amidinophenoxymethyl)benzene (**14**) was the most active against *Leishmania donovani* ($IC_{50} = 1.3$ μM). Compound **2** displayed the greatest activity against *T. b. rhodesiense* in vivo, curing three of four infected mice dosed intraperitoneally at 5 mg/kg × 4 days.

1. Introduction

The insect-vectored protozoal infections human African trypanosomiasis (HAT), malaria, and leishmaniasis continue to cause significant rates of morbidity and mortality. Both HAT and malaria are most prevalent in sub-Saharan Africa [1,2]. The epidemiology of HAT has been recently reviewed [3]. More than 90% of reported cases of HAT, found in west and central Africa, are a chronic infection due to *Trypanosoma brucei gambinse*, in which patients may be asymptomatic for months or even years, and the disease is often in an advanced state when symptoms first occur. An acute infection due to *Trypanosoma brucei rhodesiense*, found in eastern and southern Africa, represents less than 10% of reported cases [1]. Approximately 17 500 cases of HAT were reported in 2006 [4], while the World Health Organization (WHO) estimates are between 50 000 and 70 000 cases [1]. Malaria is caused by several *Plasmodium* species, with the most deadly infection due to *Plasmodium falciparum*. The WHO estimates that 40% of the world's population, mostly those living in the poorest countries, are at risk of malaria. In some African nations, malaria accounts for as much as 40% of the public health expenditure [2]. An estimated 300–500 million severe cases occur annually, with 1.5–2.0 million fatalities [2,5]. As many as 20 species of the *Leishmania* protozoa give rise to human leishmaniasis, manifested mainly as the cutaneous, mucocutaneous, and life-threatening visceral forms [6,7]. The WHO estimates that two million new cases develop annually and 350 million people are at risk [8]. Hundreds of cases individuals dually infected with HIV and visceral leishmaniasis in the South American, European, and African continents have been reported in recent years [9–14].

The need for safe, orally effective, and inexpensive drugs to combat HAT (especially the late stage disease) is great. Suramin and pentamidine, limited to treatment of early stage HAT, must be administered parenterally [1], and adverse reactions to pentamidine are well known [15]. Melarsoprol (an organoarsenical) is effective for late stage HAT, but its disadvantages include a fatal encephalopathy in up to 10% of cases and rising rates of treatment failure [4,15]. Eflornithine, a safer alternative available since 1990, is effective only against late stage *T. b. gambiense* infections, must be administered in high doses over long periods, and relapses have occurred [15,16].

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A growing problem with treatment of malaria has been drug resistance, especially to inexpensive and widely used drugs such as chloroquine and sulfadoxine–pyrimethamine [2,17]. Thus more expensive drugs or drug combinations (especially with artemisinins) must be used [14,18,19]. Various antimalarial drugs which are currently in clinical use or under development are chemically related or have similar mechanisms of action (or resistance). Thus the risk of cross-resistance and failure of new treatments are increased [14,20].

Antileishmanial therapy has been summarized recently [21]. Parenteral pentavalent antimonial compounds have been the primary therapy against visceral leishmaniasis. Resistance to anitimonial drugs in Bihar State, India (which accounts for 90% of India's and 45% of the world's visceral cases), has led to alternate therapies including amphotericin B (conventional and liposomal formulations), paromomycin, and miltefosine (the only orally administered drug).

Pentamidine (Fig. 1) has shown efficacy against all three diseases. *N*-Hydroxy and -methoxy derivatives of amidines have shown the potential to be orally active prodrugs of amidines [22,23]. More recently, DB289, an *N*-methoxy (methamidoxime) prodrug of furamidine (DB75) was in Phase III clinical trials against early stage HAT and Phase II trials against malaria [24,25]. However, the compound exhibited nephro- and hepatoxicities in a recent expanded Phase I trial [26].

The present investigation involves bis(phenoxymethyl)benzene, bis(phenoxymethyl)- and bis(benzyloxy)naphthalene derivatives **1**–**37** (Table 1). 1,3-Bis[(4-amidino)phenoxymethyl]benzene (**1**), a pentamidine analogue bearing an aromatic ring between the two oxygen atoms, is the lead compound for the series. The syntheses of 24 novel compounds are described. Activities against *T. b. rhodesiense*, *P. falciparum*, and *Leishmania donovani*, as well as toxicity to rat myoblast cells in vitro are reported for the 37 compounds. Antitrypanosomal activities in vivo are reported for select compounds.

2. Chemistry

Dicationic compounds **1**–**37** (Table 1) are an expansion of a smaller group of bis(phenoxymethyl)benzenes (xylene derivatives) and naphthalene derivatives (**1**, **6**, **14**, **17**, **20**, **21**, **23**, **25**, **29**, **30**, **33**) prepared previously in this laboratory for other purposes [27]. Preparations of analogues **4**, **8**, **14**, and **17** have also been reported previously [28–31]. The in vitro activities of compounds **18**, **25**, **28**, **31**, and **34** against *Leishmania infantum* have been recently published [32] but their syntheses (except for **25**) have not been described.

1,3-Bis(4-amidinophenoxymethyl)benzene (**1**), formally a derivative of *m*-xylene, is structurally similar to pentamidine but contains an aromatic ring in the linkage between the oxygen atoms. Such a linkage retains similar spacing between the oxygen atoms but is more rigid. Variation of the nature and position of the two cationic groups gives rise to congeners **2**–**7**. *o*-Xylene derivatives **8**–**13** have a rigid four carbon spacing between the oxygen atoms, while a six carbon spacing is present in *p*-xylene derivatives **14**–**19**.

Bis(phenoxymethyl)naphthalenes **20**–**22** (dimethylnaphthalene derivatives) differ from the xylene derivatives by having a naphthalene system in place of the central benzene ring. Bis(benzyloxy) naphthalenes **23**–**37** (dihydroxynaphthalene derivatives) differ from compounds **20**–**22** not only by having opposite ring substitution patterns on the naphthalene system but also by reversal of the carbon and oxygen atoms bridging the naphthalene system and the outer rings.

Compounds **1**–**37** were prepared as shown in Scheme 1. Williamson ether syntheses involving cyanophenols and α,α′-dibromoxylenes or bis(bromomethyl)naphthalenes [33,34] gave the previously known [27–29] dinitrile precursors to xylene derivatives **1**–**19** and dimethylnaphthalene derivatives **20**–**22**. Similar reactions involving naphthalenediols and α-bromotolunitriles gave the dicyano precursors to dihydroxynaphthalene derivatives **23**–**37**. Five of these seven intermediates had been prepared previously [27]. The nitriles underwent Pinner reactions, and the imidate intermediates were treated with the appropriate amines to give compounds **1**–**3**, **6**, **8**–**26**, and **28**–**37**. Pinner syntheses involving 1,4-bis[(3-cyano)phenoxymethyl]naphthalene failed to give the desired products, the *m*-amidino and *m*-isopropylamidino analogues of compounds **21** and **22**, but gave 3-hydroxybenzamidine and 3-hydroxy-*N*-isopropylbenzamidine. Thus, the benzylic ether linkages of the starting material were unstable to the strongly acidic reaction conditions. Williamson ether syntheses involving protected 3-hydroxybenamidines and 1,4-bis(bromomethyl)naphthalene were also unsuccessful. Four prodrugs were also prepared. Treatment of the corresponding nitrile with hydroxylamine [35] gave the known amidoxime **4** [29]. Similar methodology was used in the preparation of **7**. Amidoxime **27** was prepared from the corresponding nitrile by a Pinner synthesis, followed by reaction of the imidate ester with hydroxylamine. *O*-Methylation of amidoxime **4** using dimethyl sulfate [35] gave compound methamidoxime **5**. All cationic compounds were isolated as their dihydrochloride salts.

3. Results and discussions

3.1. In vitro activities

The compounds were assayed in vitro against *T. b. rhodesiense* STIB900 [36–39], chloroquine resistant *P. falciparum* K1 [36,40], and *L. donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes [41,42] for antiprotozoal activities, and against rat myoblast (L6) cells for cytotoxicity [43] (Table 1). Selectivity indices [44] (ratios of cytotoxic IC_{50} values to antiprotozoal IC_{50} values) are shown for each parasite. Antiprotozoal IC_{50} values and cytotoxic data are compared to those of pentamidine and furamidine. Other positive controls employed were melarsoprol (against *T. b. rhodesiense*), chloroquine and artemisinin (against *P. falciparum*), and podophyllotoxin (against L6 cells).

3.1.1. Antitrypanosomal activities

Xylene derivative **1** was the most active in vitro against *T. b. rhodesiense*, with an IC_{50} value of 2.1 nM (Table 2), followed by

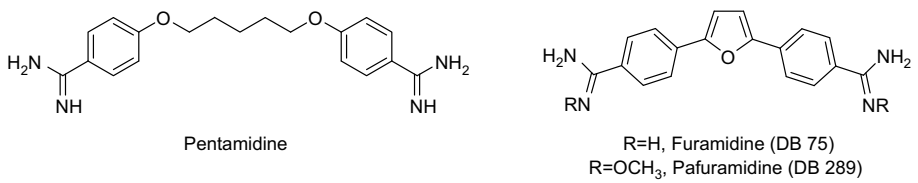


Fig. 1. Structures of 1,5-bis(4-amidinophenoxy)pentane (pentamidine), 2,5-bis(4-amidinophenyl)furan (furamidine, DB75), and 2,5-bis[4-(*N*-methoxyamidino)phenyl]furan (pafuramidine, DB289).

regioisomer **14** (21 nM), imidazoline **3** (46 nM), and regioisomer **6** (47 nM). *N*-Isopropylamidines **2** and **15** and diamidine **17** exhibited antitrypanosomal IC_{50} values between 68 and 78 nM. Of these seven compounds, all but **6**, **14** and **17** exhibited selectivity indices higher than that of furamidine, but all were less selective for the parasite than pentamidine. Compounds **8** and **11** displayed IC_{50} values around 0.14 μM, and all the other xylene derivatives were less active, with IC_{50} values ranging from 0.16 to 24 μM. The naphthalene derivatives, as a whole, were less active against *T. b. rhodesiense* than the xylene derivatives. Analogue **35** was the most active naphthalene derivative, with an IC_{50} value of 0.15 μM, followed by congeners **30** and **25** with IC_{50} values between 0.16 and 0.20 μM. A group of ten naphthalene derivatives displayed IC_{50} values between 0.2 and 0.4 μM, and all the other naphthalene derivatives were less active, with IC_{50} values between 0.8 and 2.4 μM. Selectivity indices for all of the naphthalene derivatives were less than those of furamidine or pentamidine.

3.1.2. Antiplasmodial activities

Xylene derivative **2** (the *N*-isopropyl derivative of **1**) was the most active against *P. falciparum*, displaying an IC_{50} value of 3.6 nM, followed by imidazoline **10** (10 nM) and *N*-isopropylamidine **9** (11 nM). Four other xylene derivatives (**3**, **6**, **15**, and **16**) exhibited IC_{50} values between 10 and 20 nM, while congeners **1** and **18** displayed IC_{50} values between 25 and 50 nM. Thus, nine xylene derivatives displayed IC_{50} values comparable to or lower than that of pentamidine, and the IC_{50} values of three compounds were lower than that of furamidine. The selectivity index of **2** was 100 times greater than that of furamidine and nearly 50 times greater than that of pentamidine, and those of **9**, **10**, and **15** were more than 20 times greater than that of furamidine. Compounds **9** and **10** displayed a 150- and 60-fold selectivity for *P. falciparum* over *T. b. rhodesiense*, a property of potential usefulness in treating patients with mixed infections. The IC_{50} values of analogues **14** and **17** were between 60 and 70 nM, and all the other xylene derivatives were less active, with IC_{50} values between 0.11 and 0.36 μM.

Among the naphthalene derivatives, the most active against *P. falciparum* was *N*-isopropylamidine **24**, with an IC_{50} value of 28 nM, a selectivity index three times greater than that of furamidine and a 45-fold selectivity for *P. falciparum* over *T. b. rhodesiense*. Eight other analogues (**20**, **22**, **23**, **28**, **30**, **32**, **36**, and **37**) were less active, with IC_{50} values between 50 and 90 nM. The remaining naphthalene derivatives displayed IC_{50} values between 0.11 and 0.57 μM.

3.1.3. Antileishmanial activities

Two xylene derivatives, amidine **14** and the corresponding imidazoline **16**, displayed antileishmanial IC_{50} values 1.3 and 1.9 μM, comparable to that of pentamidine but with somewhat lower selectivity indices. Congeners **1**, **3**, **8**, and **10** exhibited IC_{50} values between 3.3 and 4.0 μM, and the selectivity indices of **3** and **10** were slightly higher than that of pentamidine. The other xylene derivatives were less active, with IC_{50} values ranging from 5.5 μM to greater than 200 μM. Among the naphthalene derivatives *N*-*sec*-butyl amidine **37** was most active ($IC_{50} = 3.7$ μM), followed by amidines **25** and **35** (4.5 and 4.8 μM). The IC_{50} values of the other naphthalene derivatives ranged from 5.7 μM to greater than 200 μM.

3.1.4. Cytotoxicity and selectivity

All compounds **1**–**37** were less toxic to rat myoblast cells than furamidine ($IC_{50} = 6.4$ μM) and 17 compounds (ten xylene derivatives and seven naphthalene derivatives) were less cytotoxic than pentamidine ($IC_{50} = 47$ μM). The least toxic compound was *N*-isopropyl xylene derivative **2** ($IC_{50} > 169$ μM) and nine other *N*-isopropyl analogues (**9**, **12**, **15**, **18**, **26**, **28**, **31**, **34**, and **36**) were among the 17 least cytotoxic compounds. The most cytotoxic compound (excluding prodrugs) was naphthalene derivative **33** ($IC_{50} = 6.9$ μM). The xylene derivatives, as a whole, were more selective for the respective parasites over L6 cells relative to the naphthalene derivatives. Four xylene derivatives (**1**, **2**, **3**, and **15**) displayed antitrypanosomal selectivity indices greater than that of furamidine. The antiplasmodial selectivity indices of five analogues (**2**, **3**, **9**, **10**, and **15**) were greater than that of pentamidine. These and 13 other compounds (including eight naphthalene derivatives) displayed antiplasmodial selectivity indices greater than that of furamidine. Only two compounds, xylene derivatives **3** and **10** exhibited antileishmanial selectivity indices greater than that of pentamidine.

3.2. In vivo antitrypanosomal activities

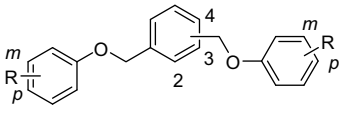
Select compounds were administered to mice infected with *T. b. rhodesiense* STIB900 [26,36,37] (Table 2). This strain proved to be more difficult to treat, relative to *T. b. rhodesiense* KETRI2537 and *T. b. brucei* STIB795 [26]. All compounds were administered once daily intraperitoneally except for the prodrugs **4**, **5**, **7**, and **27**, which were given orally. A strong correlation between in vivo activities and the in vitro data was not observed. For example, lead compound **1**, the most active against the trypanosome in the in vitro assay ($IC_{50} = 2.1$ nM) cured only one of four animals at the 4×10 mg/kg dosing, with a greater than 35 day mean survival. By contrast, the corresponding *N*-isopropyl analogue **2**, which displayed an IC_{50} value of 68 nM, cured three of four animals at both 4×10 mg/kg and 4×5 mg/kg doses, but the lower dose resulted in a lower mean survival (greater than 60 days versus greater than 53 days). The corresponding imidazoline **3** (IC_{50} value = 46 nM) cured none of the animals at 4×20 mg/kg. Compound **4**, the amidoxime prodrug of **1**, displayed moderate activity, with one of four animals cured. The corresponding methamidoxime prodrug **5** cured none of the animals. Amidine **6** (a *meta*-amidino regioisomer of **1**) and the corresponding amidoxime prodrug **7** cured no animals at the tested dosages. Of the xylene derivatives with different central ring substitution patterns, all were inactive except for **11**, which cured one of four animals. This compound, as well as **15**, proved to be lethal at higher doses. Of the naphthalene derivatives tested, all were inactive or toxic except for **25**, which cured one of four animals at 20 mg/kg.

3.3. Structure–activity relationship

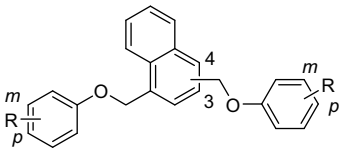
As a whole, the xylene derivatives exhibited greater activity against the three parasites and were less toxic than either group of naphthalene derivatives. Lead compound **1** was the most active against *T. b. rhodesiense* in vitro. All structural modifications resulted in decreased antitrypanosomal activity. The corresponding *p*-xylene and *o*-xylene derivatives **14** and **8** were 10 and 70 times less active, respectively. Compound **6**, a regioisomer of **1** with *meta*-amidino groups was 20 times less active than **1**. *m*-Xylene derivative **17** was only four times less active than its *para*-amidino counterpart **14**, and the *o*-xylene derived isomers **8** and **11** were equal in activity. All of the *N*-isopropylamidines and imidazolines were less active than the corresponding simple amidines, consistent with previous results from this laboratory [14,20] and elsewhere [45,46].

The introduction of a central fused ring system resulted in a 150-fold loss of activity (**20** versus **1**). A smaller (10-fold) loss of activity was observed between naphthalene derivative **21** and its xylene derivative counterpart **14**, and only a four-fold difference between **22** and **15**. No significant activity was observed with any of the dihydroxynaphthalene derivatives **21**–**37**.

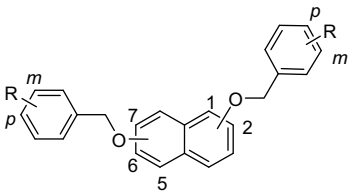
Table 1
Structures and in vitro antiprotozoal activities of compounds **1**–**37**.



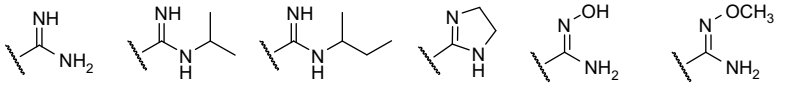
Compounds **1-19**



Compounds **20-22**

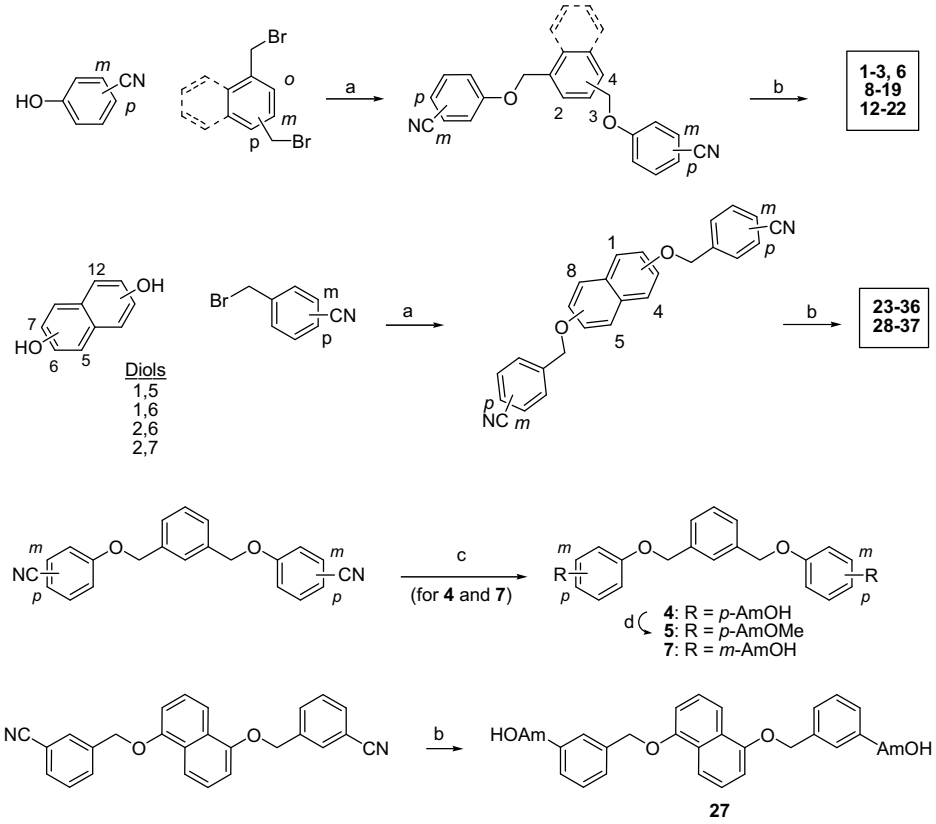


Compounds **23-37**

R = 

Am iPrAm sBuAm Im AmOH AmOMe

Compd	Position	R	*T. b. rhodesiense*[a]		*P. falciparum*[b]		*L. donovani*[c]		L6 cells[d]
			IC_{50} (μM)	SI_T[e]	IC_{50} (μM)	SI_P[f]	IC_{50} (μM)	SI_L[g]	IC_{50} (μM)
1	1,3	*p*-Am	0.0021	4350	0.0256	357	3.26	2.80	9.14
2	1,3	*p*-iPrAm	0.0680	>2490	0.0036	>46 900	7.27	>23.2	>169
3	1,3	*p*-Im	0.0462	2670	0.0180	6860	3.99	30.9	123
4	1,3	*p*-AmOH	>62.6	<0.104	>10.4	<0.628	>100	<0.0653	6.53
5	1,3	*p*-AmOMe	30.4	>1.11	>9.72	>3.46	>100	<0.336	>33.6
6	1,3	*m*-Am	0.0473	1360	0.0191	3361			64.2
7	1,3	*m*-AmOH	7.71	1.12	2.09	4.12	>50.0	<0.172	8.6
8	1,2	*p*-Am	0.144	209	0.153	197	3.82	7.87	30.1
9	1,2	*p*-iPrAm	1.68	97.0	0.0109	15 000	11.7	14.0	163
10	1,2	*p*-Im	0.647	168	0.0104	10 500	3.66	29.8	109
11	1,2	*m*-Am	0.140	602	0.109	775	5.50	15.4	84.6
12	1,2	*m*-iPrAm	1.280	>126	0.111	>1470	>30.0	<5.41	>162
13	1,2	*m*-Im	24.2	2.75	0.362	184	>100	<0.665	66.5
14	1,4	*p*-Am	0.0207	600	0.064	194	1.32	9.4	12.4
15	1,4	*p*-iPrAm	0.0748	>2230	0.017	>9820	7.99	20.9	>167
16	1,4	*p*-Im	0.380	79.5	0.0180	1680	1.90	15.9	30.2
17	1,4	*m*-Am	0.0778	103	0.0677	119	>200	<0.0401	8.02
18	1,4	*m*-iPrAm	1.20	72.4	0.0474	1830	14.8	5.89	87
19	1,4	*m*-Im	1.42	13.9	0.113	175	>200	<0.099	19.8
20	1,3	*p*-Am	0.325	212	0.0551	1250			68.8
21	1,4	*p*-Am	0.234	121	0.126	224			28.3
22	1,4	*p*-iPrAm	0.313	134	0.0669	625	9.73	4.29	41.8
23	1,5	*p*-Am	0.250	312	0.0734	1060			78
24	1,5	*p*-iPrAm	1.24	30.7	0.0280	1360	5.73	6.67	38.2
25	1,5	*m*-Am	0.182	79.2	0.575	25.1	4.51	3.2	14.4
26	1,5	*m*-iPrAm	0.359	141	0.367	138			50.7
27	1,5	*m*-AmOH	12.4	0.541	7.930	0.845	>100	<0.0670	6.70
28	1,6	*p*-iPrAm	0.298	324	0.0748	1290	8.49	11.4	96.4
29	2,6	*p*-Am	2.36	8.17	0.430	44.7	7.12	2.70	19.3
30	2,6	*m*-Am	0.162	82.2	0.0870	153	>200	<0.0665	13.3
31	2,6	*m*-iPrAm	0.311	236	0.112	654	11.9	6.17	73.4
32	2,6	*m*-sBuAm	0.960	12.4	0.0681	174	5.89	2.02	11.9
33	2,7	*p*-Am	0.219	31.7	0.163	42.6			6.95
34	2,7	*p*-iPrAm	0.223	440	0.133	739	10.8	9.07	98.0
35	2,7	*m*-Am	0.146	208	0.149	205	4.77	6.38	30.4
36	2,7	*m*-iPrAm	0.348	390	0.0738	1840	11.0	12.3	136
37	2,7	*m*-sBuAm	0.812	16.9	0.0719	191	3.69	3.71	13.7
PMD[h]			0.0028	16 600	0.0464	1004	1.84	25.4	46.6
FMD[i]			0.0032	1880	0.0138	464	2.70[j]		6.4
MLSP[k]			0.0064	1220					7.8
CQ[l]					0.1248	937			116.9
ATMS[m]					0.0043	105 000			450.5
PPT[n]									0.017



Scheme 1. Reagents and conditions: (a) K_2CO_3 or Cs_2CO_3, DMF; (b) HCl, EtOH, 1,4-dioxane, then appropriate amine; (c) $NH_2OH{\cdot}HCl$, *t*-BuOK, DMSO; (d) Me_2SO_4, NaOH, DMF.

The alteration of central ring substitutions and the positions of the amidine functions had a less pronounced impact upon the antiplasmodial activities of the xylene derivatives relative to their antitrypanosomal activities. In contrast, the introduction of alkyl groups onto the amidine nitrogen atoms generally resulted in increased antiplasmodial activity, consistent with previous results from this laboratory [14,20]. Four of the five *N*-isopropylamidines (**2**, **9**, **15**, and **18**) and three of the imidazolines (**3**, **10**, and **16**) were more active than the corresponding amidines, exhibiting antiplasmodial IC_{50} values less than 50 nM. Thus these seven compounds displayed various degrees of selectivity for *P. falciparum* over *T. b. rhodesiense*, up to 150-fold in the case of *N*-isopropylamidine **9**. The introduction of a central fused ring system resulted in decreased antiplasmodial activities, although a much smaller decrease relative to that observed with antitrypanosomal activity. For example, only a two-fold decrease in antiplasmodial activity was observed between compounds **20** and **1**, in contrast to a 150-fold decrease in antitrypanosomal activity. Among both groups of naphthalene derivatives, only *N*-isopropylamidine **24** exhibited an antiplasmodial IC_{50} value less than 50 nM. Despite their low activities, *N*-isopropylamidines **33** and **35** and *N*-*sec*-butylamidines **32** and **37** were more active than the corresponding amidines.

The *N*-isopropyl group had the most significant impact upon decreased cytotoxicity. Ten of the 12 *N*-isopropylamidines in the series were among the 17 compounds that were less cytotoxic than pentamidine. The *N*-isopropyl derivatives were less cytotoxic than the corresponding lamidines in 10 of 11 instances. Three imidazolines (all xylene derivatives) were also among the 17 least cytotoxic compounds. The *N*-*sec*-butyl group had a smaller impact upon decreased cytotoxicity. Thirty-one of the 36 analogues were less cytotoxic than lead compound **1**.

4. Conclusions

The xylene derivatives, as a whole, were more active against the three parasites in vitro and less cytotoxic than either group of the naphthalene derivatives. While lead compound **1** was more active

[a] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations (Refs. [36–39]).
[b] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations (Refs. [36,40]).
[c] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations (Refs. [41,42]).
[d] Rat myoblast cells, used as a measure of cytotoxicity. Average of duplicate determinations (Ref. [43]).
[e] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].
[f] Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].
[g] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].
[h] PMD, pentamidine.
[i] FMD, furamidine.
[j] Value reported in Ref. [7].
[k] MLSP, melarsoprol.
[l] CQ, chloroquine.
[m] ATSM, artemisinin.
[n] PPT, podophyllotoxin.

Table 2
Activities of select compounds against mice infected with *T. b. rhodesiense* (STIB900).[a]

Compd	Dose[b] (mg/kg)	Route[c]	Cured/infected[d]	MSD[e] (days)
1	4 × 10	i.p.	1/4	>35.5
2	4 × 10	i.p.	3/4	>60
	4 × 5	i.p.	3/4	>51.5
3	4 × 20	i.p.	0/4	22.8
4	4 × 100	p.o.	1/4	>38.3
5	4 × 100	p.o.	0/4	6
6	4 × 5	i.p.	0/4	17
7	4 × 100	p.o.	0/4	14.5
8	4 × 20	i.p.	0/4	13.5
11	4 × 10	i.p.	1/4	>27.3
14	4 × 20	i.p.	0/4	32
15	4 × 5	i.p.	0/4	17
16	4 × 20	i.p.	0/4	15
17	4 × 20	i.p.	0/4	19.5
20	4 × 20	i.p.	0/4	12
25	4 × 20	i.p.	1/4	>60
27	4 × 100	p.o.	0/4	7.8
28	4 × 5	i.p.	0/4	6
30	4 × 5	i.p.	0/4	13.5
34	4 × 5	i.p.	0/4	18
35	4 × 20	i.p.	0/4	17.3
MLSP[g]	4 × 4	i.p.	3/4	>60
PMD[h]	4 × 20	i.p	0/4	42.8

[f] Ref. [26].
[a] Female NMRI mice.
[b] Single daily doses.
[c] intraperitoneal (i.p.) or oral (p.o.).
[d] Mice that survived for 60 days after infection without showing a parasitemia relapse.
[e] Mean survival days post infection of the mice that showed a parasitemia relapse.
[g] MLSP, melarsoprol.
[h] PMD, pentamidine.

than pentamidine against *T. b. rhodesiense* in vitro, all structural modifications resulted in decreased activity. Analogue **2**, the *N*-isopropyl derivative of **1**, was quite active in the acute mouse model of the parasite. Disappointingly, none of the prodrugs exhibited substantial oral activity. The greatest potential for these compounds may lie in their antiplasmodial activities. Compound **2** displayed not only an IC_{50} value lower than that of all the reference compounds, but also a selectivity index 47 times greater than that of pentamidine. Nine other analogues exhibited IC_{50} values less than 50 nM (activities comparable to or greater than that of pentamidine), four of which displayed selectivity indices between five and 15 times greater than that of pentamidine. Compound **14** was the most active against *L. donovani* ($IC_{50} = 1.3$ μM), and eight other analogues displayed IC_{50} values between 1.9 and 4.8 μM.

5. Experimental

5.1. Chemistry

Uncorrected melting points were measured on a Thomas–Hoover Capillary melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) spectrometer. Spectra were in DMSO-d_6 (with 0.05% TMS) unless stated otherwise. Anhydrous EtOH was distilled over Mg/I_2 immediately prior to use. Other anhydrous solvents were purchased from Aldrich Chemical Co., Milwaukee, WI, in Sure-seal® containers and were used without further purification. Reaction mixtures were monitored by TLC on silica gel or by reverse phase HPLC. Organic layers of extraction mixtures were washed with saturated NaCl solution and dried over Na_2SO_4 or $MgSO_4$ before being evaporated under reduced pressure. Gravity and flash column chromatography were performed using Davisil grade 633, type 60A silica gel (200–425 mesh). Analytical HPLC chromatograms were recorded on a Hewlett–Packard 1090 Series II chromatograph using a Zorbax Rx C8 column (4.6 × 75 mm, 3.5 μm) and UV photodiode array detection at 230, 254, 265, 290, and 320. Wavelengths reported are those at which the strongest signals of the major products were observed. Mobile phases consisted of mixtures of CH_3CN (0–75%) in water containing formic acid (80 mM), ammonium formate (20 mM) and triethylamine (15 mM). Flow rates were maintained at 1.5 mL/min at a column temperature of 40 °C. The concentration of CH_3CN was increased linearly from 0 to 22.5% over 6 min, then from 22.5 to 56.25% over 4 min, then maintained for 1 min. Preparative reverse phase HPLC was performed on Varian ProStar Chromatography Workstation configured with two PS-215 pumps fitted with 50 mL pumpheads, a Dynamax Microsorb C18 (60 Å) column (41.4 × 25 cm, 8 μm), PS-320 variable wavelength UV–Vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of CH_3CN (0–75%) in water containing formic acid (40 mM) and ammonium formate (10 mM). Flow rates were maintained at 40 mL/min. Detector wavelengths and mobile phase gradients were optimized for the individual compounds. Select fractions were analyzed for purity using a Zorbax Rx C8 column (4.6 × 75 mm, 3.5 μm) and the latter mobile phases on an Agilent Technologies 1100 chromatograph. Pooled purified fractions were evaporated under reduced pressure, reconstituted in water, and lyophilized on a VirTis BenchTop 2K lyophilizer. Low resolution ESI^+ mass spectra were recorded on an Agilent Technologies 1100 Series LC/MSD Trap spectrometer or an Applied Biosystems Series 100 spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ±0.4% of calculated values.

5.2. General procedure for compounds **1**–**3**, **6**, **8**–**26**, and **27**–**37**

The nitrile was added to a mixture of anhydrous EtOH and 1,4-dioxane that had been saturated with hydrogen chloride at 0 °C in a dry 3-neck flask equipped with a gas inlet tube, a thermometer, and a drying tube, and cooled in an ice–salt bath. The reaction mixture was then sealed, slowly warmed to ambient temperature, and stirred until the nitrile was no longer detectable. The reaction mixture was diluted with ether. The crude imidate was filtered off under inert gas and dried under high vacuum over KOH. The imidate (or an aliquot thereof) was then reacted immediately with the appropriate ammonia or the appropriate amine in EtOH. The reaction mixture was diluted with ether, and the crude product was filtered off. The product was purified by direct recyrstallization from appropriate solvents or by preparative HPLC followed by conversion to the dihydrochloride salt using aqueous or ethanolic HCl.

5.2.1. 1,3-Bis[4-(N-isopropylamidino)phenoxymethyl]benzene dihydrochloride (**2**)

Yield, 0.76 g (55%): mp 258–259 °C; ^{1}H NMR δ 9.42 (d, $J = 7.9$ Hz, 2H), 9.32 (br s, 2H), 9.97 (br s, 2H), 7.73 (d, $J = 8.5$ Hz, 4H), 7.59 (s, 1H), 7.40 (m, 2H), 7.45 (s, 3H), 7.22 (d, $J = 8.5$ Hz, 4H), 5.26 (s, 4H), 4.05 (m, 2H), 1.26 (d, $J = 6.3$ Hz, 12H); HPLC t_R 8.25 min (98.7 area % at 254 nM). Anal. Calcd for $C_{28}H_{34}N_4O_2 \cdot 2HCl$: C, 63.27; H, 6.83; N, 10.54; Cl, 13.34. Found: C, 63.14; H, 6.64; N, 10.35; Cl, 13.34.

5.2.2. 1,3-Bis[4-(2-imidazolinyl)phenoxymethyl]benzene dihydrochloride (**3**)

Yield, 1.52 g (82%): mp 241–243 °C; ^{1}H NMR δ 10.67 (s, 4H), 8.08 (d, $J = 8.2$ Hz, 4H), 7.58 (s, 1H), 7.46 (s, 3H), 7.27 (d, $J = 8.2$, 4H), 5.28 (s, 4H), 3.95 (s, 8H); HPLC t_R 7.48 min (100 area % at 254 nM). Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 60.35; H, 5.84; N, 10.83; Cl, 13.70. Found: C, 60.28; H, 5.96; N, 10.87; Cl, 13.97.

*5.2.3. 1,2-Bis[4-(*N*-isopropylamidino)phenoxymethyl]benzene dihydrochloride (**9**)*

Yield, 0.06 g (3.7%): mp 196–198 °C; ^{1}H NMR δ 9.46 (d, J = 7.7 Hz, 2H), 9.37 (br s, 2H), 9.03 (br s, 2H), 7.74 (d, J = 8.5 Hz, 4H), 7.55 (m, 2H), 7.40 (m, 2H), 7.21 (d, J = 8.5 Hz, 4H), 5.38 (s, 4H), 4.07 (m, 2H), 1.26 (d, J = 6.3 Hz, 12H); MS m/z 459 (MH^+ of free base); HPLC t_R 8.21 min (100 area % at 254 nM). Anal. Calcd for $C_{28}H_{34}N_4O_2 \cdot 2HCl \cdot 1.4H_2O$: C, 60.41; H, 7.02; N, 10.06; Cl, 12.74. Found: C, 60.40; H, 6.88; N, 10.03; Cl, 12.60.

*5.2.4. 1,2-Bis[4-(2-imidazolinyl)phenoxymethyl]benzene dihydrochloride (**10**)*

Yield, 0.99 g (81%): mp 185–190 °C; ^{1}H NMR δ 10.67 (s, 4H), 8.07 (dd, J = 8.7 and 2.1 Hz, 4H), 7.56 (m, 2H), 7.41 (m, 2H), 7.26 (dd, J = 8.7 and 2.1 Hz, 4H), 5.38 (s, 4H), 3.95 (s, 8H); MS m/z 427 (MH^+ of free base); HPLC t_R 7.39 min (96.02 area % at 254 nM). Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2HCl \cdot 0.7H_2O$: C, 60.99; H, 5.79; N, 10.94; Cl, 13.83. Found: C, 60.94; H, 5.75; N, 10.91; Cl, 13.98.

*5.2.5. 1,2-Bis (3-amidinophenoxymethyl)benzene dihydrochloride (**11**)*

Yield, 0.19 g (14%): mp 238–240 °C.; ^{1}H NMR δ 9.48 (br s, 4H), 9.13 (br s, 4H), 7.61 (m, 2H), 7.57 (m, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.41 (m, 6H); MS m/z 375 (MH^+ of free base); HPLC t_R 6.53 min (97 area % at 230 nM). Anal. Calcd for $C_{22}H_{22}N_4O_2 \cdot 2HCl \cdot 0.7H_2O$: C, 57.45; H, 5.57; N, 12.18. Found: C, 57.42; H, 5.63; N, 12.00.

*5.2.6. 1,2-Bis[3-(*N*-isopropylamidino)phenoxymethyl]benzene dihydrochloride (**12**)*

Yield, 0.35 g (24%): mp indef.; ^{1}H NMR δ 9.68 (d, J = 7.7 Hz, 2H), 9.54 (br s, 2H), 9.18 (br s, 2H), 7.58 (m, 2H), 7.42 (m, 4H), 7.36 (m, 6H), 5.41 (s, 4H), 4.07 (m, 2H), 1.27 (d, J = 6.3 Hz, 12H); MS m/z 459 (MH^+ of free base); HPLC t_R 8.00 min (100 area % at 230 nM). Anal. Calcd for $C_{28}H_{34}N_4O_2 \cdot 2HCl \cdot 1.3H_2O$: C, 60.60; H, 7.01; N, 10.10; Cl, 12.78. Found: C, 60.31; H, 6.97; N, 10.25; Cl, 12.13.

*5.2.7. 1,2-Bis[3-(2-imidazolinyl)phenoxymethyl]benzene dihydrochloride (**13**)*

Yield, 0.77 g (65%): mp 289 °C; ^{1}H NMR δ 10.67 (s, 4H), 7.94 (br s, 2H), 7.57 (m, 6H), 7.42 (m, 4H), 5.41 (s, 4H), 4.00 (s, 8H);MS m/z 427 (MH^+ of free base); HPLC t_R 7.23 min (100 area % at 254 nM). Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2HCl \cdot 1.4H_2O$: C, 59.52; H, 5.92; N, 10.68; Cl, 13.51. Found: C, 59.46; H, 5.92; N, 10.63; Cl, 13.63.

*5.2.8. 1,4-Bis[4-(*N*-isopropylamidino)phenoxymethyl]benzene dihydrochloride (**15**)*

Yield, 0.95 g (36%): mp 318 °C dec.; ^{1}H NMR δ 9.42 (d, J = 8.8 Hz, 2H), 9.32 (br s, 2H), 8.99 (br s, 2H), 7.73 (d, J = 8.8 Hz, 4H), 7.50 (s, 4H), 7.21 (d, J = 8.8 Hz, 4H), 5.25 (s, 4H), 4.07 (m, 2H), 1.26 (d, J = 6.3 Hz, 12H); HPLC t_R 8.20 min (97.8 area % at 254 nM). Anal. Calcd for $C_{28}H_{34}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 62.22; H, 6.90; N, 10.36; Cl, 13.12. Found: C, 62.41; H, 6.79; N, 10.28; Cl, 12.99.

*5.2.9. 1,4-Bis[4-(2-imidazolinyl)phenoxymethyl]benzene dihydrochloride (**16**)*

Yield, 0.70 g (28%): mp 268–170 °C; ^{1}H NMR δ 10.60 (s, 4H), 8.05 (d, J = 8.8 Hz, 4H), 7.51 (s, 4H), 7.28 (d, J = 8.8 Hz, 4H), 5.27 (s, 4H), 3.96 (s, 8H); HPLC t_R 7.35 min (97.5 area % at 265 nM). Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$: C, 59.32; H, 5.94; N, 10.64; Cl, 13.47. Found: C, 59.07; H, 6.02; N, 10.45; Cl, 13.19.

*5.2.10. 1,4-Bis[3-(*N*-isopropylamidino)phenoxymethyl]benzene dihydrochloride (**18**)*

Yield, 0.99 g (73%): mp 173 °C; ^{1}H NMR δ 9.60 (d, J = 7.9 Hz, 2H), 9.48 (br s, 2H), 9.19 (br s, 2H), 7.52 (s, 4H), 7.42 (s, 2H), 7.38 (m, 6H), 5.24 (s, 4H), 4.07 (m, 2H), 1.27 (d, J = 6.3 Hz, 12H); HPLC t_R 8.09 min (100 area % at 254 nM). Anal. Calcd for $C_{28}H_{34}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 62.22; H, 6.90; N, 10.36; Cl, 13.12. Found: C, 62.20; H, 6.88; N, 10.23; Cl, 12.97.

*5.2.11. 1,4-Bis[3-(2-imidazolinyl)phenoxymethyl]benzene dihydrochloride (**19**)*

Yield, 0.90 g (70%): mp 293 °C, dec; ^{1}H NMR δ 10.83 (s, 4H), 7.82 (s, 2H), 7.55 (m, 6H), 7.51 (s, 4H), 5.24 (s, 4H), 4.00 (s, 8H); HPLC t_R 7.25 min (100 area % at 254 nM). Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$: C, 59.32; H, 5.94; N, 10.64; Cl, 13.47. Found: C, 59.38; H, 6.09; N, 10.56; Cl, 13.23.

*5.2.12. 1,4-Bis[4-(*N*-isopropylamidino)phenoxymethyl]naphthalene dihydrochloride (**22**)*

Yield 0.11 g (15%): mp 294–295 °C; ^{1}H NMR δ 9.42 (d, J = 7.6 Hz, 2H), 9.32 (br s, 2H), 8.96 (br s, 2H), 8.17 (m, 2H), 7.74 (d, J = 8.8 Hz, 4H), 7.73 (s, 2H), 7.66 (m, 2H), 7.32 (d, J = 8.8 Hz, 4H), 5.71 (s, 4H), 4.04 (m, 2H), 1.27 (d, J = 6.3 Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.83 min (100 area % at 254 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot 0.8H_2O$: C, 64.49; H, 6.70; N, 9.40; Cl, 11.90. Found: C, 64.35; H, 6.66; N, 9.29; Cl, 12.07.

*5.2.13. 1,5-Bis[4-(*N*-isopropylamidino)benzyloxy]naphthalene dihydrochloride (**24**)*

Yield 0.39 g (30%): mp 304–305 °C; ^{1}H NMR δ 9.63 (d, J = 8.0 Hz, 2H), 9.49 (br s, 2H), 9.19 (br s, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.3 Hz, 4H), 7.76 (d, J = 8.3 Hz, 4H), 7.44 (t, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 5.45 (s, 4H), 4.08 (m, 2H), 1.28 (d, J = 6.4 Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.79 min (97.8 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl$: C, 66.09; H, 6.59; N, 9.63; Cl, 12.19. Found: C, 65.89; H, 6.50 N, 9.44; Cl, 12.11.

*5.2.14. 1,5-Bis[3-(*N*-isopropylamidino)benzyloxy]naphthalene dihydrochloride (**26**)*

Yield, 0.16 g (12%): mp 249–250 °C; ^{1}H NMR δ 9.71 (d, J = 7.7 Hz, 2H), 9.56 (br s, 2H), 9.25 (br s, 2H), 7.94 (s, 2H), 7.87 (m, 4H), 7.74 (d, J = 8.0 Hz, 2H), 7.67 (t, J = 7.6 Hz, 2H), 7.45 (t, J = 8.0 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 5.39 (s, 4H), 4.13 (m, 2H), 1.29 (d, J = 6.6 Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.80 min (100 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 64.10; H, 6.72; N, 9.34; Cl, 11.83. Found: C, 64.06 H, 6.66 N, 9.25; Cl, 11.87.

*5.2.15. 1,5-Bis[3-(*N*-hydroxyamidino)benzyloxy]naphthalene dihydrochloride (**27**)*

The crude imidate ester was prepared from the corresponding nitrile (1.50 g, 8.85 mmol) following the general procedure, then suspended in ethanol (10 mL). To this mixture was added a solution of hydroxylamine (50 mL of a 0.68 M ethanolic solution, prepared from hydroxylamine hydrochloride and sodium ethoxide, followed by filtration). The mixture was refluxed for 1 h and allowed to cool. The amidoxime base was precipitated by dilution with ether, then converted to the HCl salt using ethanolic HCl. Yield, 0.31 g (15%): mp > 200 °C (dec.); ^{1}H NMR δ 11.27 (s, 1H), 9.05 (2, 2H), 7.93 (s, 2H), 7.87 (m, 4H), 7.74 (d, J = 8.0 Hz, 2H), 7.67 (t, J = 7.7 Hz, 2H), 7.45 (t, J = 8.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 5.38 (s, 4H); MS m/z 457 (MH^+ of free base); HPLC t_R 3.68 min (97.3% at 254 nM). Anal. Calcd for $C_{26}H_{24}N_4O_4 \cdot 2HCl \cdot 0.6H_2O \cdot 0.3C_2H_5OH$: C, 57.66; H, 5.28; N, 10.11; Cl, 12.80. Found: C, 57.68; H, 5.04; N, 10.13; Cl, 12.57.

*5.2.16. 1,6-Bis[4-(*N*-isopropylamidino)benzyloxy]naphthalene dihydrochloride (**28**)*

Yield, 1.08 g (61%): mp 218–220 °C; ^{1}H NMR δ 9.61 (d, J = 8.4 Hz, 2H), 9.48 (br s, 2H), 9.16 (br s, 2H), 8.15 (d, J = 9.2 Hz,

1H), 7.75 (m, 8H), 7.41 (d, $J = 2.5$ Hz, 1H), 7.37 (d, $J = 4.4$ Hz, 2H), 7.28 (dd, $J = 9.2$ and 2.4 Hz, 1H), 6.92 (t, $J = 4.3$ Hz, 1H), 5.43 (s, 2H), 5.37 (s, 2H), 4.07 (m, 2H), 1.27 (d, $J = 6.2$ Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.84 min (100 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot 1.6H_2O$: C, 62.97; H, 6.80; N, 9.18; Cl, 11.62. Found: C, 63.01; H, 6.70; N, 9.14; Cl, 11.74.

5.2.17. 2,6-Bis[3-(N-*isopropylamidino*)benzyloxy]naphthalene dihydrochloride (**31**)

Yield, 0.24 g (24%): mp 194–197 °C; ^1H NMR δ 9.67 (d, $J = 8.5$ Hz, 2H), 9.50 (br s, 2H), 9.14 (br s, 2H), 7.83 (m, 6H), 7.68 (m, 4H), 7.45 (d, $J = 2.4$ Hz, 2H), 7.25 (dd, $J = 8.9$ and 2.4 Hz, 2H), 5.28 (s, 4H), 4.07 (m, 2H), 1.28 (d, $J = 6.4$ Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.72 min (97.1 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 64.10; H, 6.72; N, 9.34; Cl, 11.83. Found: C, 64.17; H, 6.69; N, 9.20; Cl, 11.61.

5.2.18. 2,6-Bis[3-(N-sec-*butylamidino*)benzyloxy]naphthalene dihydrochloride (**32**)

Yield, 0.20 g (19%): mp 258–259 °C; ^1H NMR δ 9.65 (br s, 2H), 9.53 (br s, 2H), 9.20 (br s, 2H), 7.88 (m, 2H), 7.85 (d, $J = 7.9$ Hz, 2H), 7.79 (dd, $J = 9.0$ and 1.9 Hz, 2H), 7.72 (dm, $J = 7.7$ Hz, 2H), 7.66 (t, $J = 7.6$ Hz, 2H), 7.45 (s, 2H), 7.25 (dd, $J = 9.0$ and 1.9 Hz), 5.28 (s, 4H), 3.89 (m, 2H), 1.65 (m, 4H), 1.25 (d, $J = 6.3$ Hz, 6H), 0.94 (t, $J = 7.3$ Hz, 6H); MS m/z 537 (MH^+ of free base); HPLC t_R 9.31 min (100 area % at 230 nM). Anal. Calcd for $C_{34}H_{40}N_4O_2 \cdot 2HCl \cdot 1.2H_2O$: C, 64.69; H, 7.09; N, 8.88. Found: C, 64.47; H, 6.86; N, 8.92.

5.2.19. 2,7-Bis[4-(N-*isopropylamidino*)benzyloxy]naphthalene dihydrochloride (**34**)

Yield, 1.23 g (52%): mp 236–238 °C; ^1H NMR δ 9.60 (d, $J = 8.0$ Hz, 2H), 9.47 (br s, 2H), 9.14 (br s, 2H), 7.78 (d, $J = 9.1$ Hz, 2H), 7.77 (d, $J = 8.4$ Hz, 4H), 7.71 (d, $J = 8.4$ Hz, 4H), 7.29 (d, $J = 2.3$ Hz, 2H), 7.11 (dd, $J = 8.8$ and 2.3 Hz, 2H), 5.34 (s, 4H), 4.06 (m, 2H), 1.27 (d, $J = 6.4$ Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.94 min (100 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 65.08; H, 6.66; N, 9.29; Cl, 12.01. Found: C, 65.25; H, 6.48; N, 9.29; Cl, 11.83.

5.2.20. 2,7-Bis[(3-amidino)benzyloxy]naphthalene dihydrochloride (**35**)

Yield, 0.54 g (64%): mp 265–268 °C; ^1H NMR δ 9.48 (br s, 4H), 9.22 (br s, 4H), 8.01 (s, 2H), 7.83 (m, 6H), 7.67 (t, $J = 7.7$ Hz, 2H), 7.39 (d, $J = 2.2$ Hz, 2H), 7.11 (dd, $J = 8.9$ and 2.3 Hz, 2H), 5.30 (s, 4H); MS m/z 425 (MH^+ of free base); HPLC t_R 7.86 min (100 area % at 230 nM). Anal. Calcd for $C_{26}H_{24}N_4O_2 \cdot 2HCl \cdot 0.7H_2O$: C, 61.23; H, 5.41; N, 10.98; Cl, 13.90. Found: C, 61.36; H, 5.47; N, 10.91; Cl, 13.80.

5.2.21. 2,7-Bis[3-(N-*isopropylamidino*)benzyloxy]naphthalene dihydrochloride (**36**)

Yield, 0.53 g (54%): mp indef.; ^1H NMR δ 9.71 (d, $J = 8.1$ Hz, 2H), 9.55 (br s, 2H), 9.20 (s, 2H), 7.90 (s, 2H), 7.84 (d, $J = 7.6$ Hz, 2H), 7.79 (d, $J = 8.9$ Hz, 2H), 7.72 (d, $J = 7.7$ Hz, 2H), 7.65 (t, J = 7.7 Hz, 2H), 7.41 (d, $J = 2.2$ Hz, 2H), 7.11 (dd, $J = 8.8$ and 1.9 Hz, 2H), 5.29 (s, 4H), 4.10 (m, 2H), 1.29 (d, $J = 6.3$ Hz, 12H); MS m/z 509 (MH^+ of free base); HPLC t_R 8.89 min (97.5 area % at 230 nM). Anal. Calcd for $C_{32}H_{36}N_4O_2 \cdot 2HCl \cdot 0.9H_2O$: C, 64.29; H, 6.71; N, 9.37; Cl, 11.86. Found: C, 64.18; H, 6.76; N, 9.37; Cl, 12.04.

5.2.22. 2,7-Bis[3-(N-sec-*butylamidino*)benzyloxy]naphthalene dihydrochloride (**37**)

Yield, 0.25 g (24%): mp 187–194 °C (dec.); ^1H NMR δ 9.66 (d, $J = 8.1$ Hz, 1.8H), 9.52 (br s, 2H), 9.19 (br s, 2H), 7.89 (s, 2H), 7.85 (d, $J = 7.5$ Hz, 2H), 7.80 (d, $J = 9.1$ Hz, 2H), 7.72 (d, $J = 7.5$ Hz, 2H), 7.66 (t, $J = 7.5$ Hz, 2H), 7.41 (s, 2H), 7.11 (dd, $J = 8.6$ and 2.0 Hz, 2H), 5.30 (s, 4H), 3.90 (m, 2H), 1.68 (m, 4H), 1.25 (d, $J = 6.3$ Hz, 6H), 0.94 (t, $J = 6.3$ Hz, 6H); MS m/z 537 (MH^+ of free base); HPLC t_R 9.44 min (100 area % at 230 nM). Anal. Calcd. for $C_{34}H_{40}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 65.25; H, 7.05; N, 8.95. Found: C, 65.16; H, 6.99; 8.86.

5.3. 1,3-Bis[4-(N-*methoxyamidino*)phenoxymethyl]benzene dihydrochloride (**5**)

Dimethyl sulfate (1.18 g, 9.39 mmol) was added dropwise to a solution of 1,3-bis[(4-{*N*-hydroxy}amidino)phenoxymethyl]benzene (**4**) [29] in DMSO (30 mL) and 2 M NaOH (10 mL) at 0 °C. After removal of the ice bath, the mixture was stirred at ambient temperature for 5 h and diluted with ice water (300 mL). The resulting precipitate was filtered off, washed with water (100 mL) and dried overnight. The crude product was purified by flash chromatography (EtOAc/hexanes [2:3]), followed by recrystallization from EtOAc/hexanes to give 0.80 g (59%) of free base. The product was recrystallized from EtOH/1 M HCl to give 0.89 g of dihydrochloride (**5**): mp 218–220 °C; ^1H NMR δ 9.05 (br s, 4H), 7.79 (d, $J = 9.0$ Hz, 4H), 7.58 (s, 1H), 7.45 (s, 3H), 7.21 (d, $J = 9.0$, 4H), 5.25 (s, 4H), 3.85 (s, 6H); HPLC t_R 10.31 min (100 area % at 254 nM). Anal. Calcd for $C_{24}H_{26}N_4O_4 \cdot 2HCl \cdot 0.4H_2O$: C, 56.01; H, 5.64; N, 10.89; Cl, 13.78. Found: C, 56.11; H, 5.51; N, 10.81; Cl, 13.61.

5.4. 1,3-Bis[3-(N-*hydroxyamidino*)phenoxymethyl]benzene dihydrochloride (**7**)

Potassium *tert*-butoxide (7.80 g, 69.5 mmol) was added to a solution of hydroxylamine hydrochloride (5.40 g, 77.7 mmol) in DMSO (100 mL). The mixture was stirred at ambient temperature for 1 h prior to the addition of 1,3-bis(3-cyanophenoxymethyl)benzene (3.75 g, 11.0 mmol). The mixture was stirred at ambient temperature overnight and poured over ice water. The resulting precipitate was filtered off and dried to give an off-white solid as the free base (4.28 g, 96% crude). ^1H NMR δ 9.64 (s, 2H), 7.56 (s, 1H), 7.43 (s, 3H), 7.33 (s, 2H), 7.29 (s, 2H), 7.28 (s, 2H), 7.03 (m, 2H), 5.82 (s, 4H), 5.15 (s, 4H). An aliquot of the free base (1.02 g, 2.50 mmol) was suspended in warm ethanol (20 mL) and treated with concentrated ethanolic HCl (10 mL). The suspension was heated and water (5 mL) was added until solids dissolved. Upon cooling, white crystals precipitated as the dihydrochloride salt (0.88 g, 74%): mp 208–210 °C (dec.): ^1H NMR δ 11.15 (br s, 1.1H), 8.94 (br s, 2.5H), 7.53 (br s, 1H), 7.49 (dd, $J = 8.0$ and 8.0 Hz, 2H), 7.43 (m, 3H), 7.39 (m, 2H), 7.30 (dd, $J = 8.0$ and 8.0 Hz, 4H), 5.19 (s, 4H); MS m/z 407 (MH^+ of free base), HPLC t_R 7.88 min (100 area % at 254 nM). Anal. Calcd for $C_{22}H_{22}N_4O_4 \cdot 2HCl$: C, 55.12; H, 5.05; N, 11.69; Cl, 14.79. Found: C, 55.40; H, 5.03; N, 11.66; Cl, 14.72.

5.5. General method for nitrile precursors to compounds **23**–**37**

A solution of the α-bromo-tolunitrile in DMF was added to a mixture of the naphthalenediol and K_2CO_3 or Cs_2CO_3 in DMF under N_2 at 90–120 °C. The reaction mixture was maintained until starting materials were no longer detectable. The cooled reaction mixture was paritioned between water and CH_2Cl_2. The product was isolated by column chromatography eluting with CH_2Cl_2 or $CHCl_3$ followed by recrystallization from an appropriate solvent.

5.5.1. 1,6-Bis[(4-cyano)benzyloxy]naphthalene

Yield, 6.22 g (64%): mp 188–189 °C; ^1H NMR ($CDCl_3$) δ 8.26 (d, $J = 9.4$ Hz, 1H), 7.72 (d, $J = 8.3$ Hz, 2H), 7.71 (d, $J = 8.3$ Hz, 2H), 7.62 (t, $J = 8.3$ Hz, 4H), 7.35 (s, 1H), 7.33 (d, $J = 1.6$ Hz, 1H), 7.24 (dd, $J = 9.1$ and 2.5 Hz, 1H), 7.16 (d, $J = 2.4$ Hz, 1H), 6.72 (m, 1H), 5.30 (s, 2H), 5.26 (s, 2H). Anal. Calcd for $C_{26}H_{18}N_2O_2$: C, 79.98; H, 4.65; N, 7.17. Found: C, 80.01; H, 4.72; N, 7.09.

5.5.2. 2,7-Bis[(3-cyano)benzyloxy]naphthalene

Yield, 6.02 g (62%): mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 2H), 7.72 (d, $J = 8.4$ Hz, 4H), 7.63 (dt, $J = 7.8$ and 2.6 Hz, 2H) 7.52 (t, $J = 7.7$ Hz, 2H), 7.10 (m, 4H), 5.19 (s, 4H). Anal. Calcd for $C_{26}H_{18}N_2O_2$: C, 79.98; H, 4.65; N, 7.17. Found: C, 80.09; H, 4.68; N, 7.25.

5.6. Biological assays

In vitro antitrypanosomal [36–39], antiplasmodial [36,40], antileishmanial [41,42], and cytotoxic [43] assays, and in vivo antitrypanosomal assays [26,36,37] were performed following established protocols.

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References

[1] African trypanosomiasis (sleeping sickness), http://www.who.int/mediacentre/factsheets/fs259/en/print.html.
[2] Malaria, http://www.who.int/nediacentre/factsheets/fs094/en/print.html.
[3] P.P. Simmarro, J. Jannin, P. Cattand, PLoS Med. 5 (2008) 174–180.
[4] Anonymous, Wkly. Epidemiol. Rec. 81 (2006) 71–80.
[5] P.B. Boland, Drug resistance in malaria, 2001. http://www.who.int/csr/resources/publications/drugresist/malaria.pdf.
[6] P. Escobar, S. Matu, C. Marques, S.L. Croft, Acta Trop. 81 (2002) 151.
[7] C.E. Stephens, R. Brun, M.M. Salem, K.A. Werbovetz, F. Tanious, W.D. Wilson, D.W. Boykin, Bioorg. Med. Chem. Lett. 13 (2003) 2065–2069.
[8] Leishmaniasis, www.who.int/topics/leishmaniasis/en.
[9] D. Wolday, N. Berhe, H. Akuffo, P. Desjus, S. Britton, Med. Microbiol. Immunol. 190 (2001) 65–67.
[10] A. Rabello, M. Orsini, J. Disch, Ann. Trop. Med. Parasitol. 97 (Suppl. 1) (2003) 17–28.
[11] V. Pintado, R. Lopez-velez, Clin. Microbiol. Infect. 7 (2003) 291–300.
[12] P. Desjeux, J. Alvar, Ann. Trop. Med. Parasitol. 97 (Suppl. 1) (2003) 3–15.
[13] I. Cruz, J. Nieto, J. Moreno, C. Canavate, P. Desjeux, J. Alvar, Indian J. Med. Res. 123 (2006) 357–388.
[14] S.M. Bakunova, S.A. Bakunov, T. Wenzler, T. Barszcz, K.A. Werbovetz, R. Brun, J.E. Hall, R.R. Tidwell, J. Med. Chem. 50 (2007) 5807–5823.
[15] D. Legros, G. Ollivier, M. Gasteullu-Etchegorry, C. Paquet, C. Burri, J. Jannin, P. Buscher, Lancet Infect. Dis. 2 (2002) 437–440.
[16] S. Croft, M. Barrett, J. Urbina, Trends Parasitol. 21 (2005) 508–512.
[17] B. Greenwood, T. Mutabingwa, Nature 415 (2002) 670–672.
[18] C.J. Woodrow, S. Krishna, Cell. Mol. Life Sci. 63 (2006) 1586–1596.
[19] G.A. Biagini, P.M. O'Neill, P.B. Bray, S.A. Ward, Curr. Opin. Pharmacol. 5 (2005) 473–478.
[20] D.A. Patrick, S.A. Bakunov, S.M. Bakunova, E.V.K.S. Kumar, R.J. Lombardy, S.K. Jones, A.S. Bridges, O. Zhirnov, J.E. Hall, T. Wenzler, R. Brun, R.R. Tidwell, J. Med. Chem. 50 (2007) 2468–2485.
[21] H.W. Murray, J.D. Berman, C.R. Davies, N.G. Saravia, Lancet 366 (2005) 1561–1577.
[22] D.A. Patrick, J.E. Hall, B.C. Bender, D.R. McCurdy, W.D. Wilson, F.A. Tanious, S. Saha, R.R. Tidwell, Eur. J. Med. Chem. 34 (1999) 575–583.
[23] J.E. Hall, J.E. Kerrigan, K. Ramachandran, B.C. Bender, J.P. Stanko, S.K. Jones, D.A. Patrick, R.R. Tidwell, Antimicrob. Agents Chemother. 42 (1998) 666–674.
[24] C. Yeates, IDrugs 6 (2003) 1086–1093.
[25] P. Yeramian, S.R. Meshnick, S. Krudsood, K. Chalermrut, U. Silachamroon, N. Tangpukdee, J. Allen, R. Brun, J.J. Kwiek, R. Tidwell, S. Looareesuwan, J. Infect. Dis. 192 (2005) 319–322.
[26] J.K. Thuita, S.M. Karanja, T. Wenzler, R.E. Mdachi, J.M. Ngotho, J.M. Kagira, R. Tidwell, R. Brun, Acta. Trop. 108 (2008) 6–10.
[27] J.D. Geratz, M.C.-F. Cheng, R.R. Tidwell, J. Med. Chem. 19 (1976) 634–639.
[28] J.J. Vanden Eynde, A. Mayence, T.L. Huang, M.S. Collins, S. Rebholz, P.D. Walzer, M.T. Cushion, Bioorg. Med. Chem. Lett. 14 (2004) 4545–4548.
[29] P.M.S. Chauhan, R.N. Iyer, D.S. Bhakuni, V. Shankhdhar, P.Y. Guru, A.B. Sen, Indian J. Chem., Sect. B 27 (1988) 38–42.
[30] S. Hamano, T. Kanazawa, S. Kitamura, Amidino Compounds. Jpn. Kokai Tokkyo Koho (1976) 8.
[31] J.N. Ashley, H.J. Barber, A.J. Ewins, G. Newbery, A.D.H. Self, J. Chem. Soc. (1942) 103–116.
[32] A.C. Rosypal, J.E. Hall, S.M. Bakunova, S.A. Bakunov, D.A. Patrick, C.E. Stephens, A. Kumar, D.W. Boykin, R.R. Tidwell, Vet. Parasitol. 145 (2007) 207–216.
[33] M.W. Haenel, Chem. Ber. 115 (1982) 1425–1436.
[34] R.H. Mitchell, R.J. Carruthers, L. Mazuch, T.W. Dingle, J. Am. Chem. Soc. 104 (1982) 2544–2551.
[35] M. Anbazhagan, J.Y. Saulter, J.E. Hall, D.W. Boykin, Heterocycles 60 (2003) 1133–1145.
[36] F. Rodriguez, I. Rozas, M. Kaiser, R. Brun, B. Nguyen, W.D. Wilson, R.N. Garcia, C. Daronville, J. Med. Chem. 51 (2008) 909–923.
[37] M.A. Ismail, R. Brun, J.D. Easterbrook, F.A. Tanious, W.D. Wilson, D.W. Boykin, J. Med. Chem. 46 (2003) 4761–4769.
[38] B. Raz, M. Iten, Y. Grether-Buhler, R. Kaminsky, R. Brun, Acta Trop. 68 (1997) 139–147.
[39] T. Baltz, D. Baltz, C. Giroud, J. Crockett, EMBO J. 4 (1985) 1273–1277.
[40] H. Matile, J.R.L. Pink, in: I. Lefkovits, B. Pernis (Eds.), Immunological Methods, Academic Press, San Diego, 1990, pp. 221–234.
[41] K.A. Werbovetz, D. Sackett, D. Delfin, G. Bhattacharya, M. Salem, T. Obrzut, D. Rattendi, C. Bacchi, Mol. Pharmacol. 64 (2003) 1325–1333.
[42] K.A. Werbovetz, J. Brendle, D. Sackett, Mol. Biochem. Parasitol. 98 (1999) 53–65.
[43] N.R. Sperandeo, R. Brun, Chem. Biochem. 4 (2003) 69–73.
[44] R. Kaminsky, C. Schmid, R. Brun, In Vitro Toxicol. 9 (1996) 315–324.
[45] I.O. Donkor, T.L. Huang, B. Tao, D. Rattendi, S. Lane, M. Vargas, B. Goldberg, C. Bacchi, J. Med. Chem. 46 (2003) 1041–1048.
[46] I.O. Donkor, H. Assefa, D. Rattendi, S. Lane, M. Vargas, B. Goldberg, C. Bacchi, Eur. J. Med. Chem. 36 (2001) 531–538.