Structure−Activity Study of Pentamidine Analogues as Antiprotozoal Agents

Svetlana M. Bakunova,[†] Stanislav A. Bakunov,[†] Donald A. Patrick,[†] E. V. K. Suresh Kumar,[†] Kwasi A. Ohemeng,[†] Arlene S. Bridges,[†] Tanja Wenzler,[‡] Todd Barszcz,[§] Susan Kilgore Jones,[†] Karl A. Werbovetz,[§] Reto Brun,[‡] and Richard R. Tidwell*,[†]

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, CH-4002 Basel, Switzerland

Received December 8, 2008

Diamidine **1** (pentamidine) and 65 analogues (**2**−**66**) have been tested for in vitro antiprotozoal activities against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani*, and for cytotoxicity against mammalian cells. Dications **32**, **64**, and **66** exhibited antitrypanosomal potencies equal or greater than melarsoprol ($IC_{50} = 4$ nM). Nine congeners (**2**−**4**, **12**, **27**, **30**, and **64**−**66**) were more active against *P. falciparum* than artemisinin ($IC_{50} = 6$ nM). Eight compounds (**12**, **32**, **33**, **44**, **59**, **62**, **64**, and **66**) exhibited equal or better antileishmanial activities than **1** ($IC_{50} = 1.8$ μM). Several congeners were more active than **1** in vivo, curing at least 2/4 infected animals in the acute mouse model of trypanosomiasis. The diimidazoline **66** was the most promising compound in the series, showing excellent in vitro activities and high selectivities against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* combined with high antitrypanosomal efficacy in vivo.

Introduction

Throughout history, protozoan infections transmitted by insects accompanied mankind, causing human suffering and death. Malaria, human African trypanosomiasis (HAT[a] or sleeping sickness), and leishmaniasis are major protozoan diseases that are prevalent in subtropical countries. Today, malaria remains one of the leading causes of morbidity and mortality in the world.[1−5] The majority of those deaths occurs in sub-Saharan Africa and is the result of the most severe and life-threatening form of disease caused by *Plasmodium falciparum*.[4−6] Poverty and nutrient deficiency, wars and civil disturbances, environmental and climatic changes, population growth in malaria-endemic areas and traveling to these regions are the factors contributing to the increased incidence of malaria. Devastating epidemics of HAT depopulated many areas in Africa at the beginning of the twentieth century.[7] Nowadays, sleeping sickness, which is typically fatal without treatment, is endemic in approximately 20 sub-Saharan countries.[8−11] The clinical manifestations of leishmaniasis depend on the species of infecting *Leishmania* parasites and can range from self-healing cutaneous lesions to life-threatening visceral infections.[12−16] While the vast majority of leishmanial infections occur in the developing world, leishmaniasis now exerts an increasing effect on developed nations as well.[17] In addition, the overlap of human immunodeficiency virus (HIV) with parasitic infections[18−22] results in growing number of cases of dually infected individuals in different parts of the globe.[23−28]

Advances in antiprotozoal chemotherapy in the middle of the twentieth century helped to limit and control the spread of parasitic infections, keeping the situation stable for decades. Recently a growing problem of treatment failures due to developing of resistance to common antiprotozoal drugs has emerged around the world.[29−39] Development of resistance to the most commonly used antimalarial drugs, such as chloroquine and sulfadoxine-pyrimethamine, represents a major obstacle in controlling malaria. To slow the emergence of resistance, many affected countries now rely on therapies that are more expensive or the use of drug combinations (particularly with artemisinins),[29,31,32,34] which increases the economic burden of the disease. Current treatments for HAT and leishmaniasis require a long course of parenteral administration and suffer from unacceptable toxicity and poor efficacy.[8,11,16,40,41] Unresponsiveness to these medications and growing number of treatment failures is becoming more prevalent because of development of parasite resistance, although new options for treatment of visceral leishmaniasis have recently become available.[42,43] Unfortunately, many drug formulations showing promise in treating parasitic infections are too costly for routine chemotherapy in the developing world. Therefore, there is a need for safe and affordable antiprotozoal treatments capable of overcoming parasite resistance.

1,5-Bis(4-amidinophenoxy)pentane (pentamidine)[44] has been used for more than half a century against early stage *Trypanosoma brucei gambiense* related HAT,[11,36,45] antimony resistant leishmaniasis caused by *Leishmania donovani*,[14,15,38] and HIV related pneumonia caused by the opportunistic pathogen *Pneumocystis jirovec*i (formerly *Pneumocystis carinii*).[46−48] In early studies, compound **1** (pentamidine) also displayed a curative effect on *Plasmodium* infected monkeys[49] and exhibited in vitro activities against selected strains of the pathogen,[49−51] but availability of other effective drugs hindered its antimalarial development. At physiological pH, both amidine groups in the dication **1** are positively charged, thus decreasing membrane permeability of the drug and diminishing its oral activity.[36,52] Consequently, compound **1** requires parenteral administration, which makes the treatment less practical, especially in remote areas where most cases of HAT and leishmaniasis take place. It is fairly well tolerated by patients although some adverse

---

* To whom correspondence should be addressed. Phone: (919) 966-4294. Fax: (919) 966-0704. E-mail: Tidwell@med.unc.edu.

[†] The University of North Carolina.

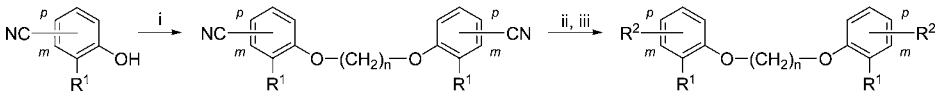
[‡] Swiss Tropical Institute.

[§] The Ohio State University.

[a] Abbreviations: HAT, human African trypanosomiasis; WHO, World Health Organization; HIV, human immunodeficiency virus; MAP, melarsoprol; CQ, chloroquine; ATM, artemisinin; PPT, podophyllotoxin.

10.1021/jm801547t CCC: $40.75

Scheme 1[a]



R^1 = H, Cl, OMe

67 n = 3, *p*-CN, R^1 = H
68 n = 4, *p*-CN, R^1 = H
69 n = 5, *p*-CN, R^1 = H
70 n = 6, *p*-CN, R^1 = H
71 n = 3, *m*-CN, R^1 = H
72 n = 4, *m*-CN, R^1 = H
73 n = 5, *m*-CN, R^1 = H
74 n = 6, *m*-CN, R^1 = H
75 n = 3, *p*-CN, R^1 = Cl
76 n = 3, *p*-CN, R^1 = OMe
77 n = 5, *p*-CN, R^1 = OMe

67 $\xrightarrow{iv}$ **78** n = 3, *p*-CN, R^1 = NO_2
68 $\xrightarrow{v}$ **79** n = 4, *p*-CN, R^1 = NO_2
69 $\xrightarrow{v}$ **80** n = 5, *p*-CN, R^1 = NO_2

1 n = 5, R^1 = H, R^2 = *p*-Am
2 n = 5, R^1 = H, R^2 = *p*-MeAm
3 n = 5, R^1 = H, R^2 = *p*-*i*-PrAm
9 n = 5, R^1 = H, R^2 = *p*-Am-NH_2
13 n = 3, R^1 = H, R^2 = *p*-*i*-PrAm
20 n = 3, R^1 = H, R^2 = *m*-Am
21 n = 3, R^1 = H, R^2 = *m*-*i*-PrAm
22 n = 3, R^1 = H, R^2 = *m*-Im
23 n = 4, R^1 = H, R^2 = *m*-Am
24 n = 4, R^1 = H, R^2 = *m*-*i*-PrAm
25 n = 4, R^1 = H, R^2 = *m*-Im
26 n = 5, R^1 = H, R^2 = *m*-Am
27 n = 5, R^1 = H, R^2 = *m*-*i*-PrAm
28 n = 5, R^1 = H, R^2 = *m*-Im
29 n = 6, R^1 = H, R^2 = *m*-Am
30 n = 6, R^1 = H, R^2 = *m*-*i*-PrAm
31 n = 6, R^1 = H, R^2 = *m*-Im
32 n = 3, R^1 = Cl, R^2 = *p*-Am
34 n = 3, R^1 = NO_2, R^2 = *p*-Am
38 n = 3, R^1 = OMe, R^2 = *p*-MeAm
41 n = 4, R^1 = NO_2, R^2 = *p*-Am
44 n = 5, R^1 = NO_2, R^2 = *p*-Am
46 n = 5, R^1 = OMe, R^2 = *p*-Am
47 n = 5, R^1 = OMe, R^2 = *p*-Im

[a] Reagents and conditions: (i) dibromoalkane, K_2CO_3, DMF, reflux; (ii) HCl gas, EtOH, dioxane, 0 °C−ambient temp; (iii) appropriate amine, EtOH, ambient temp; (iv) fuming HNO_3; (v) 70% HNO_3, TFA, Ac_2O.

effects, including hypotension, abdominal pain, vertigo, hypersalivation, hypoglycemia, nausea, and mild nephrotoxicity, have been reported upon the drug administration.[52−54]

Since the discovery of the antiprotozoal properties of the diamidine **1**, a large number of structurally related congeners showing potency against a wide range of microorganisms,[55−64] including *P. jiroveci*[65−77] as well as fungi and bacteria,[78,79] have been synthesized. Aromatic diamidines also have been tested as enzyme inhibitors[80−85] and demonstrated antitumor potential.[86−90] Over the years, a wealth of data on biological activity of compounds related to **1** have been accumulated. Nevertheless, sometimes an analysis of structure−activity relationships of antiprotozoal properties of aromatic dications is difficult because compounds in a series may have been screened against either different pathogens or different strains of the same pathogens or have not been tested at all.

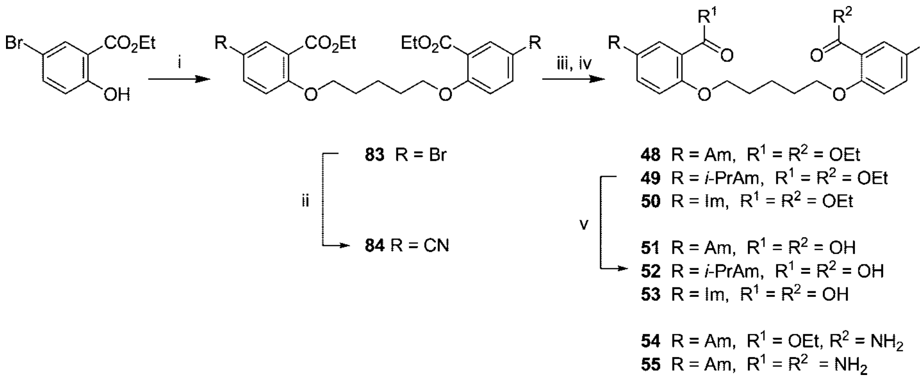
The purpose of the current study was to evaluate diamidine **1** and a series of its congeners, both newly synthesized and available from our in-house library, in the standard screens that the Consortium for Parasitic Drug Development (CPDD) uses to identify its antiprotozoal drug candidates. To analyze structure−activity relationships with respect to antiprotozoal activity, cytotoxicity, and parasite selectivity, dications **1−66** were screened in vitro against *Trypanosoma brucei rhodesiense* (STIB900), chloroquine resistant *Plasmodium falciparum* (K1), and axenic amastigotes of *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) and evaluated for cytotoxicity against rat myoblast cells (L6). Compounds exhibiting high antitrypanosomal activities in vitro underwent in vivo testing in the STIB900 acute mouse model of infection.

Chemistry. Compounds **4**,[66,91] **5**,[58,67] **11**,[92,93] **12**,[66] **14**,[50,66] **17**,[44,66] **18**,[67] **19**,[66] **33**,[83] **35**,[66] **37**,[66] **39**,[67,94] **40**,[66] **42**,[66] **43**,[67,94] and **44**[66] were prepared earlier in this laboratory for other purposes.

The previously described dications **1**,[44] **20**,[66] **22**,[67] **23**,[66] **25**,[67] **26**,[66] **29**,[66] **32**, **41**,[66] **45**,[66] **46**,[66] **47**,[67,94] and **64** as well as the newly synthesized analogues **2**, **3**, **9**, **13**, **21**, **24**, **27**, **28**, **30**, **31**, **34**, **38**, **65**, and **66** were obtained using a modified Pinner reaction.[95] In this method, the dinitrile precursors **67−80** were converted to imidate esters, which reacted with the appropriate amines at ambient temperature to give corresponding dications (Scheme 1).

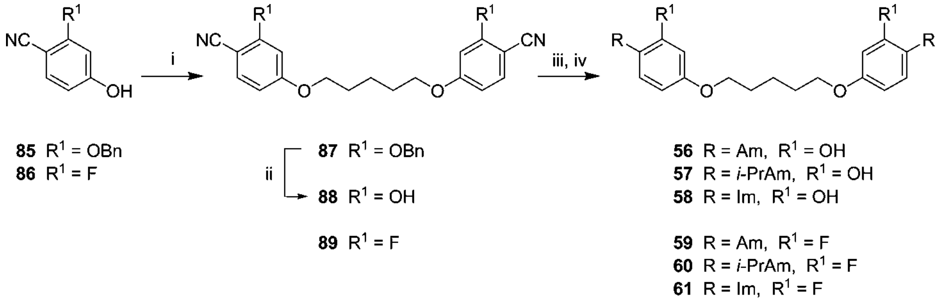
Dinitriles **67**,[44,66] **68**,[44] **69**,[44,66] **70**,[44,66] **76**,[94] and **77**[94,96] have been reported before. Novel dinitriles **71−75** were obtained from appropriate cyano phenols and dibromoalkanes by Williamson ether syntheses. Compounds **78**,[66] **79**,[66] and **80**[66] have been prepared by the nitration of the dinitriles **67**,[44,66] **68**,[44] and **69**[44,66] following the published protocol. 1,5-Bis[4-(1*H*-tetrazol-5-yl)phenoxy]pentane (**6**) was synthesized from **69** and sodium azide in the solution of acetic acid in DMF under reflux in 82% yield. The dications **7** and **8** were obtained by reaction of 1,5-bis(4-aminophenoxy)pentane[97] with two equivalents of the hydrobromide salts of 2-naphthalenylmethyl esters of benzenecarboximidothioic[98] and ethanimidothioic[99] acids in 77 and 17% yields, respectively, according to the published procedure.[98] 1-(4-Amidinophenoxy)-5-(4-aminophenoxy)pentane (**10**) was synthesized as previously described.[83] A Williamson ether synthesis involving 4-(4,5-dihydro-1*H*-imidazol-2-yl)-phenol[100] and 1-bromo-3-(4-cyanophenoxy)propane[44,96,101] afforded 4-cyanophenoxy-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenoxy]propane (**81**), which was subjected to Pinner conditions[95] to give 4-amidinophenoxy-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenoxy]propane (**15**) in 64% yield. Similarly, 4-cyanophenoxy-(4-ethoxycarbonylphenoxy)propane (**82**), prepared by reaction of commercially available ethyl 4-hydroxybenzoate and 1-bromo-3-(4-cyanophenoxy)propane[44,96,101] in 54% yield, was converted to 4-amidinophenoxy-(4-carboxyphenoxy)propane (**16**) by a two-step process involving a Pinner reaction[95] followed by acid catalyzed hydrolysis of an ester group. The synthesis of 1,3-bis[2-amino-4-(4,5-dihydro-1*H*-imidazol-2-yl)phenoxy]propane (**36**) also involved a two-step procedure. First, 1,3-bis(4-cyano-2-nitrophenoxy)propane (**78**) was converted to the corresponding 2,2′-dinitro substituted diimidazoline by a Pinner method, which then underwent catalytic hydrogenation of its

Scheme 2[a]



[a] Reagents and conditions: (i) dibromopentane, K_2CO_3, DMF, 100 °C; (ii) CuCN, DMF, reflux; (iii) HCl gas, EtOH, 1,4-dioxane, 0 °C–ambient temp; (iv) ammonia or amine, EtOH, ambient temp; (v) 10% aq HCl, 70 °C.

Scheme 3[a]



[a] Reagents and conditions: (i) dibromopentane, K_2CO_3, DMF, 100 °C; (ii) H_2, 10% Pd/C, EtOH; (iii) HCl gas, EtOH, 1,4-dioxane, 0 °C–ambient temp; (iv) appropriate amine, EtOH, ambient temp.

nitro groups to form the 2,2′-diamino substituted congener **36** in 23% overall yield.

The synthesis of compounds **48**–**55** is depicted in Scheme 2. Ethyl 5-bromo-2-hydroxybenzoate, prepared by acid-catalyzed esterification of commercially available 5-bromo-2-hydroxybenzoic acid, reacted with 1,5-dibromopentane to afford 1,5-bis(4-bromo-2-ethoxycarbonylphenoxy)pentane (**83**) in 48% yield. Compound **83** was converted to 1,5-bis(4-cyano-2-ethoxycarbonylphenoxy)pentane (**84**) by reaction with CuCN in DMF under reflux in 62% yield. An imidate ester prepared from the dinitrile **84** by modified Pinner method reacted with ammonia or appropriate amines to give dications **48**–**50**. The congeners **51**–**53** were synthesized by hydrolysis of the ester groups in compounds **48**–**50** with aqueous HCl. Compounds **54** and **55** bearing one or two amide substituents were isolated as byproducts formed during the reaction of the aforementioned imidate ester with ammonia.

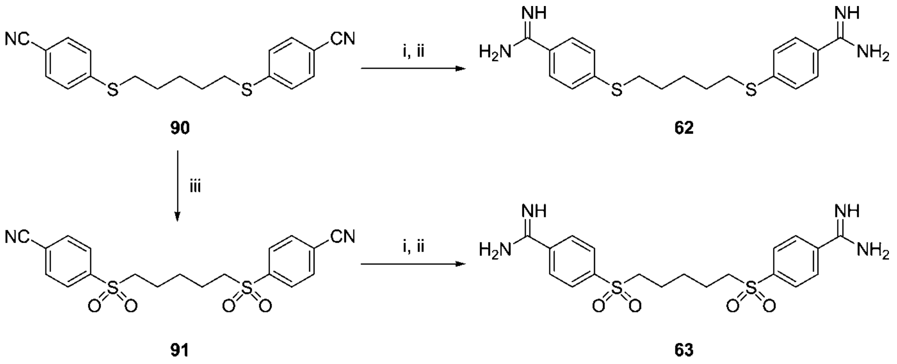
The synthesis of the 3,3′-dihydroxy and 3,3′-difluoro substituted dications **56**–**61** is shown in Scheme 3. Both 2-benzyloxy-4-hydroxybenzonitrile (**85**), prepared by diazotization of 4-amino-2-(benzyloxy)-benzonitrile[102] in 52% yield, and commercially available 2-fluoro-4-hydroxybenzonitrile (**86**) reacted with 1,5-dibromopentane to give 1,5-bis(4-cyano-3-benzyloxyphenoxy) pentane (**87**) and 1,5-bis(4-cyano-3-fluorophenoxy)pentane (**89**) in 67 and 51% yields, respectively. The protection of phenol groups in compound **87** was removed by catalytic hydrogenation over 10% palladium on charcoal to afford 1,5-bis(4-cyano-3-hydroxyphenoxy)pentane (**88**) in 97% yield. Dinitriles **88** and **89** underwent a Pinner reaction[95] followed by interaction with ammonia, *i*-propylamine, and ethylenediamine to yield the dications **56**–**58** and **59**–**61**, respectively.

The syntheses of the thio diamidine **62**[91,103] and the sulfone **63** are outlined in Scheme 4. 4-Mercaptobenzonitrile[104] reacted with 1,5-dibromopetane in the presence of potassium carbonate in DMF to give 1,5-bis[(4-cyano)phenylthio]pentane (**90**), which was oxidized with periodic acid and chromium trioxide in acetonitrile[105] to give the dinitrile sulfone **91** in 70% overall yield. The dinitriles **90** and **91** were converted to the diamidines **62** and **63** using modified Pinner methodology.[95]

Results and Discussion

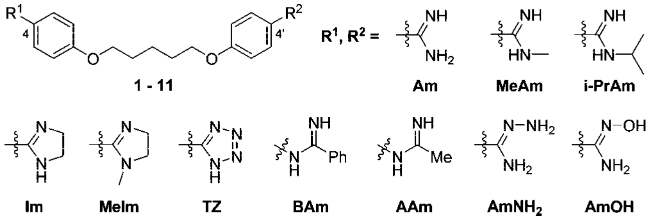
The effect of structural variations of aromatic dications on activities against *Trypanosoma*, *Plasmodium*, and *Leishmania* species have been previously investigated by others[44,91,96,97,106–111] and us.[50,112] However, a majority of these studies have been conducted with different strains of parasites and many derivatives have simply not been tested against all pathogens. Here, we report a continued investigation of structure–activity relationship of analogues related to compound **1**. Structural modifications of dications used in this study included substitution on the cationic groups, increasing the length of the aliphatic chain between the two aromatic rings from three to six carbon atoms, varying the position of attachment of the cationic substituents, introduction of substituents on the aromatic rings, and replacement of oxygen atoms in the alkyl linker with isosteric sulfur or nitrogen atoms. Diamidine **1** as a hydrochloride salt prepared in our laboratory following the published procedure,[44] and its congeners **2**–**66** were evaluated in vitro for their activity against bloodstream form trypomastigotes of *T. b. rhodesiense* (STIB900), chloroquine resistant *P. falciparum* (K1), axenic amastigotes of *L. donovani* (MHOM/SD/62/1S-$CL2_D$), and for cytotoxicity against rat myoblast cells (L6). The results of the screening are summarized in Tables 1–7 and compared to the data of melarsoprol (*T. b. rhodesiense*), chloroquine and artemisinin (*P. falciparum*), and podophyllotoxin (L6). In addition to activities, three selectivity indexes,[113]

Scheme 4[a]



[a] Reagents and conditions: (i) HCl gas, EtOH, dioxane, 0 °C−ambient temp; (ii) EtOH saturated with ammonia, ambient temp; (iii) H_5IO_6, CrO_3, MeCN.

Table 1. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **1**−**11**



compd	R1	R2	cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
			IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
1[a]	Am	Am	3.80	0.007	543	0.014	271	1.83	2
2	MeAm	MeAm	152	0.024	6333	0.004	38000	4.69	32
3	*i*-PrAm	*i*-PrAm	150	0.027	5556	0.003	50000	17.8	8
4	Im	Im	>178	0.053	>3358	0.003	>59333	2.77	>64
5	MeIm	MeIm	>176	>19.6	9	1.65	107	>100	2
6	TZ	TZ	>229	>229	1	8.31	>28	>100	2
7	BAm	BAm	3.1	0.147	21	0.201	15	>100	<1
8	AAm	AAm	>202	3.04	>66	0.048	>4208	>100	2
9	$AmNH_2$	$AmNH_2$	139	9.67	14	1.41	99	126	1
10	Am	NH_2	120	1.31	91	2.24	54	>100	1
11	Am	AmOH	11.6	0.289	40	0.234	50	55.6	<1
MAP[b]			7.78	0.004	1945				
CQ[c]			117			0.124	944		
ATM[d]			450			0.006	75000		
PPT[e]			0.01						

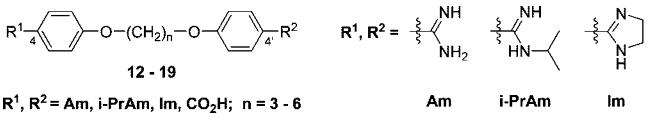
[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[113]

reflecting the inhibition of the particular parasite with respect to the L6 cells, were analyzed: antitrypanosomal selectivity index SI_T, expressed as the ratio [IC_{50} (L6-cells)/IC_{50} (*T. b. rhodesiense*)]; antiplasmodial selectivity index SI_P, expressed as the ratio [IC_{50} (L6-cells)/IC_{50} (*P. falciparum*)]; and antileishmanial selectivity index SI_L, expressed as the ratio [IC_{50} (L6-cells)/IC_{50} (*L. donovani*)]. Dications exhibiting high antitrypanosomal activities in vitro underwent in vivo screening in the STIB900 acute mouse model of trypanosomiasis (Table 8).

Structure−Activity Study. Modifications of Cationic Moieties. The antiprotozoal activities of analogues bearing various cationic substituents in the 4,4′-positions of aromatic rings are presented in Table 1. Amidine groups in compound 1[44] were replaced with (*N*-methyl)amidine (compound **2**), (*N*-isopropyl)amidine (compound **3**), imidazoline (compound **4**),[66,91] (*N*-methyl)imidazoline (compound **5**),[58,67] tetrazole (compound **6**), and amidrazone (compound **9**) motifs. Cationic substituents of congeners **7** and **8** were attached to the 1,5-diphenoxypentane motif through the nitrogen atoms on the amidine groups rather than through the carbons. In the dications **10**[83] and **11**,[92,93] one of the amidine moieties was replaced with amino or (*N*-hydroxy)imidoyl groups, respectively.

The N-substitution on the cationic fragments significantly reduced cytotoxicity of the dications **2**−**6** and **9** compared to the compound **1**, corroborating previously published results.[66,68] For example, the bis(*N*-methyl)amidine **3** was 40-fold less cytotoxic than diamidine **1**. Similarly, cytotoxicity of congeners **4**−**6** bearing cyclic cationic scaffolds decreased 46- to 60-fold compared to that of the compound **1**. Cytotoxicity of compounds **7** and **8** depended on the type of the substituents on the cationic fragments. While the phenyl substituted dication **7** exhibited cytotoxicity comparable to that of **1**, the corresponding methyl analogue **8** was 50-fold less cytotoxic than the compound **1**. Cytotoxicity of congener **11** bearing amidine and (*N*-hydroxy)-imidoyl groups was only slightly lower than that of diamidine **1**, while compound **10** with amidine and amino substituents was at least 30-fold less cytotoxic than dication **1**.

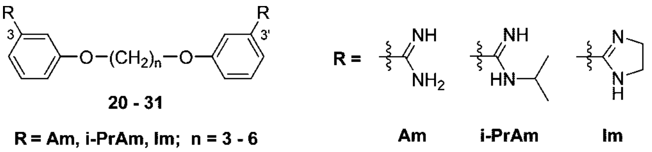
Table 2. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **12**−**19**



				cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R^1	R^2	*n*	IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
12	Am	Am	3	8.80	0.007	1257	0.006	1467	1.50	6
13	*i*-PrAm	*i*-PrAm	3	>192	0.093	2065	0.025	7680	7.11	>27
14	Im	Im	3	75.3	0.110	685	0.010	7530	288	26
15	Am	Im	3	71.6	0.018	3978	0.018	3978	2.98	24
16	Am	CO_2H	3	167	>257	0.6	>2.80	60	>100	<2
17	Am	Am	4	4.70	0.010	470	0.021	224	NT[m]	NT[m]
18	Im	Im	4	62.7	0.244	257	0.031	2023	3.06	20
1[a]	Am	Am	5	3.80	0.007	543	0.014	271	1.83	2
19	Am	Am	6	10.5	0.036	292	0.015	700	1.97	5
MAP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATM[d]				450			0.006	75000		
PPT[e]				0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[113] [m] NT, not tested.

Table 3. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **20**−**31**

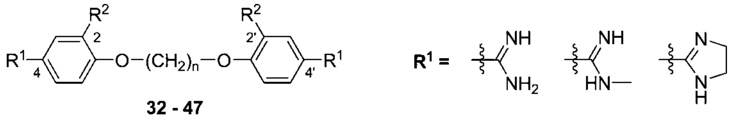


			cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R	*n*	IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
20	Am	3	16.2	0.052	312	0.017	953	6.07	3
21	*i*-PrAm	3	>183	0.543	>337	0.018	>10167	16.2	>11
22	Im	3	43.5	4.30	10	5.39	8	30.8	1
23	Am	4	16.0	0.056	286	0.021	762	4.46	4
24	*i*-PrAm	4	>171	0.619	>276	0.013	>13154	19.3	>9
25	Im	4	>185	1.94	>95	0.107	>1729	39.5	5
1[a]	Am	5	3.80	0.007	543	0.014	271	1.83	2
26	Am	5	18.4	0.065	283	0.019	968	3.19	6
27	*i*-PrAm	5	>175	0.416	421	0.004	43750	15.4	>11
28	Im	5	100	1.49	67	0.024	4167	22.4	4
29	Am	6	17.1	0.075	228	0.013	1315	2.38	7
30	*i*-PrAm	6	107	0.258	415	0.006	17833	11.6	9
31	Im	6	99.0	0.875	113	0.020	4950	21.9	5
MAP[b]			7.78	0.004	1945				
CQ[c]			117			0.124	944		
ATM[d]			450			0.006	75000		
PPT[e]			0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[113]

In this study, the N-alkylation of cationic fragments reduced the activity of congeners **2**−**11** against *T. b. rhodesiense* and *L. donovani* compared to dication **1**. Among compounds **2**−**11**, only bis(*N*-methyl)amidine **2**, bis(*N*-isopropyl)amidine **3**, and diimidazoline **4** exhibited antitrypanosomal IC_{50} values less than 100 nM and antileishmanial potencies less than 20 μM. Previous reports also demonstrated that N-substitution on the amidine groups reduced antitrypanosomal and antileishmanial activities of aromatic diamidines.[50,91,107,108,110,112,114] While diimidazoline **4** was less potent against *T. b. rhodesiense* compared to bis(*N*-methyl)amidine **2** and bis(*N*-isopropyl)amidine **3**, it exhibited greater antileishmanial activity than both congeners **2** and **3**. Derivatives **2**−**4** displayed selectivity against *T. b. rhodesiense* 6- to 12-fold greater than that of compound **1**. They were also 4 to 32 times more selective against *L. donovani* relative to **1**. Contrary to the antitrypanosomal and antileishmanial results, the N-alkyl substitution on the amidine groups improved activities of N-alkyl amidines **2** and **3** and diimidazoline **4** against *P. falciparum*. For example, dications **2**−**4** displayed antiplasmodial IC_{50} values less than that of diamidine **1** (IC_{50}

Table 4. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **32**−**47**

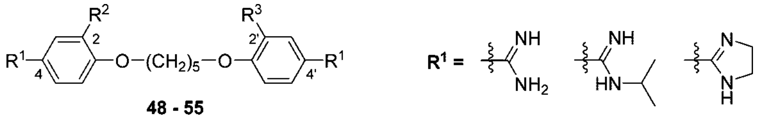


R^1 = Am, MeAm, Im; R^2 = Br, Cl, OMe, NO_2, NH_2; n = 3 - 5

				cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R^1	R^2	*n*	IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
32	Am	Cl	3	24.8	0.004	6200	0.019	1305	1.56	16
33	Am	Br	3	6.40	0.019	337	0.016	400	1.17	5
34	Am	NO_2	3	22.8	0.188	121	0.035	651	30.9	0.7
35	Am	NH_2	3	68.7	0.156	440	0.027	2544	NT[m]	NT[m]
36	Im	NH_2	3	>167	0.813	>205	0.070	2386	NT[m]	NT[m]
37	Am	OCH_3	3	114	0.023	4957	0.062	1839	6.89	17
38	MeAm	OCH_3	3	177	0.057	3105	0.641	276	25.3	7
39	Im	OCH_3	3	105	0.090	1167	0.055	1909	34.6	3
40	Am	Cl	4	10.9	0.042	260	0.019	574	2.13	5
41	Am	NO_2	4	42.8	0.181	236	0.018	2378	15.1	3
42	Am	OCH_3	4	>187	0.270	>693	0.044	>4250	38.1	>5
43	Im	OCH_3	4	>164	0.068	>2412	0.020	8200	38.9	>4
1[a]	Am	H	5	3.80	0.007	543	0.014	271	1.83	2
44	Am	Cl	5	11.2	0.044	255	0.026	431	1.45	8
45	Am	NO_2	5	37.3	0.098	381	0.024	1554	11.4	3
46	Am	OCH_3	5	162	0.010	16200	0.021	7714	24.0	7
47	Im	OCH_3	5	>158	0.040	>3950	0.019	>8316	36.3	>4
MAP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATM[d]				450			0.006	75000		
PPT[e]				0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparu*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[113] [m] NT, not tested.

Table 5. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **48**−**55**



R^1 = Am, i-PrAm, Im; R^2, R^3 = CO_2Et, CO_2H, $CONH_2$

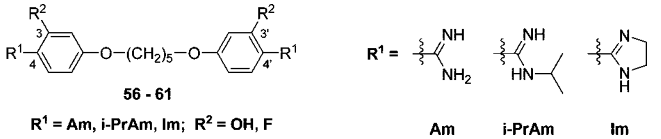
				cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R^1	R^2	R^3	IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
1[a]	Am	H	H	3.80	0.007	543	0.014	271	1.83	2
48	Am	CO_2Et	CO_2Et	>152	12.1	>13	0.021	>7238	>50	3
49	*i*-PrAm	CO_2Et	CO_2Et	>140	0.051	>2745	0.013	>10769	55.9	2
50	Im	CO_2Et	CO_2Et	>138	>15.3	9	0.027	>5111	13.6	10
51	Am	CO_2H	CO_2H	>165	>18.3	9	71.8	2	NT[m]	NT[m]
52	*i*-PrAm	CO_2H	CO_2H	>144	>16.0	9	>1.60	90	>200	0.7
53	Im	CO_2H	CO_2H	>151	>16.8	9	>1.68	90	>200	0.8
54	Am	CO_2Et	$CONH_2$	>165	0.011	>15000	0.014	>11786	52.1	3
55	Am	$CONH_2$	$CONH_2$	>168	0.045	>3733	0.038	>4421	>50	3
MAP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATM[d]				450			0.006	75000		
PPT[e]				0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[113] [m] NT, not tested.

= 14 nM) and artemisinin (ATM) (IC_{50} = 6 nM). Compounds **2**−**4** exhibited antiplasmodial selectivity indexes SI_P that were 140- to nearly 220-fold greater than that of dication **1**. These findings correlate with previously published results.[112,114]

In the case of "reversed" amidines, phenyl derivative **7** was 20-fold more active against *T. b. rhodesiense* than the corresponding methyl analogue **8**. However, because of its higher cytotoxicity, antitrypanosomal selectivity of the dication **7** was

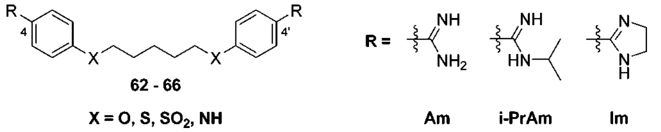
Table 6. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **56**−**61**



			cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R^1	R^2	IC$_{50}$ (μM)	IC$_{50}$ (μM)	SI$_T$[h]	IC$_{50}$ (μM)	SI$_P$[j]	IC$_{50}$ (μM)	SI$_L$[l]
1[a]	Am	H	3.80	0.007	543	0.014	271	1.83	2
56	Am	OH	>195	7.96	>24	1.29	>151	>100	2
57	*i*-PrAm	OH	>156	14.7	>11	0.581	>269	>100	2
58	Im	OH	>169	3.70	>46	0.098	>1724	>100	2
59	Am	F	6.90	0.013	531	0.015	460	1.88	4
60	*i*-PrAm	F	131	0.259	506	0.007	18714	15.0	9
61	Im	F	23.2	4.46	5	0.544	43	52.6	0.4
MAP[b]			7.78	0.004	1945				
CQ[c]			117			0.124	944		
ATM[d]			450			0.006	75000		
PPT[e]			0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI$_T$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI$_P$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI$_L$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*L. donovani*)].[113]

Table 7. In Vitro Antiprotozoal Activity and Cytotoxicity of Congeners **62**−**66**



			cytotoxicity[f]	*T. b. rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R	X	IC$_{50}$ (μM)	IC$_{50}$ (μM)	SI$_T$[h]	IC$_{50}$ (μM)	SI$_P$[j]	IC$_{50}$ (μM)	SI$_L$[l]
1[a]	Am	O	3.80	0.007	543	0.014	271	1.83	2
62	Am	S	7.80	0.005	1560	0.019	411	1.27	6
63	Am	SO$_2$	>174	28.1	>6	0.098	>1776	>100	2
64	Am	NH	0.95	<0.001	>950	0.006	158	0.502	2
65	*i*-PrAm	NH	125	0.050	2500	0.002	62500	5.75	22
66	Im	NH	34.5	0.001	34500	0.001	34500	0.254	136
MAP[b]			7.78	0.004	1945				
CQ[c]			117			0.124	944		
ATM[d]			450			0.006	75000		
PPT[e]			0.01						

[a] Pentamidine hydrochloride. [b] MAP, melarsoprol. [c] CQ, chloroquine. [d] ATM, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[128] [g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[129] [h] Selectivity index for *T. b. rhodesiense* (SI$_T$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*T. b. rhodesiense*)].[113] [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[130,131] [j] Selectivity index for P. *falciparum* (SI$_P$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*P. falciparum*)].[113] [k] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[132,133] [l] Selectivity index for *L. donovani* (SI$_L$), expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*L. donovani*)].[113]

3 times lower than that of the compound **8**. Furthermore, methyl analogue **8** displayed antimalarial potency that was 4 times superior to that of phenyl congener **7**. Antiplasmodial selectivity index SI$_P$ of the compound **8** was 15-fold greater than that of dication **1** and more than 280 times superior to that of the dication **7**. Both congeners **7** and **8** were inactive against *L. donovani*, despite the previous report in which “reversed” diamidines possessing a diphenylfuran linker exhibited excellent antileishmanial potencies.[115]

Bis(*N*-methyl)imidazoline **5**, ditetrazole **6**, diamidrazone **9**, and congener **10** were the least active compounds in the group against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. Dication **5** demonstrated lower DNA binding affinity compared to compound **1**,[116] which could explain its reduced potency toward the pathogens. Although a direct correlation between DNA binding and antiprotozoal potency for analogues of diamidine **1** has not been observed, it was concluded that a possession of $\Delta T_{\rm m}$ values above a certain threshold is required for an activity.[108,117] The reduced antiprotozoal potency of the congener **6** compared to dication **1** is probably a result of decreased basicity of tetrazole motifs, which are isosteres of carboxyl groups. Among derivatives **10** and **11** bearing a single amidine groups, dication **11** was 4 times more active against *T. b. rhodesiense*, 10 times more effective against *P. falciparum*, and 2 times more potent against *L. donovani* than compound **10**. These results can be explained by the fact that at physiological pH only the amidine group in congener **10** is protonated, although presence of at least two cationic fragments is required for pronounced antiprotozoal activity.[110,114] While being significantly less active in vitro compared to corresponding amidines,[118−121] aromatic amidoximes have been shown to be rapidly converted to amidines in vivo.[122] The use of amidoximes as prodrugs for amidines creates an opportunity for developing orally bioavailable drug candidates.[118,123−125]

Table 8. Activity of Select Dications in the STIB900 Mouse Model of Trypanosomiasis

		in vivo[b]		
compd	in vitro IC_{50} (nM)[a]	dose (mg/kg)[c]	cures[d]	survival (days)[e]
melarsoprol	4	4 × 8	4/4	>60
		4 × 2	4/4	>60
		4 × 1	2/4	>51.5
1	7	4 × 20	2/4	>44.3
		4 × 10	2/4	>43.5
2	24	4 × 20	4/4	>60.0
		4 × 5	2/4	>41.0
3	27	4 × 5	1/4	>41.3
4	53	4 × 20	0/4	>39.0
7	147	4 × 20	0/4	7.0
12	7	4 × 20	0/4	34.0
13	93	4 × 10	2/4	>38.5
14	110	4 × 20	3/4	>51.0
		4 × 10	0/4	22.8
15	18	4 × 10	1/4	>26.8
17	10	4 × 20	0/4	24.8
18	244	4 × 20	2/4	>41.5
		4 × 5	0/4	7.8
19	36	4 × 20	3/4	>51.8
		4 × 5	1/4	>44.3
20	52	4 × 20	1/4	>32.8
		4 × 10	2/4	>40.0
23	56	4 × 20	2/4	>43.0
		4 × 10	0/4	20.0
26	65	4 × 20	0/4	18.5
29	75	4 × 20	1/4	>36.0
32	4	4 × 5	4/4	>60
33	19	4 × 5	0/4	13.0
35	156	4 × 20	1/4	>40.0
37	23	4 × 20	3/3	>60
		4 × 5	2/4	>52.8
39	90	4 × 20	3/4	>60.0
		4 × 10	1/4	>32.8
40	42	4 × 20	2/4	>41.5
		4 × 10	0/4	22.0
42	270	4 × 10	0/4	17.5
43	68	4 × 10	0/4	18.3
44	44	4 × 20	0/4	29.5
46	10	4 × 5	0/4	26.0
47	40	4 × 10	0/4	>31.8
49	51	4 × 20	0/4	5.5 (toxic)
55	45	4 × 20	0/4	26.5
59	13	4 × 10	1/4	>33.0
62	5	4 × 5	0/4	32.3
64	<1	4 × 5	1/4	>29.3
65	50	4 × 5	0/4	18.0
66	1	4 × 5	4/4	>60

[a] Average of duplicate determinations.[129] [b] STIB900 acute mouse model.[134] [c] Intraperitoneal administration. [d] Number of mice that survive and are parasite free for 60 days. [e] Average days of survival; untreated controls expired between day 7 and 10 post infection.

Alteration of the Length of Carbon Linkers. Antiprotozoal activities of congeners bearing cationic substituents in the 4,4′-positions of aromatic rings and an alkyl chain of three to six carbon atoms are presented in Table 2. Previously, the nature of the linker between the cationic groups had been shown to affect the antiprotozoal properties of dications.[44,58,111,112] In the present study, there was no apparent correlation between the number of methylene groups in the linker and the cytotoxicity of compounds **12**−**19** with respect to analogues **1**, **3**, and **4**. Thus, diamidines **12**, **14**, and **19** bearing three-, four-, and six-carbon chains were less cytotoxic than compound **1**. Similarly, bis(*N*-isopropyl)amidine **13** containing a propylene bridge exhibited lower cytotoxicity compared to dication **3**. On the other hand, diimidazolines **14** and **18** with three- and four-carbon chains, respectively, were more cytotoxic than congener **4**.

Among compounds with linkers of the same length between aromatic rings, substitution on the cationic fragments decreased cytotoxicity of the dications **12**−**19**.[112] For example, bis(*N*-isopropyl)amidine **13** and diimidazoline **14** bearing a propylene bridge demonstrated cytotoxicity 22 and 9 times lower than that of diamidine **12**. Similarly, diimidazoline **18** possessing a four-carbon chain was 13 times less cytotoxic compared to dication **17**. Replacement of a single amidine group in dication **12** with an imidazoline fragment (compound **15**) caused a significant reduction in cytotoxicity. Likewise, congener **16**, bearing amidine and carboxyl substituents, was 19 times less cytotoxic than diamidine **12**.

Alterations of the length of carbon chains connecting the aromatic rings in compound **1** and its derivatives **3** and **4** reduced antiprotozoal activities and selectivities of congeners **13**−**19** against *T. b. rhodesiense*, *P. falciparum*, and, except for the bis(*N*-isopropyl)amidine **13**, against *L. donovani*. For example, dication **13** exhibited antileishmanial activity 2 times greater and was 3 times more selective against the pathogen than bis(*N*-isopropyl)amidine **3**. On the other hand, congener **12** was as active as dication **1** against *T. b. rhodesiense* and demonstrated superior potencies against *P. falciparum* and *L. donovani*, at the same time exhibiting greater selectivity to all three pathogens than compound **1**. These results correlate with the previously published data on antiprotozoal properties of select aromatic diamidines.[50]

Among compounds with carbon chains of the same length, N-substitution or replacement of the amidine groups reduced activities of the dications **12**−**19** against *T. b. rhodesiense*,[108,112] *P. falciparum*, and *L. donovani*. For example, the antitrypanosomal potencies of bis(*N*-isopropyl)amidine **13** and diimidazoline **14**, possessing a trimethylene linker, decreased 13- and 15-fold, respectively, compared to diamidine **12**. In addition, diimidazoline **18** bearing a tetramethylene bridge was 24 times less active against *T. b. rhodesiense* than dication **17**. Smaller declines in the antiplasmodial and the antileishmanial activities of the congeners **13** and **14** with respect to compound **12** were observed. Thus, bis(*N*-isopropyl)amidine **13** displayed 4 times lower activities against both *P. falciparum* and *L. donovani*, and diimidazoline **14** was half as active against the parasites as diamidine **12**. At the same time, the dications **13** and **14** were 5 times more selective against *P. falciparum* and 4 times more selective against *L. donovani* than the compound **12**. Interestingly, dication **15**, bearing amidine and imidazoline substituents, was less active against *P. falciparum* than both diamidine **12** and diimidazoline **14**. Congener **16**, bearing amidine and carboxyl substituents, exhibited almost no activity against all three pathogens used in this study.

Change of the Position of Cationic Substituents. The cytotoxicity and antiprotozoal activities of congeners bearing cationic substituents in the 3,3′-positions of aromatic rings and alkyl linkers ranging from three to six carbon atoms are presented in Table 3.

Regardless of the length of the carbon bridge, placement of the amidine groups in the 3,3′-positions reduced the cytotoxicities of diamidines **20**, **23**, **26**, and **29** relative to compound **1**. Bis(*N*-isopropyl)amidines **21**, **24**, and **27**, bearing three- to five-carbon chains were less cytotoxic than dication **3**. Further elongation of the linker between the aromatic rings in compound **30** increased its cytotoxicity relative to congener **3**. All the other derivatives, **22**, **28**, and **31**, except for the diimidazoline **25**, possessing a tetramethylene linker, were more cytotoxic than the dication **4**. Within the groups of 3,3′-substituted dications with the same number of carbon atoms in the linker, the bis(*N*-

isopropyl)amidines and diimidazolines exhibited lower cytotoxicities than diamidines.

The 3,3′-substituted diamidines **20**, **23**, **26**, and **29** exhibited lower activities against *T. b. rhodesiense* than dication **1**, as did bis(*N*-isopropyl)amidines **21**, **24**, **27**, and **30**, as well as diimidazolines **22**, **25**, **28**, and **31** compared to 4,4′-substituted bis(*N*-isopropyl)amidine **3** and diimidazoline **4**, respectively. Also, the 3,3′-substituted dications **20**−**22**, **23**, **25**, and **29** displayed lower antitrypanosomal potencies and were less selective against *T. b. rhodesiense* than the corresponding 4,4′-substituted isomers **12**−**14**, **17**, **18**, and **19**. Within groups of congeners with aliphatic linkers of the same length, the antitrypanosomal properties of 3,3′-substituted dications **20**−**31** decreased in the order Am > *i*-PrAm > Im. Elongation of the carbon chain between the aromatic rings reduced activities of the 3,3′-substituted diamidines **20**, **23**, **26**, and **29** against *T. b. rhodesiense*. On the other hand, antitrypanosomal potencies of bis(*N*-isopropyl)amidines **21**, **24**, **27**, and **30** as well as diimidazolines **22**, **25**, **28**, and **31** improved as the number of carbon atoms in the bridge increased. The 3,3′-substituted diamidines and bis(*N*-isopropyl)amidines demonstrated comparable levels of antitrypanosomal selectivity, while diimidazolines were significantly less selective against the pathogen.

Antiplasmodial properties of 3,3′-substituted diamidines **20**, **23**, and **26** connected by the three-, four-, and five-carbon chains, respectively, decreased with respect to diamidine **1**, while the analogue **29** bearing the hexamethylene linker was more active against *P. falciparum* than **1**. Contrary to the previously published results,[50] 3,3′-substituted dications **20**, **22**, **25**, and **29** were less active against *P. falciparum* than the corresponding 4,4′-substituted derivatives **12**, **14**, **18**, and **19**. At the same time, bis(*N*-isopropyl)amidine **21** exhibited greater antimalarial potency than the corresponding 4,4′-substituted analogue **13**, and diamidine **23** was as effective against the pathogen as congener **17**. The 3,3′-substituted diamidines **23**, **26**, and **29** as well as bis(*N*-isopropyl)amidine **21** were more selective for *P. falciparum* relative to the 4,4′-substituted congeners **17**, **1**, **19**, and **13**, respectively. Among the 3,3′-substituted dications bearing four-, five-, and six-carbon linkers, the bis(*N*-isopropyl)amidines exhibited the highest activity against *P. falciparum*, followed by the diamidines and diimidazolines. Antiplasmodial selectivity of these congeners decreased in the order *i*-PrAm > Im > Am. At the same time, diamidine **20** and bis(*N*-isopropyl)amidine **21**, bearing propylene bridge, displayed similar antiplasmodial potencies, while the corresponding diimidazoline **22** was inactive against the pathogen. Although activities of 3,3′-substituted diamidines **20**, **23**, **26**, and **29** against *P. falciparum* underwent only minor changes as number of carbon atoms in the linker increased, the antiplasmodial potencies of bis(*N*-isopropyl)amidines **21**, **24**, **27**, and **30** increased with elongation of the carbon chain. A similar improvement on an even greater scale was observed for the diimidazolines **22**, **25**, **28**, and **31**.

Except for bis(*N*-isopropyl)amidines **21**, **27**, and **30**, the 3,3′-substituted congeners exhibited reduced activity against *L. donovani* compared to diamidine **1** and its N-substituted derivatives **3** and **4**. Antileishmanial properties of the dications **20**−**22**, **25**, and **29** also diminished with respect to their 4,4′-substituted counterparts **12**−**14**, **18**, and **19**,[50] as did the selectivity of the compounds **20**−**22** and **25** against the pathogen. Among the 3,3′-substituted pentamidine congeners with alkyl linkers of identical length, the antileishmanial properties decreased in the order Am > *i*-PrAm > Im. On the other hand, activities of the 3,3′-substituted congeners **20**−**31** against *L. donovani* usually increased with the number of carbon atoms in the linker.

Aromatic Ring Substituents. The cytotoxicity and antiprotozoal activities of congeners bearing substituents in the 2,2′- or 3,3′-positions of aromatic rings and with alkyl linker chains of three to five carbon atoms are presented in Tables 4−6.

2,2′-Substituted diamidines **32**−**35**, **37**, **40**−**42**, and **44**−**46**, as well as bis(*N*-methyl)amidine **38** (Table 4) exhibited reduced cytotoxicities relative to the corresponding dications **12** and **17** as well as to diamidine **1** in all cases except for the 2,2′-dibromo substituted diamidine **33**, which was slightly more cytotoxic than compound **12**. All 2,2′-substituted diimidazolines **36**, **39**, **43**, and **47** demonstrated increased cytotoxicity relative to analogue **4**, whereas congeners **36**, **39**, and **43** were less cytotoxic than the corresponding diimidazolines **14** and **18** containing the three- and four-carbon chains, respectively. Within groups of dications with aliphatic linkers of the same length, all 2,2′-dimethoxy substituted diimidazolines **39**, **43**, and **47** were more cytotoxic than the corresponding diamidines **37**, **42**, and **46**, respectively. Conversely, the 2,2′-diamino diimidazoline **36** exhibited lower cytotoxicity compared to the diamidine **35**.

The cytotoxicities of 2,2′-diethoxycarbonyl, -dicarboxy, and -diaminocarbonyl diamidines **48**, **51**, **54**, and **55** (Table 5) were significantly lower than that of compound **1**, while bis(*N*-isopropyl)amidine and diimidazoline congeners **49**, **50**, **52**, and **53** were slightly more cytotoxic relative to the corresponding analogues **3** and **4**, lacking any 2,2′-substituents. Regardless of the type of substituents in the 2,2′-positions, the diamidines **48**−**55** demonstrated comparable cytotoxicities. At the same time, the introduction of the hydroxy or fluoro groups in the 3,3′-positions produced different effects on cytotoxic properties of dications **56**−**61** with regard to congeners **1**, **3**, and **4** (Table 6). Thus, cytotoxicity of the 3,3′-dihydroxy substituted diamidine **56** decreased more than 50-fold compared to diamidine **1**, while the bis(*N*-isopropyl)amidine **57** and the diimidazoline **58** displayed cytotoxicities similar to those of compounds **3** and **4**. On the other hand, the addition of 3,3′-difluoro groups in diamidine **59** resulted in only a minor reduction of its cytotoxicity relative to dication **1**, whereas the congeners **60** and **61** were more cytotoxic than bis(*N*-isopropyl)amidine **3** and diimidazoline **4**, respectively. Among the 3,3′-dihydroxy substituted dications **56**−**58**, diamidine **56** exhibited the lowest cytotoxicity, while in the group of 3,3′-difluoro congeners **59**−**61**, diamidine **59** was the most cytotoxic compound.

The antitrypanosomal properties of congeners **33**−**46** and **48**−**61** bearing substituents on the aromatic rings were diminished compared to compound **1**, its N-substituted derivatives **3** and **4**, diamidines **12** and **17**, and their imidazoline analogues **14** and **18** (Tables 4−6). The only compounds that were more active against *T. b. rhodesiense* than the corresponding derivatives without 2,2′-substituents were 2,2′-dichloro diamidine **32** and 2,2′-dimethoxy diimidazolines **39**, **43**, and **47**. The 2,2′-diethoxycarbonyl dications **48** and **50**, 2,2′-dicarboxy congeners **51**−**53**, and the 3,3′-dihydroxy compounds **56**−**58** displayed significantly lower antitrypanosomal activities in the micromolar range. While the 3,3′-difluoro diamidine **59** was nearly as active as dication **1** against *T. b. rhodesiense*, bis(*N*-isopropyl)amidine **60** and diimidazoline **61** were almost 10- and 80-fold less potent against the pathogen than the corresponding congeners **3** and **4**, respectively. The antitrypanosomal activities of the dications **59**−**61** decreased in the order Am > *i*-PrAm > Im. The diamidine **32** displayed improved antitrypanosomal properties and was 11- and 5-fold more selective for the *T. b. rhodesiense* than the dications **1** and **12**, respectively. Similarly, the 2,2′-dimethoxy substituted congeners as well as aminocarbonyl-substituted dications **54** and **55** demonstrated greater antitrypanosomal selectivity compared to the corresponding compounds without 2,2′-substituents. Thus, the 2,2′-dimethoxy diamidine **46** and 2-aminocarbonyl-2′-ethoxycarbonyl diamidine **54** were nearly 30- and 28-fold more selective against the pathogen than compound **1**.

Antiplasmodial activities of the 2,2′- and 3,3′-disubstituted congeners **32**−**61** decreased relative to those of dications **1**, **3**, and **4**, although the potency against *P. falciparum* as well as the parasite selectivity of the compounds **40**, **41**, and **43** bearing four-carbon linkers improved compared to that of the diamidine **17** and diimidazoline **18**.[50] Substitution on the amidine fragments often afforded derivatives with increased activities against the pathogen. Thus, among dications **48**−**53** and **59**−**61**, bis(*N*-isopropyl)amidines **49**, **52**, and **60** displayed higher antiplasmodial activities compared to the corresponding diamidines and diimidazolines. By contrast, in the case of the 2,2′-dimethoxy and 3,3′-dihydroxy congeners, the diimidazolines **39**, **43**, **47**, and **58** were the most active against *P. falciparum* in the respective groups. While the antiplasmodial properties of the 2,2′-dichloro substituted congeners **32**, **40**, and **44** decreased with the elongation of the alkyl linker, the activity of the 2,2′-dimethoxy dications **37**, **39**, **42**, **43**, **46**, and **47** versus *P. falciparum* was enhanced as the length of the linker increased. Also, the introduction of 2,2′-dimethoxy substituents resulted in improved selectivity of diamidines **37**, **42**, and **46** for the pathogen relative to the corresponding unsubstituted analogues. Thus, congeners **42** and **46** were 19- and 28-fold more selective for *P. falciparum* than diamidines **17** and **1**, respectively.

The antileishmanial activities of congeners **34**−**43** and **45**−**47** bearing nitro, amino, and methoxy substituents on the aromatic rings were lower relative to those of the unsubstituted dications **1**, **4**, **12**, **14**, and **18** (Tables 4−6). Compounds **48**−**58** possessing 2,2′-diethoxycarbonyl, 2,2′-dicarboxy, 2,2′-diaminocarbonyl, and 3,3′-dihydroxy substituents were completely inactive against the pathogen. Conversely, the 2,2′-dichloro or 2,2′-dibromo substituted congeners **32**, **33**, and **44** bearing three- and five-carbon chains were as active against *L. donovani* as dication **1**.[50] The 3,3′-difluoro substituted diamidine **59** and bis(*N*-isopropyl)amidine **60** exhibited antileishmanial IC_{50} values comparable to those of the congeners **1** and **3**, respectively, while the corresponding diimidazoline **61** was less active against *L. donovani* than its unsubstituted counterpart **4**. The antileishmanial selectivity of dications was not significantly affected by the introduction of nitro, ethoxycarbonyl, carboxyl, and aminocarbonyl groups to the 2,2′-positions of the aromatic rings. However, 2,2′-dichloro or 2,2′-dimethoxy substituted diamidines **32**, **37**, **44**, and **46** displayed higher antileishmanial selectivities compared to both diamidines **12** and **1**.

Replacement of Oxygen Atoms in the Linker Region. The cytotoxicity and antiprotozoal activities of dications, in which oxygen atoms in the linker region were replaced with sulfur atoms (compound **62**), sulfonyl groups (compound **63**), and amino groups (compounds **64**, **65**, and **66**) are presented in Table 7. The oxygen atoms in the aliphatic linker are part of the recognition motif for P2 transporter in *Trypanosoma* species[11,49,64,126] and are important for the activity of diamidine **1** against the pathogen.[127]

The replacement of oxygens produced opposite effects on cytotoxicities of the sulfur- and nitrogen-containing congeners **62**−**66**. While cytotoxicity of the thio-analogue **62** declined only slightly compared to diamidine **1**, the sulfone derivative **63** was 46-fold less cytotoxic than compound **1**. In contrast, the aza-analogues **64**−**66** were up to 4 times more cytotoxic than the corresponding dications **1**, **3**, and **4**. N-Substitution on the amidine groups reduced cytotoxicities of the congeners **65** and **66** relative to the diamidine **64**. Thus, bis(*N*-isopropyl)amidine **65** and diimidazoline **66** were nearly 130 and about 35 times less cytotoxic than dication **64**.

The thio-analogue **62** demonstrated improved antitrypanosomal and antileishmanial properties and a slight decline in antiplasmodial potency compared to diamidine **1**. Because of its lower cytotoxicity, dication **62** displayed greater selectivity toward *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* than compound **1**. In contrast, the sulfone derivative **63** was inactive against both *T. b. rhodesiense* and *L. donovani.* Although the compound **63** exhibited only moderate antiplasmodial activity ($IC_{50} = 98$ nM), it was 6 times more selective against *P. falciparum* than diamidine **1**.

Replacement of the linker oxygens in compound **1** with nitrogen atoms afforded the aza-analogue **64**, which was active against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. The most significant improvements in antiprotozoal properties were observed in the antitrypanosomal and antileishmanial assays, which is consistent with the previously reported data.[50] Compound **64** exhibited subnanomolar activity against *T. b. rhodesiense*, being nearly twice as selective against the pathogen as diamidine **1**. It was also almost 4 times more potent against *L. donovani* relative to dication **1**, at the same time showing similar parasite selectivity. While diamidine **64** displayed improved antiplasmodial properties compared to compound **1**, it demonstrated lower selectivity toward *P. falciparum* than the dication **1**.

Compounds **65** and **66**, the N-substituted congeners of **64**, displayed slightly different antiprotozoal properties. Thus, bis(*N*-isopropyl)amidine **65** demonstrated lower antitrypanosomal and antileishmanial activities while at the same time showing improved selectivity for *T. b. rhodesiense* and *L. donovani* with respect to diamidines **1** and **64**. On the other hand, the antiplasmodial activity of **65** increased 7- and 3-fold relative to that of **1** and **64**, respectively. Dication **65**, which was significantly less cytotoxic than diamidine **64**, exhibited the highest antiplasmodial selectivity among all congeners ($SI_P = 62500$). It was 230 times more selective for *P. falciparum* than compound **1**. Conversely, diimidazoline **66** displayed high activities against all three parasites used in this study. Compound **66** not only exhibited the second highest antitrypanosomal activity in the series with the IC_{50} value of 1 nM but also demonstrated the highest parasite selectivity ($SI_T = 34500$), being 63 times more selective against *T. b. rhodesiense* than diamidine **1**. Diimidazoline **66** was also the most active compound in the group against *P. falciparum* ($IC_{50} = 1$ nM) and *L. donovani*, where the dications **64** and **66** were the only two congeners with antileishmanial IC_{50} values less than 1 μM. The compound **66** possessed antileishmanial selectivity index ($SI_L = 136$), which was 68-fold greater than that of dication **1**.

The aza-analogues **64**−**66** exhibited higher activity against *P. falciparum* and *L. donovani* relative to compound **1** and its bis(*N*-isopropyl)amidine and diimidazoline congeners **3** and **4**. While diimidazoline **4** displayed greater antiplasmodial selectivity compared to its counterpart **66**, the (*N*-isopropyl)amidine **65** was more selective for the parasite than the corresponding derivative **3**. At the same time, the bis(*N*-isopropyl)amidine **65** and the diimidazoline **66** were 2−3 times more selective against *L. donovani* than the congeners **3** and **4**. Compound **65** exhibited 2-fold decrease in antileishmanial activity and selectivity for the pathogen relative to dication **3**. Conversely, diamidine **64** and diimidazoline **66** were more potent against *T. b. rhodesiense* than dications **1** and **4**, respectively. Aza-analogue **66** displayed antitrypanosomal selectivity index SI_L more than 10-fold greater than that of the corresponding congener **4**. Overall, diimidazoline **66** was the most promising compound in the series, showing excellent activities and high selectivities against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*.

In Vivo Antitrypanosomal Activity. Select dications exhibiting promising in vitro activities against *T. b. rhodesiense* were evaluated in vivo in the STIB900 mouse model of African trypanosomiasis (Table 8). The screening was conducted using intraperitoneal dosing at 20 mg/kg daily for four days. Diamidine **1** and 12 congeners (**2**, **13**, **14**, **18**, **19**, **20**, **23**, **32**, **37**, **39**, **40**, and **66**) displayed promising in vivo efficacies, curing at least 2 out of 4 mice. Because of the significant curative rate and substantial increase in the survival time of the infected animals, the administered daily dosage was reduced to 10 mg/kg or even further to 5 mg/kg. At the 10 mg/kg daily for 4 days regimen, six dications (**2**, **13**, **20**, **32**, **37**, and **66**) provided cures to at least 2 out of 4 mice, while at the 5 mg/kg daily for four days treatment, four congeners (**2**, **32**, **37**, and **66**) cured 2 or more out of 4 animals.

In several cases, N-substitution on the cationic fragments increased antitrypanosomal efficacies of tested compounds. For example, the bis(*N*-methyl)amidine **2** provided 4/4 cures when administered intraperitoneally at 20 mg/kg for four days, while diamidine **1** cured only 2/4 animals at this dosage. Similarly, the diimidazolines **14**, **18**, and **66** displayed better in vivo activities against *T. b. rhodesiense* compared to the corresponding diamidines **12**, **17**, and **64**, respectively. The position of attachment of cationic substituents and the length of the alkyl bridge influenced the efficacies of dications in the acute mouse model of trypanosomiasis. Thus, the 3,3′-substituted diamidines (compounds **20**, **23**, **26**, and **29**) were more effective in vivo than the 4,4′-substituted analogues (dications **12**, **17**, **1**, and **19**) when connected by the tri- or tetramethylene linkers and displayed lower activities when the aromatic rings were connected by five- or six-carbon chains. Similarly, the introduction of 2,2′-dichloro substituents improved the efficacies of diamidines **32** and **40** against *T. b. rhodesiense* compared to that of dications **12** and **17**, while the same structural variation reduced the in vivo activity of diamidine **44** compared to compound **1**. The 2,2′-dimethoxy analogues **37** and **39** with a three-carbon linker were more active than their unsubstituted counterparts **12** and **14**. However, the presence of methoxy groups with longer four- and five-carbon linkers had no effect on activity (**42**, **43**, and **47** versus **17**, **18**, and **4**, respectively). The antitrypanosomal efficacies of the unsubstituted diimidazolines **14**, **18**, and **4**, the 2,2′-dichloro substituted diamidines **32**, **40**, and **44**, and the 2,2′-dimethoxy substituted diamidines and diimidazolines **37**, **39**, **42**, **43**, **46**, and **47** decreased as the length of the carbon linker between the aromatic moieties increased. Replacement of the oxygen atoms in the aliphatic linker with sulfur atoms afforded the diamidine **62**, which, despite promising antitrypanosomal activities in vitro, was inactive in the in vivo model. Among the congeners **64**−**66** possessing secondary amino groups in place of oxygen atoms, the diamidine **64** cured only 1 out of 4 mice at 4 × 5 mg/kg and bis(*N*-isopropyl)amidine **65** was inactive at this dosage. Conversely, diimidazoline **66** displayed excellent antitrypanosomal activity both in vitro and in vivo, providing cures to 4/4 animals when administered at 5 mg/kg daily for four days.

Conclusions

In this study, a series of dications has been tested in vitro against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* as well as for cytotoxicity against mammalian cells. Generally, N-substituted congeners were less cytotoxic compared to the unsubstituted diamidines. Conversely, the substitution on aromatic rings increased cytotoxicity of select analogues. Thus, the cytotoxicity of 2,2′-substituted dications increased in the order OMe, CO_2Et, CO_2H, $CONH_2 < NO_2 <$ Cl. Replacement of oxygens in the aliphatic linker of compound **1** with sulfur atoms or sulfone groups reduced cytotoxicity of dications as well.

Unsubstituted amidines were more active in vitro against *T. b. rhodesiense* and *L. donovani* compared to the N-substituted derivatives. In several cases, bis(*N*-isopropyl)amidines exhibited superior antiplasmodial potency relative to the corresponding diamidines and diimidazolines. Diamidine **12** (possessing a trimethylene linker) demonstrated improved activities against all three pathogens compared to compound **1**. Placement of the cationic fragments in the 3,3′-position of the aromatic rings reduced antitrypanosomal and antileishmanial properties of dications, while their activity against *P. falciparum* was similar to that of the 4,4′-substituted isomers. Introduction of 2,2′-dichloro substituents further improved antitrypanosomal properties of diamidine **12**. Similarly, placement of the halogen groups in the 2,2′-positions also increased activities of dications against *L. donovani*. The replacement of oxygen atoms in the alkyl chain with secondary amino groups improved activities of the resulting congeners against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. 2,2′-Dichloro substituted diamidine **32** and diimidazoline **66** exhibited excellent in vivo efficacies in the acute mouse model of trypanosomiasis, providing cures of all infected animals.

Experimental Section

Biology. Preparation of Compounds. Compounds were dissolved in 100% dimethylsulfoxide (DMSO) and finally diluted in culture medium prior to the assay. The DMSO concentration never exceeded 1% in the in vitro assays. For in vivo experiments, the compounds were dissolved in DMSO and further diluted with distilled H_2O to a final DMSO concentration of 10% prior to injection into the animals.

In Vitro Cytotoxicity Assay (L6 Rat Myoblast Cells). IC_{50} values were determined using the Alamar blue assay[128] and were carried out twice independently and in duplicate. Briefly, 4000 L6 cells were seeded in RPMI 1640 medium supplemented with L-glutamine 2 mM, HEPES 5.95 g/L, $NaHCO_3$ 2 g/L, and 10% fetal bovine serum in 96-well microtiter plates. The serial drug dilutions were incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin [Sigma] dissolved in 100 mL phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2−3 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength 536 nm and an emission wavelength 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of *T. b. rhodesiense* (STIB900). IC_{50} values were determined using the Alamar blue assay and were carried out twice independently and in duplicate. Briefly, the compounds were tested in minimum essential medium with Earle's salts, supplemented as previously described[129] with the following modifications: 2-mercaptoethanol 0.2 mM, sodium pyruvate 1 mM, hypoxanthine 0.5 mM, and 15% heat-inactivated horse serum. Serial drug dilutions were prepared in 96-well microtiter plates and each well inoculated with 2000 bloodstream forms and incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin [Sigma] dissolved in 100 mL phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2−6 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of P. falciparum (K1). The determination of IC_{50} values against erythrocytic stages of *P. falciparum* was carried out twice independently and in duplicate using the [^{3}H]-hypoxanthine incorporation assay.[130,131] Briefly, the compounds were tested in RPMI 1640 medium 10.44 g/L, supplemented with Hepes 5.94 g/L, Albumax II 5 g/L, sodium bicarbonate 2.1 g/L, and neomycin 100 mg/L in 96-well microtiter plates. Infected human red blood cells in medium (hematocrit 1.25%, parasitemia 0.3%) were incubated with the drug dilutions in an atmosphere of 93% N_2, 4% CO_2, and 3% O_2 at 37 °C. After 48 h, [^{3}H]-hypoxanthine (0.5 μCi/well) was added and the plates were incubated for additional 24 h under the same conditions. The wells were harvested with a Betapalte cell harvester and transferred on a glass fiber filter. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The IC_{50} values were calculated from the sigmoidal inhibition curves using MS Excel.

Antileishmanial Assay. Axenic amastigotes of *L. donovani* (WHO designation MHOM/SD/62/1S-CL2_D) were adapted from promastigotes and grown in the amastigote medium described previously[132] at 37 °C. In a final volume of 60 μL, 6×10^4 parasites were added to each well of a 96-well plate except for negative control wells. Standard and test compounds were added as appropriate using 2-fold dilutions to allow a range of concentrations to be tested. Plates were then incubated at 37 °C for 72 h in a humidified environment containing 5% CO_2. The tetrazolium dye-based CellTiter reagent (Promega, Madison, WI) was used to assess parasite growth.[133] Several hours after adding 12 μL of the CellTiter reagent to each well of the plate, absorbance readings were taken at 490 nm using a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA). SoftMax Pro software (Amersham Biosciences, Piscataway, NJ) was used to calculate IC_{50} values by employing the dose−response equation $y = [(a - d)/(1 + (x/c)^b)] + d$, where x = compound concentration, y = absorbance at 490 nm, a = upper asymptote, b = slope, $c = IC_{50}$ value, and d = lower asymptote.

STIB900 Acute Mouse Model of Trypanosomiasis. Experiments were performed as previously reported[134] with minor modifications. Briefly, female NMRI mice were infected intraperitoneally (ip) with 2×10^4 STIB900 bloodstream forms. Experimental groups of four mice were treated ip with tested dications on 4 consecutive days from day 3 to day 6 postinfection. A control group was infected but remained untreated. The tail blood of all mice was checked for parasitemia until 60 days postinfection. Surviving and aparasitemic mice at day 60 were considered cured and then euthanized. Death of animals (including the aparasitemic mice, >60) was recorded to calculate the mean survival time in days.

Chemistry. General Experimental Information. All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Acros Organics and were used without further purification. Uncorrected melting points were measured on a Thomas−Hoover capillary melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 spectrometer operating at 300 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane. Anhydrous ethanol was distilled over Mg/I_2 immediately prior to use. Reaction mixtures were monitored by TLC using Whatman silica gel 250 μm UV_{254} plates 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. Flash column chromatography was performed using Davisil grade 633, type 60A silica gel (200−425 mesh). Analytical HPLC chromatograms were recorded on an Agilent 1090 or 1200 chromatograph using an Agilent Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. The column temperature was maintained at 40 °C. Mobile phases consisted of mixtures of acetonitrile (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. In method A, the concentration of acetonitrile was increased linearly from 0 to 22.5% over 6 min, then from 22.5 to 56.25% over 4 min, and finally maintained for 1 min. In method B, the concentration of acetonitrile was increased linearly from 22.5 to 75% over 10 min and then maintained for 2 min.

Preparative Reverse Phase HPLC. Preparative reverse phase HPLC was performed on a Varian ProStar Chromatography Workstation configured with two PS-215 pumps fitted with 50 mL pump heads, a Dynamax Microsorb C18 (60 Å) column (41.4 mm × 250 mm, 8 μm), PS-320 variable wavelength UV−vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of acetonitrile (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 as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 2K or 6K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Flash Chromatography of Amidines on C_{18} Reversed Phase Silica Gel. The chromatographic column was half-filled with acetonitrile and packed with a slurry of C_{18} silica gel (70 g) in acetonitrile (70−100 mL). The excess acetonitrile was drained out, and the top of the column was covered with a 2 cm pad of sand. The column was equilibrated with 150 mL of initial mobile phase consisting of water containing formic acid (40 mM) and ammonium formate (10 mM). A concentrated reaction mixture was dissolved in the initial mobile phase. In case of low solubility, heating of the mixture and/or addition of a small amount of methanol as a cosolvent were performed. After the reaction mixture was applied to the column, the elution began with initial mobile phase (150 mL) to remove the excess amine and then with a mobile phase consisting of a mixture of acetonitrile (0−75%) in water containing formic acid (40 mM) and ammonium formate (10 mM). Acetonitrile concentrations varied for each individual compound and contained 50−70% of the calculated amount of acetonitrile at the point of the retention time of the compound in analytical method A. After the purification was completed, the column was washed with acetonitrile (3 × 100 mL), ethanol (100 mL), deionized water (2 × 100 mL), and kept in acetonitrile or acetonitrile−water mixture. Select fractions were analyzed for purity as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 6K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Low resolution ESI mass spectra were recorded on an Agilent Technologies 1100 series LC/MSD Trap spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ±0.4% of calculated values.

General Procedure for Syntheses of Diamidines (1−5, 9−53, 55−60, 64−66). 1,5-Bis(benzamidine-4-oxy)pentane (Pentamidine) Dihydrochloride (1).[44] A mixture of dry 1,4-dioxane (230 mL) and dry EtOH (20 mL) in a 3-neck 500 mL flask equipped with a gas inlet tube, a thermometer, and a drying tube was saturated with gaseous HCl at 0 °C. 1,5-Bis(4-cyanophenoxy)pentane (**69**) (6.00 g, 20.0 mmol) was added in one portion, the flask was sealed, and the mixture was stirred at room temperature until the starting material was no longer detectable by HPLC. The reaction mixture was diluted with dry diethyl ether and cooled in a freezer overnight. The resulting precipitate was filtered off under Ar, washed with dry diethyl ether, and dried under high vacuum for 3 h to yield a diimidate ester (9.10 g, 98%), which was reacted immediately with ammonia, appropriate amines, or hydrazine.

The diimidate ester (9.10 g, 19.0 mmol) was suspended in dry EtOH (80 mL) and saturated ethanolic ammonia (70 mL) was added. The reaction mixture was sealed and stirred at ambient temperature. The progress of the reaction was monitored by HPLC. After 2 days, the mixture was diluted with dry diethyl ether and cooled in a freezer. A formed precipitate was filtered off, dried under high vacuum, and recrystallized from 1 N HCl/EtOH and then from water/*i*-PrOH to yield the compound **1** (5.10 g, 63%): mp 245.5 °C (lit.[44] 233−234 °C dec). ^{1}H NMR (DMSO-d_6), δ 9.25 (s, 4H), 9.03 (s, 4H), 7.85 (d, J = 8.2 Hz, 4H), 7.15 (d, J = 8.2 Hz, 4H), 4.13 (t, J = 6.2 Hz, 4H), 1.82 (m, 4H), 1.61 (m, 2H). HPLC (method A) t_R = 6.28 min (100 area %). Anal. ($C_{19}H_{24}N_4O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis[(*N*-methyl)benzamidine-4-oxy]pentane Dihydrochloride (2). Compound **2** was prepared from 1,5-bis(4-cyanophenoxy)pentane (**69**)[44] as a white solid (3.00 g, 91%): mp 205−207 °C. ^{1}H NMR (DMSO-d_6), δ 9.99 (s, 2H), 9.48 (s, 2H), 8.95 (s, 2H), 7.85 (d, J = 8.8 Hz, 4H), 7.10 (d, J = 8.8 Hz, 4H), 4.10 (t, J = 6.2 Hz, 4H), 2.99 (s, 6H), 1.81 (m, 4H), 1.60 (m, 2H). HPLC (method A) t_R = 6.80 min (98.5 area %); m/z 369.2 (MH$^+$ of free base). Anal. ($C_{21}H_{28}N_4O_2 \cdot 2HCl$) C, H, N.

1,5-Bis[(*N*-isopropyl)benzamidine-4-oxy]pentane Dihydrochloride (3). Compound **3** was prepared from 1,5-bis(4-cyanophenoxy)pentane (**69**).[44] The oily crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **3** (0.30 g, 71%): mp 195 °C (dec). ^{1}H NMR (DMSO-d_6), δ 9.41 (d, J = 7.9 Hz, 2H), 9.31 (s, 2H), 8.97 (s, 2H), 7.73 (d, J = 8.8 Hz, 4H), 7.13 (d, J = 8.8 Hz, 4H), 4.11 (t, J = 6.2 Hz, 4H), 4.06 (m, 2H), 1.82 (m, 4H), 1.59 (m, 2H), 1.27 (d, J = 6.3 Hz, 12H). HPLC (method A) t_R = 7.98 min (100 area %). Anal. ($C_{25}H_{36}N_4O_2 \cdot 2.4HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis-[(1*H*-tetrazolyl-5)benzene-4-oxy]pentane (6). A suspension of 1,5-bis(4-cyanophenoxy)pentane (**69**)[44] (0.20 g, 0.65 mmol), sodium azide (0.13 g, 2.00 mmol), and glacial acetic acid (0.12 g, 2.00 mmol) in DMF (2 mL) was refluxed overnight. The mixture was cooled to ambient temperature, diluted with water (5 mL), and treated with 1 N NaOH (2 mL) to dissolve a formed precipitate. The solution was extracted with benzene (2 × 10 mL) and then acidified with 1N HCl to yield a white precipitate, which was filtered off, washed with water (3 × 15 mL), and dried under high vacuum. The crude product was recrystallized from *i*-PrOH to yield compound **6** (0.21 g, 82%): mp 246−247 °C (dec). ^{1}H NMR (DMSO-d_6), δ 7.96 (d, J = 8.7 Hz, 4H), 7.16 (d, J = 8.7 Hz, 4H), 4.10 (t, J = 6.2 Hz, 4H), 1.84 (m, 4H), 1.61 (m, 2H). HPLC (method B) t_R = 4.69 min (98.6 area %). Anal. ($C_{19}H_{20}N_8O_2$) C, H, N.

1,5-Bis[4-(benzimidoylamino)-benzene-1-oxy]pentane Dihydrochloride (7). To an ice-cold solution of 1,5-bis(4-aminophenoxy)pentane[97] (0.20 g, 0.70 mmol) in dry MeCN (5 mL) and dry EtOH (15 mL) was added hydrobromide salt of benzenecarboximidothioic acid 2-naphthalenylmethyl ester[98] (0.80 g, 2.20 mmol). The mixture was stirred at ambient temperature overnight. The resulting solution was concentrated to give an oily residue, which was triturated with diethyl ether to yield a yellow precipitate. The solid was taken in a hot water (200 mL) and washed with diethyl ether (2 × 100 mL). The water layer was concentrated to give a white solid, which was recrystallized from 1 N HCl to yield compound **7** (0.31 g, 77%): mp 143−145 °C. ^{1}H NMR (DMSO-d_6), δ 11.23 (s, 2H), 9.71 (s, 2H), 8.24 (s, 2H), 7.90 (dd, J_1 = 7.1 Hz, J_2 = 2.1 Hz, 4H), 7.79 (tt, J_1 = 7.4 Hz, J_2 = 2.1 Hz, 2H), 7.67 (dd, J_1 = 7.6 Hz, J_2 = 7.1 Hz, 4H), 7.40 (d, J = 8.8 Hz, 4H), 7.13 (d, J = 8.8 Hz, 4H), 4.72 (t, J = 6.2 Hz, 4H), 1.82 (m, 4H), 1.59 (m, 2H). HPLC (method B) t_R = 2.84 min (98.0 area %); m/z 493.0 (MH$^+$ of free base). Anal. ($C_{31}H_{32}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis[4-(acetimidoylamino)-benzene-1-oxy]pentane Dihydrochloride (8). Compound **8** was prepared from 1,5-bis(4-aminophenoxy)pentane[97] and hydrobromide salt of ethanimidothioic acid 2-naphthalenylmethyl ester[99] following the procedure described above for the compound **7**. A crude product was purified by preparative HPLC and recrystallized from 1 N HCl to yield compound **8** (0.10 g, 17%): mp 208−211 °C. ^{1}H NMR (DMSO-d_6), δ 11.56 (s, 2H), 9.53 (s, 2H), 8.33 (s, 2H), 7.23 (d, J = 8.8 Hz, 4H), 7.05 (d, J = 8.8 Hz, 4H), 4.03 (t, J = 6.0 Hz, 4H), 2.35 (s, 6H), 1.80 (m, 4H), 1.58 (m, 2H). HPLC (method A) t_R = 6.46 min (100 area %); m/z 368.7 (MH$^+$ of free base). Anal. ($C_{21}H_{28}N_4O_2 \cdot 2HCl \cdot 0.25H_2O$) C, H, N, Cl.

1,5-Bis(benzamidrazone-4-oxy)pentane Dihydrochloride (9). Compound **9** was prepared from 1,5-bis(4-cyanophenoxy)pentane (**69**)[44] as a white solid (7.00 g, 81%): mp 195 °C. ^{1}H NMR (DMSO-d_6), δ 7.78 (d, J = 8.8 Hz, 4H), 7.14 (d, J = 8.8 Hz, 4H), 6.7 (br s, 10H), 4.11 (t, J = 6.2 Hz, 4H), 1.80 (m, 4H), 1.05 (m, 2H). HPLC (method A) t_R = 6.19 min (100 area %); m/z 371.5 (MH$^+$ of free base). Anal. ($C_{19}H_{26}N_6O_2 \cdot 2HCl \cdot 1.4H_2O$) C, H, N, Cl.

1-(4-Amidinophenoxy)-5-(4-aminophenoxy)pentane Dihydrochloride (10).[83] Compound **10** was prepared according to the published procedure as a white solid (2.63 g, 83%): mp 218−220 °C (lit.[83] 125−126 °C). ^{1}H NMR (DMSO-d_6), δ 10.22 (br s, 3H), 9.24 (s, 2H), 9.02 (s, 2H), 7.85 (d, J = 9.0 Hz, 2H), 7.31 (d, J = 8.9 Hz, 2H), 7.15 (d, J = 9.0 Hz, 2H), 7.03 (d, J = 9.0 Hz, 2H), 4.12 (t, J = 6.4 Hz, 2H), 4.00 (t, J = 6.3 Hz, 2H), 1.79 (m, 4H), 1.57 (m, 2H). HPLC (method A) t_R = 6.64 min (100 area %). Anal. ($C_{18}H_{23}N_3O_2 \cdot 2HCl$) C, H, N, Cl.

1,3-Bis[(*N*-isopropyl)benzamidine-4-oxy]propane Dihydrochloride (13). Compound **13** was prepared from 1,3-bis(4-cyanophenoxy)propane (**67**).[44] A crude product was recrystallized from 1 N HCl to yield compound **13** (1.08 g, 96%): mp 263−266 °C. ^{1}H NMR (DMSO-d_6), δ 9.50 (m, 4H), 9.20 (s, 2H), 7.85 (d, J = 8.8 Hz, 4H), 7.15 (d, J = 8.8 Hz, 4H), 4.30 (t, J = 6.2 Hz, 4H), 4.20 (m, 2H), 2.25 (m, 2H), 1.27 (d, J = 6.3 Hz, 12H); m/z 397.2 (MH$^+$ of free base). Anal. ($C_{23}H_{32}N_4O_2 \cdot 2HCl$) C, H, N.

1-(4-Amidinophenoxy)-3-[(2-imidazolinyl)benzene-4-oxy]propane Dihydrochloride (15). Compound **15** was prepared from 1-(4-cyanophenoxy)-3-[(2-imidazolinyl)benzene-4-oxy]propane (**81**). A crude product was recrystallized from 1 N HCl to yield compound **15** (0.47 g, 64%): mp 166−170 °C. ^{1}H NMR (DMSO-d_6), δ 10.79 (s, 2H), 9.36 (s, 2H), 9.17 (s, 2H), 8.13 (d, J = 8.8 Hz, 2H), 7.89 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 4.29 (t, J = 5.5 Hz, 2H), 4.25 (t, J = 5.5 Hz, 2H), 3.95 (s, 4H), 2.24 (m, 2H). HPLC (method A) t_R = 5.39 min (98.3 area %); m/z 339.4 (MH$^+$ of free base). Anal. ($C_{19}H_{22}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$) C, H, N.

1-(4-Amidinophenoxy)-3-(4-carboxyphenoxy)propane Hydrochloride (16). 1-(4-Cyanophenoxy)-3-(4-ethoxycarbonylphenoxy)propane (**87**) was subjected to Pinner conditions followed by reaction with ammonia as described above for **1** to yield 1-(4-amidinophenoxy)-3-(4-ethoxycarbonylphenoxy)propane (0.45 g, 69%); mp 153−154 °C. ^{1}H NMR (DMSO-d_6), δ 9.31 (s, 2H), 9.14 (s, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.90 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 4.29 (m, 6H), 2.25 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H).

A suspension of the 1-(4-amidinophenoxy)-3-(4-ethoxycarbonylphenoxy)propane in 2 N aqueous HCl was kept at 70 °C overnight. The mixture was concentrated and a residue was recrystallized from 1 N HCl to yield compound **16** (0.30 g, 45%); mp 278−280 °C. ^{1}H NMR (DMSO-d_6), δ 12.65 (br s. 1H), 9.29 (s, 2H), 9.12 (s, 2H), 7.87 (m, 4H), 7.16 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 4.25 (m, 4H), 2.23 (m, 2H). HPLC (method A) t_R = 8.06 min (100 area %); m/z 315.1 (MH$^+$ of free base). Anal. ($C_{17}H_{18}N_2O_4 \cdot 1HCl$) C, H, N, Cl.

1,3-Bis(benzamidine-3-oxy)propane Dihydrochloride (20).[66] Compound **20** was prepared from 1,3-bis(3-cyanophenoxy)propane (**71**).[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **20** (0.38 g, 66%); mp 189−191 °C (lit.[66] 300 °C). ^{1}H NMR (DMSO-d_6), δ 9.47 (s, 4H), 9.30 (s, 4H), 7.53 (t, J = 7.7 Hz, 2H), 7.48 (s, 2H), 7.43 (d, J = 7.7 Hz, 2H), 7.32 (d, J = 7.7 Hz, 2H), 4.27 (t, J = 6.0 Hz, 4H), 2.24 (m, 2H). HPLC (method A) t_R = 4.36 min (100 area %). Anal. ($C_{17}H_{20}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,3-Bis[(*N*-isopropyl)benzamidine-3-oxy]propane Dihydrochloride (21). Compound **21** was prepared from 1,3-bis(3-cyanophenoxy)propane (**71**).[66] An oily crude material was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **21** (0.35 g, 50%); mp 146 °C (dec). ^{1}H NMR (DMSO-d_6), δ 9.65 (s, 2H), 9.53 (s, 2H), 9.27 (s, 2H), 7.50 (t, $J = 7.7$ Hz, 2H), 7.30 (m, 6H), 4.27 (br s, 4H), 4.13 (br s, 2H), 2.24 (br s, 2H), 1.27 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 6.20$ min (100 area %). Anal. ($C_{23}H_{32}N_4O_2 \cdot 2.1HCl \cdot 1H_2O$) C, H, N, Cl.

1,3-Bis-[(2-imidazolinyl)benzene-3-oxy]propane Dihydrochloride (22). Compound **22** was prepared from 1,3-bis(3-cyanophenoxy)propane (**71**).[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **36** (0.48 g, 73%); mp 306−307 °C. ^{1}H NMR (DMSO-d_6), δ 10.93 (s, 4H), 7.78 (s, 2H), 7.66 (d, $J = 7.1$ Hz, 2H), 7.55 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.1$ Hz, 2H), 7.35 (d, $J = 8.2$ Hz, 2H), 4.28 (t, $J = 5.5$ Hz, 4H), 3.99 (s, 8H), 2.23 (m, 2H). HPLC (method A) $t_R = 5.17$ min (100 area %). Anal. ($C_{21}H_{24}N_4O_2 \cdot 2HCl \cdot 2.2H_2O$) C, H, N, Cl.

1,4-Bis(benzamidine-3-oxy)butane Dihydrochloride (23).[66] Compound **23** was prepared from 1,4-bis(3-cyanophenoxy)butane (**72**).[66] A crude product was recrystallized from 1 N HCl to yield compound **23** (0.85 g, 98%); mp 245−246 °C (lit.[66] 257 °C). ^{1}H NMR (DMSO-d_6), δ 9.45 (s, 4H), 9.27 (s, 4H), 7.52 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.46 (s, 2H), 7.42 (d, $J = 7.6$ Hz, 2H), 7.30 (d, $J = 8.2$ Hz, 2H), 4.17 (s, 4H), 1.92 (s, 4H). HPLC (method A) $t_R = 5.16$ min (100 area %). Anal. ($C_{18}H_{22}N_4O_2 \cdot 2HCl \cdot 1.9H_2O$) C, H, N, Cl.

1,4-Bis[(*N*-isopropyl)benzamidine-3-oxy]butane Dihydrochloride (24). Compound **24** was prepared from 1,4-bis(3-cyanophenoxy)butane (**72**).[66] An oily crude material was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **24** (0.86 g, 81%); mp 163−165 °C. ^{1}H NMR (DMSO-d_6), δ 9.59 (d, $J = 6.6$ Hz, 2H), 9.47 (s, 2H), 9.17 (s, 2H), 7.51 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.30 (m, 6H), 4.16 (br s, 4H), 4.08 (m, 2H), 1.92 (br s, 4H), 1.27 (d, $J = 6.6$ Hz, 12H). HPLC (method A) $t_R = 6.98$ min (100 area %). Anal. ($C_{24}H_{34}N_4O_2 \cdot 2.3HCl \cdot 1.7H_2O$) C, H, N, Cl.

1,4-Bis-[(2-imidazolinyl)benzene-3-oxy]butane Dihydrochloride (25).[67] Compound **25** was prepared from 1,4-bis(3-cyanophenoxy)butane (**72**).[66] A crude product was recrystallized from 1 N HCl/EtOH to yield compound **25** (0.97 g, 98%); mp 333 °C (dec) (lit.[67] >300 °C). ^{1}H NMR (DMSO-d_6), δ 10.86 (s, 4H), 7.75 (s, 2H), 7.62 (d $J = 8.2$ Hz, 2H), 7.55 (t, $J = 8.2$ Hz, 2H), 7.33 (d, $J = 8.2$ Hz, 2H), 4.18 (br s, 4H), 3.99 (s, 8H), 1.92 (br s, 4H). HPLC (method A) $t_R = 5.99$ min (100 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis(benzamidine-3-oxy)pentane Dihydrochloride (26).[66] Compound **26** was prepared from 1,5-bis(3-cyanophenoxy)pentane (**73**).[44] A crude product was recrystallized from 1 N HCl to yield compound **26** (1.12 g, 77%); mp 88−92 °C (lit.[66] 133−134 °C). ^{1}H NMR (DMSO-d_6), δ 9.46 (s, 4H), 9.26 (s, 4H), 7.52 (t, $J = 8.2$ Hz, 2H), 7.44 (s, 2H), 7.42 (d, $J = 8.2$ Hz, 2H), 7.29 (d, $J = 8.2$ Hz, 2H), 4.11 (t, $J = 6.2$ Hz, 4H), 1.83 (m, 4H), 1.58 (m, 2H). HPLC (method A) $t_R = 5.81$ min (100 area %). Anal. ($C_{19}H_{24}N_4O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis[(*N*-isopropyl)benzamidine-3-oxy]pentane Dihydrochloride (27). Compound **27** was prepared from 1,5-bis(3-cyanophenoxy)pentane (**73**).[44] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **27** (0.43 g, 58%); mp 152−155 °C. ^{1}H NMR (DMSO-d_6), δ 9.56 (d, $J = 8.2$ Hz, 2H), 9.45 (s, 2H), 9.17 (s, 2H), 7.47 (t, $J = 7.7$ Hz, 2H), 7.25 (m, 6H), 4.08 (m, 6H), 1.78 (m, 4H), 1.57 (m, 2H), 1.24 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 7.49$ min (100 area %). Anal. ($C_{25}H_{36}N_4O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis-[(2-imidazolinyl)benzene-3-oxy]pentane Dihydrochloride (28). Compound **28** was prepared from 1,5-bis(3-cyanophenoxy)pentane (**73**).[44] A crude product was recrystallized from 1 N HCl to yield compound **28** (0.75 g, 85%); mp 91−93 °C. ^{1}H NMR (DMSO-d_6), δ 10.93 (br s, 4H), 7.75 (s, 2H), 7.63 (br s, 2H), 7.54 (t, $J = 8.2$ Hz, 2H), 7.31 (d $J = 8.2$ Hz, 2H), 4.11 (t, $J = 6.0$ Hz, 4H), 3.99 (s, 8H), 1.83 (m, 4H), 1.60 (m, 2H). HPLC (method A) $t_R = 6.72$ min (100 area %). Anal. ($C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,6-Bis(benzamidine-3-oxy)hexane Dihydrochloride (29).[66] Compound **29** was prepared from 1,6-bis(3-cyanophenoxy)hexane (**74**). A crude product was recrystallized from 1 N HCl to yield compound **29** (0.75 g, 95%); mp 247−249 °C (lit.[66] 268 °C). ^{1}H NMR (DMSO-d_6), δ 9.41 (s, 4H), 9.24 (s, 4H), 7.52 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.42 (s, 2H), 7.40 (d, $J = 7.6$ Hz, 2H), 7.29 (d, $J = 8.2$ Hz, 2H), 4.09 (t, $J = 6.0$ Hz, 4H), 1.78 (m, 4H), 1.51 (br s, 4H). HPLC (method A) $t_R = 6.79$ min (100 area %). Anal. ($C_{20}H_{26}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

1,6-Bis[(*N*-isopropyl)benzamidine-3-oxy]hexane Dihydrochloride (30). Compound **30** was prepared from 1,6-bis(3-cyanophenoxy)hexane (**74**). A crude product was recrystallized from 1 N HCl to yield compound **30** (0.65 g, 69%); mp 150−153 °C. ^{1}H NMR (DMSO-d_6), δ 9.60 (d, $J = 8.2$ Hz, 2H), 9.49 (s, 2H), 9.21 (s, 2H), 7.50 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.23 (m, 6H), 4.15 (m, 2H), 4.09 (t, $J = 6.0$ Hz, 4H), 1.78 (br s, 4H), 1.51 (br s, 4H), 1.27 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 7.94$ min (100 area %). Anal. ($C_{26}H_{38}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$) C, H, N, Cl.

1,6-Bis-[(2-imidazolinyl)benzene-3-oxy]hexane Dihydrochloride (31). Compound **31** was prepared from 1,6-bis(3-cyanophenoxy)hexane (**74**). A crude product was recrystallized from 1 N HCl to yield compound **31** (0.85 g, 96%); mp 243−244 °C. ^{1}H NMR (DMSO-d_6), δ 10.84 (s, 4H), 7.70 (s, 2H), 7.61 (d $J = 7.6$ Hz, 2H), 7.54 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.31 (d $J = 8.2$ Hz, 2H), 4.09 (t, $J = 6.0$ Hz, 4H), 3.99 (s, 8H), 1.78 (br s, 4H), 1.50 (br s, 4H). HPLC (method A) $t_R = 7.33$ min (100 area %). Anal. ($C_{24}H_{30}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,3-Bis(3-chlorobenzamidine-4-oxy)propane Dihydrochloride (32). Compound **32** was prepared from 1,3-bis(2-chloro-4-cyanophenoxy)propane (**75**).[66] A crude product was recrystallized from 1 N HCl to yield compound **32** (1.07 g, 84%); mp 299−301 °C. ^{1}H NMR (DMSO-d_6), δ 9.42 (s, 4H), 9.24 (s, 4H), 8.05 (d, $J = 1.6$ Hz, 2H), 7.91 (dd, $J_1 = 8.8$ Hz, $J_2 = 1.6$ Hz, 2H), 7.44 (d, $J = 8.8$ Hz, 2H), 4.40 (t, $J = 5.5$ Hz, 4H), 2.32 (m, 2H). HPLC (method A) $t_R = 5.92$ min (98.4 area %). Anal. ($C_{17}H_{18}N_4O_2Cl_2 \cdot 2HCl$) C, H, N, Cl.

1,3-Bis(3-nitrobenzamidine-4-oxy)propane Dihydrochloride (34). Compound **34** was prepared from 1,3-bis(2-nitro-4-cyanophenoxy)propane (**78**).[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **34** (0.12 g, 22%); mp 281−283 °C. ^{1}H NMR (DMSO-d_6), δ 9.54 (s, 4H), 9.30 (s, 4H), 8.46 (d, $J = 1.6$ Hz, 2H), 8.18 (dd, $J_1 = 9.3$ Hz, $J_2 = 1.6$ Hz, 2H), 7.61 (d, $J = 9.3$ Hz, 2H), 4.47 (t, $J = 5.5$ Hz, 4H), 2.29 (m, 2H). HPLC (method A) $t_R = 4.42$ min (100 area %); m/z 403.2 (MH^+ of free base). Anal. ($C_{17}H_{18}N_6O_6 \cdot 2HCl \cdot 0.3H_2O$) C, H, N, Cl.

1,3-Bis-[3-amino-(2-imidazolinyl)benzene-4-oxy]propane Tetrahydrochloride (36). 1,3-Bis(2-nitro-4-cyanophenoxy)propane (**78**)[66] (1.80 g, 4.90 mmol) underwent Pinner reaction followed by the interaction with ethylenediamine as described above for compound **1**. A crude material was recrystallized from 1 N aqueous HCl to afford 1,3-bis-[3-nitro-(2-imidazolinyl)benzene-4-oxy]propane dihydrochloride (0.68 g, 34%). ^{1}H NMR (DMSO-d_6), δ 10.65 (s, 4H), 8.59 (d, $J = 1.8$ Hz, 2H), 8.25 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.8$ Hz, 2H), 7.67 (d, $J = 8.2$ Hz, 2H), 4.47 (t, $J = 6.0$ Hz, 4H), 3.98 (s, 8H), 2.30 (m, 2H); m/z 455.0 (MH^+ of free base).

A suspension of 1,3-bis-[3-nitro-(2-imidazolinyl)benzene-4-oxy]propane dihydrochloride (0.68 g, 1.50 mmol) and 10% Pd/C (0.1 g) in MeOH (150 mL) was stirred at 50 psi of H_2 for 1 h. The mixture was filtered through a pad of celite and a filtrate was concentrated to give a crude product, which was recrystallized from 1 N HCl/EtOH to yield compound **36** (0.40 g, 67%); mp 283−286 °C. ^{1}H NMR (DMSO-d_6), δ 10.45 (s, 4H), 7.51 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.8$ Hz, 2H), 7.41 (d, $J = 1.8$ Hz, 2H), 7.21 (d, $J = 8.2$ Hz, 2H), 4.37 (t, $J = 6.0$ Hz, 4H), 4.11 (br s, 6H), 3.99 (s, 8H), 2.29 (m, 2H). HPLC (method A) $t_R = 4.88$ min (98.2 area %); m/z 395.2 (MH^+ of free base). Anal. ($C_{21}H_{26}N_6O_2 \cdot 4HCl$) C, H, N.

1,3-Bis[3-methoxy-(*N*-methyl)benzamidine-4-oxy]propane Dihydrochloride (38). Compound **38** was prepared from 1,5-bis(2-methoxy-4-cyanophenoxy)pentane (**76**).[94] A crude material was recrystallized from EtOH/diethyl ether followed by treatment with 3 N HCl to yield compound **38** (1.20 g, 50%); mp 167−169 °C. ^{1}H NMR (DMSO-d_6) (free base), δ 9.50 (br s, 4H), 7.57 (s, 1H), 7.51 (d, $J = 8.2$ Hz, 1H), 7.40 (d, $J = 6.7$ Hz, 1H), 7.39 (s, 1H), 7.18 (d, $J = 8.0$ Hz, 1H), 7.17 (d, $J = 8.2$ Hz, 1H), 4.21 (t, $J = 5.0$ Hz, 4H), 3.88 (s, 3H), 3.81 (s, 3H), 3.40 (s, 6H), 2.22 (m, 2H). HPLC (method A) $t_R = 5.17$ min (98.2 area %); *m*/*z* 401.2 (MH$^+$ of free base). Anal. ($C_{21}H_{28}N_4O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N.

1,4-Bis(3-nitrobenzamidine-4-oxy)butane Dihydrochloride (41).[66] Compound **41** was prepared from 1,5-bis(2-nitro-4-cyanophenoxy)butane (**79**).[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **41** (0.35 g, 49%); mp 300−301 °C (lit.[66] 298 °C). ^{1}H NMR (DMSO-d_6), δ 9.53 (s, 4H), 9.28 (s, 4H), 8.46 (d, $J = 2.2$ Hz, 2H), 8.18 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 2H), 7.67 (d, $J = 8.8$ Hz, 2H), 4.38 (br s, 4H), 1.94 (br s, 4H). HPLC (method A) $t_R = 4.96$ min (100 area %); *m*/*z* 417.3 (MH$^+$ of free base). Anal. ($C_{18}H_{20}N_6O_6 \cdot 2HCl$) C, H, N, Cl.

1,5-Bis(3-nitrobenzamidine-4-oxy)pentane Dihydrochloride (45).[66] Compound **45** was prepared from 1,5-bis(2-nitro-4-cyanophenoxy)pentane (**80**).[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **45** (0.30 g, 33%); mp 261−263 °C (lit.[66] 255 °C). ^{1}H NMR (DMSO-d_6), δ 9.49 (s, 4H), 9.23 (s, 4H), 8.44 (d, $J = 2.2$ Hz, 2H), 8.14 (dd, $J_1 = 9.3$ Hz, $J_2 = 2.2$ Hz, 2H), 7.63 (d, $J = 9.3$ Hz, 2H), 4.32 (t, $J = 6.0$ Hz, 4H), 1.84 (m, 4H), 1.58 (m, 2H). HPLC (method A) $t_R = 6.02$ min (100 area %); *m*/*z* 431.7 (MH$^+$ of free base). Anal. ($C_{19}H_{22}N_6O_6 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis(3-methoxybenzamidine-4-oxy)pentane Dihydrochloride (46).[66] Compound **46** was prepared from 1,5-bis(2-methoxy-4-cyanophenoxy)pentane (**77**).[94] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **46** (0.45 g, 51%); mp 233−234 °C (lit.[66] 258−259 °C). ^{1}H NMR (DMSO-d_6), δ 9.29 (s, 4H), 9.04 (s, 4H), 7.51 (d, $J = 8.2$ Hz, 2H), 7.50 (s, 2H), 7.18 (d, $J = 8.2$ Hz, 2H), 4.10 (m, 4H), 3.86 (s, 6H), 1.83 (m, 4H), 1.57 (m, 2H). HPLC (method A) $t_R = 5.91$ min (100 area %). Anal. ($C_{21}H_{28}N_4O_4 \cdot 2HCl \cdot 0.1H_2O$) C, H, N, Cl.

1,5-Bis-[3-methoxy-(2-imidazolinyl)benzene-4-oxy]pentane Dihydrochloride (47).[67,94] Compound **47** was prepared from 1,5-bis(2-methoxy-4-cyanophenoxy)pentane (**77**).[94] A crude material was recrystallized from 1 N HCl to yield compound **47** (2.40 g, 62%); mp 175 °C (lit.[94] 188 °C). ^{1}H NMR (DMSO-d_6), δ 9.45 (s, 4H), 7.83 (s, 2H), 7.75 (d, $J = 8.2$ Hz, 2H), 7.20 (d, $J = 8.2$ Hz, 2H), 4.17 (m, 4H), 4.00 (s, 8H), 3.85 (s 6H), 1.80 (m 6H). HPLC (method A) $t_R = 6.97$ min (98.4 area %); *m*/*z* 453.2 (MH$^+$ of free base). Anal. ($C_{25}H_{32}N_4O_4 \cdot 2HCl \cdot 2.5H_2O$) C, H, N.

1,5-Bis(4-amidino-2-ethoxycarbonylphenoxy)pentane Dihydrochloride (48). Compound **48** was synthesized from 1,5-bis(4-cyano-2-ethoxycarbonylphenoxy)pentane (**84**). A reaction product, containing a mixture of amides **48**, **54**, and **55**, was separated by preparative HPLC. The purified component was recrystallized from 1 N HCl/EtOH to give compound **48** (0.08 g, 3%); mp 133−135 °C. ^{1}H NMR (DMSO-d_6), δ 9.34 (s, 4H), 9.06 (s, 4H), 8.14 (s, 2H), 8.03 (d, $J = 8.8$ Hz, 2H), 7.40 (d, $J = 8.8$ Hz, 2H), 4.29 (q, $J = 7.1$ Hz, 2H), 4.27 (q, $J = 7.1$ Hz, 2H), 4.20 (t, $J = 5.5$ Hz, 4H), 1.84 (m, 4H), 1.65 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H), 1.27 (t, $J = 7.1$ Hz, 3H). HPLC (method A) $t_R = 7.31$ min (100 area %). Anal. ($C_{25}H_{32}N_4O_6 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis[3-ethoxycarbonyl(*N*-isopropyl)benzamidine-4-oxy-]pentane Dihydrochloride (49). Compound **49** was prepared from 1,5-bis(4-cyano-2-ethoxycarbonylphenoxy)pentane (**84**). A very hygroscopic crude material was obtained, which was recrystallized from dry EtOH saturated with HCl to yield compound **49** (0.49 g, 53%); mp 220−222 °C. ^{1}H NMR (DMSO-d_6), δ 9.53 (d, $J = 8.2$ Hz, 2H), 9.40 (s, 2H), 9.02 (s, 2H), 7.99 (d, $J = 2.2$ Hz, 2H), 7.90 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 2H), 7.37 (d, $J = 8.8$ Hz, 2H), 4.29 (q, $J = 7.1$ Hz, 4H), 4.18 (t, $J = 6.0$ Hz, 4H), 4.03 (m, 2H), 1.84 (m, 4H), 1.65 (m, 2H), 1.28 (m, 18H). HPLC (method B) $t_R = 2.74$ min (98.5 area %). Anal. ($C_{31}H_{44}N_4O_6 \cdot 2HCl$) C, H, N, Cl.

1,5-Bis[3-ethoxycarbonyl-(2-imidazolinyl)benzene-4-oxy]pentane Dihydrochloride (50). Compound **50** was prepared from 1,5-bis(4-cyano-2-ethoxycarbonylphenoxy)pentane (**84**). A crude material was recrystallized from EtOH saturated with HCl to yield compound **50** (0.65 g, 33%); mp 232−235 °C. ^{1}H NMR (DMSO-d_6), δ 10.58 (s, 4H), 8.31 (d, $J = 2.7$ Hz, 2H), 8.19 (dd, $J_1 = 9.3$ Hz, $J_2 = 2.7$ Hz, 2H), 7.45 (d, $J = 9.3$ Hz, 2H), 4.29 (q, $J = 7.1$ Hz, 4H), 4.21 (t, $J = 6.0$ Hz, 4H), 3.98 (s, 8H), 1.83 (m, 4H), 1.65 (m, 2H), 1.27 (t, $J = 7.1$ Hz, 6H). HPLC (method B) $t_R = 2.12$ min (100 area %). Anal. ($C_{29}H_{36}N_4O_6 \cdot 2HCl \cdot 2.5H_2O$) C, H, N, Cl.

Syntheses of amidinoacids (51−53). 1,5-Bis(4-amidino-2-carboxyphenoxy)pentane (51). A suspension of 1,5-bis(4-amidino-2-ethoxycarbonylphenoxy)pentane dihydrochloride (**48**) (0.07 g, 0.13 mmol) in 3 N HCl was kept at 70 °C overnight. The solvent was evaporated and a light-brown residue was recrystallized from 1 N HCl to yield compound **51** (0.015 g, 24%); mp 233−235 °C. ^{1}H NMR (DMSO-d_6), δ 9.26 (s, 4H), 8.85 (s, 4H), 8.14 (d, $J = 2.2$ Hz, 2H), 7.97 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 2H), 7.37 (d, $J = 8.8$ Hz, 2H), 4.18 (br s, 4H), 1.83 (m, 4H), 1.61 (m, 2H). HPLC (method A) $t_R = 3.72$ min (100 area %). Anal. ($C_{21}H_{24}N_4O_6 \cdot 2HCl \cdot 2.5H_2O$) C, H, N.

1,5-Bis[3-carboxy-(*N*-isopropyl)benzamidine-4-oxy]pentane Dihydrochloride (52). Following the procedure described above for the compound **51**, **52** was prepared from 1,5-bis[3-ethoxycarbonyl(*N*-isopropyl)benzamidine-4-oxy]pentane (**49**). A crude product was recrystallized from 1 N HCl/EtOH to yield compound **52** (0.12 g, 66%); mp 198−200 °C. ^{1}H NMR (DMSO-d_6), δ 9.49 (d, $J = 6.6$ Hz, 2H), 9.35 (s, 2H), 8.97 (s, 2H), 8.00 (s, 2H), 7.87 (d, $J = 8.2$ Hz, 2H), 7.34 (d, $J = 8.2$ Hz, 2H), 4.16 (br s, 4H), 4.01 (m, 2H), 1.82 (m, 4H), 1.62 (m, 2H), 1.26 (d, $J = 4.9$ Hz, 12H). HPLC (method A) $t_R = 5.50$ min (100 area %). Anal. ($C_{27}H_{36}N_4O_6 \cdot 2HCl \cdot 0.9H_2O \cdot 0.5EtOH$) C, H, N, Cl.

1,5-Bis[3-carboxy-(2-imidazolinyl)benzene-4-oxy]pentane Dihydrochloride (53). Following the procedure described above for the compound **51**, **53** was prepared from 1,5-bis[3-ethoxycarbonyl-(2-imidazolinyl)benzene-4-oxy]pentane (**50**). A crude product was recrystallized twice from 1 N HCl to yield compound **53** (0.12 g, 26%); mp 187−189 °C. ^{1}H NMR (DMSO-d_6), δ 10.56 (s, 4H), 8.30 (s, 2H), 8.17 (d, $J = 8.2$ Hz, 2H), 7.42 (d, $J = 8.2$ Hz, 2H), 4.18 (br s, 4H), 3.97 (s, 8H), 1.82 (m, 4H), 1.63 (m, 2H). HPLC (method A) $t_R = 4.44$ min (100 area %). Anal. ($C_{25}H_{28}N_4O_6 \cdot 2.2HCl \cdot 2H_2O$) C, H, N, Cl.

1-(4-Amidino-2-ethoxycarbonylphenoxy)-5-(4-amidino-2-aminocarbonylphenoxy)pentane Dihydrochloride (54). The reaction mixture described above for the compound **48** was separated by preparative HPLC and a product was recrystallized from 1 N HCl to yield compound **54** (0.10 g, 4%); mp 168−170 °C. ^{1}H NMR (DMSO-d_6), δ 9.30 (s, 4H), 8.94 (s, 2H), 8.90 (s, 2H), 8.25 (s, 1H), 8.13 (s, 1H), 8.01 (m, 2H), 7.70 (m, 2H), 7.39 (m, 2H), 4.28 (q, $J = 7.1$ Hz, 2H), 4.19 (m, 4H), 1.84 (m, 4H), 1.63 (m, 2H), 1.29 (t, $J = 7.1$ Hz, 3H). HPLC (method B) $t_R = 0.80$ min (100 area %); *m*/*z* 456.4 (MH$^+$ of free base). Anal. ($C_{23}H_{29}N_5O_5 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis(4-amidino-2-aminocarbonylphenoxy)pentane Dihydrochloride (55). The reaction mixture described above for the compound **48** was separated by preparative HPLC and a product was recrystallized from 1 N HCl to yield compound **55** (0.13 g, 6%); mp 208−210 °C. ^{1}H NMR (DMSO-d_6), δ 9.33 (s, 4H), 9.02 (s, 4H), 8.24 (d, $J = 2.7$ Hz, 2H), 7.96 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.7$ Hz, 2H), 7.76 (s, 2H), 7.64 (s, 2H), 7.38 (d, $J = 8.8$ Hz, 2H), 4.25 (m, 4H), 1.88 (m, 4H), 1.60 (m, 2H). HPLC (method B) $t_R = 0.57$ min (100 area %); *m*/*z* 427.4 (MH$^+$ of free base). Anal. ($C_{21}H_{26}N_6O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis(2-hydroxybenzamidine-4-oxy)pentane Dihydrochloride (56). Compound **56** was prepared from 1,5-bis(4-cyano-3-hydroxyphenoxy)pentane (**88**). A crude product was separated by preparative HPLC and treated with ethanol saturated with HCl to yield compound **56** (0.13 g, 27%); mp 145−148 °C. ^{1}H NMR (DMSO-d_6), δ 9.00 (br s, 6H), 8.70 (s, 4H), 7.61 (d, $J = 8.8$ Hz, 2H), 6.62 (d, $J = 2.3$ Hz, 2H), 6.55 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.3$ Hz, 2H), 4.03 (t, $J = 5.5$ Hz, 4H), 1.75 (m, 4H), 1.53 (m, 2H). HPLC (method A) $t_R = 6.24$ min (100 area %); m/z 373.7 (MH$^+$ of free base). Anal. ($C_{19}H_{24}N_4O_4 \cdot 2HCl \cdot 0.9H_2O$) C, H, N, Cl.

1,5-Bis[2-hydroxy-(*N*-isopropyl)benzamidine-4-oxy]pentane Dihydrochloride (57). Compound **57** was prepared from 1,5-bis(4-cyano-3-hydroxyphenoxy)pentane (**88**). A crude product was separated by preparative HPLC and treated with ethanol saturated with HCl to yield compound **57** (0.11 g, 29%); mp 123−125 °C. ^{1}H NMR (DMSO-d_6), δ 11.02 (br s, 2H), 9.18 (d, $J = 7.6$ Hz, 2H), 8.97 (s, 2H), 8.81 (s, 2H), 7.34 (d, $J = 8.6$ Hz, 2H), 6.68 (d, $J = 2.3$ Hz, 2H), 6.54 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.3$ Hz, 2H), 3.99 (t, $J = 6.5$ Hz, 4H), 3.97 (m, 2H), 1.75 (m, 4H), 1.53 (m, 2H), 1.22 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 7.87$ min (100 area %); m/z 457.3 (MH$^+$ of free base). Anal. ($C_{25}H_{36}N_4O_4 \cdot 2HCl \cdot 2.6H_2O$) C, H, N, Cl.

1,5-Bis-[2-hydroxy(2-imidazolinyl)benzene-4-oxy]pentane Dihydrochloride (58). Compound **58** was prepared from 1,5-bis(4-cyano-3-hydroxyphenoxy)pentane (**88**). A crude product was separated by preparative HPLC and treated with ethanol saturated with HCl to yield compound **58** (0.11 g, 29%); mp 128−131 °C. ^{1}H NMR (DMSO-d_6), δ 12.12 (s, 2H), 9.74 (s, 4H), 7.85 (d, $J = 9.0$ Hz, 2H), 6.79 (d, $J = 2.4$ Hz, 2H), 6.63 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H), 4.04 (t, $J = 6.4$ Hz, 4H), 3.89 (s, 8H), 1.78 (m, 4H), 1.53 (m, 2H). HPLC (method A) $t_R = 7.10$ min (100 area %); m/z 425.3 (MH$^+$ of free base). Anal. ($C_{23}H_{28}N_4O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis(2-fluorobenzamidine-4-oxy)pentane Dihydrochloride (59). Compound **59** was prepared from 1,5-bis(4-cyano-3-fluorophenoxy)pentane (**89**). A crude product was separated by preparative HPLC and treated with ethanol saturated with HCl to yield compound **59** (0.10 g, 9%); mp 245−247 °C. ^{1}H NMR (DMSO-d_6), δ 9.30 (s, 4H), 9.24 (s, 4H), 7.63 (m, 2H), 7.70 (d, $J = 11.8$ Hz, 2H), 6.98 (d, $J = 10.2$ Hz, 2H), 4.11 (t, $J = 6.2$ Hz, 4H), 1.81 (m, 4H), 1.55 (m, 2H). HPLC (method A) $t_R = 6.27$ min (100 area %); m/z 377.2 (MH$^+$ of free base). Anal. ($C_{19}H_{22}F_2\ N_4O_2 \cdot 2HCl$) C, H, N, Cl, F.

1,5-Bis[2-fluoro-(*N*-isopropyl)benzamidine-4-oxy]pentane Dihydrochloride (60). Compound **60** was prepared from the compound (**89**). A crude product was separated by preparative HPLC and treated with ethanol saturated with HCl to yield compound **60** (0.23 g, 20%); mp 254−256 °C. ^{1}H NMR (DMSO-d_6), δ 9.69 (d, $J = 7.6$ Hz, 2H), 9.46 (s, 2H), 9.29 (s, 2H), 7.56 (t, $J = 8.5$ Hz, 2H), 7.12 (dd, $J_1 = 12.8$ Hz, $J_2 = 2.2$ Hz, 2H), 6.96 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.0$ Hz, 2H), 4.10 (t, $J = 6.5$ Hz, 4H), 4.06 (m, 2H), 1.79 (m, 4H), 1.57 (m, 2H), 1.24 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 8.02$ min (100 area %); m/z 461.8 (MH$^+$ of free base). Anal. ($C_{25}H_{34}F_2N_4O_2 \cdot 2HCl \cdot 0.85H_2O$) C, H, N, Cl, F.

1,5-Bis-[2-fluoro(2-imidazolinyl)benzene-4-oxy]pentane Dihydrochloride (61). Compound **61** was prepared from the dinitrile **89**. A crude product was recrystallized from 1 N HCl to yield compound **61** (0.27 g, 27%); mp 216−218 °C. ^{1}H NMR (DMSO-d_6), δ 10.40 (s, 4H), 7.98 (t, $J = 8.8$ Hz, 2H), 7.18 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 2H), 7.05 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.2$ Hz, 2H), 4.15 (t, $J = 6.4$ Hz, 4H), 3.95 (s, 8H), 1.83 (m, 4H), 1.56 (m, 2H). HPLC (method A) $t_R = 7.19$ min (98.0 area %). Anal. ($C_{23}H_{26}F_2N_4O_2 \cdot 2HCl \cdot 0.9H_2O$) C, H, N, Cl, F.

1,5-Bis(benzamidine-4-thio)pentane Dihydrochloride (62).[91,103] Compound **62** was prepared from 1,5-bis[(4-cyano)phenylthio]pentane (**90**) as a white solid (1.88 g, 41%); mp 150 °C (dec). ^{1}H NMR (DMSO-d_6), δ 9.34 (s, 4H), 9.09 (s, 4H), 7.77 (d, $J = 8.7$ Hz, 4H), 7.49 (d, $J = 8.7$ Hz, 4H), 3.10 (t, $J = 6.8$ Hz, 4H), 1.64 (m, 6H). HPLC (method A) $t_R = 7.45$ min (99.4 area %); m/z 373.3 (MH$^+$ of free base). Anal. ($C_{19}H_{24}N_4S_2 \cdot 2HCl \cdot 0.75H_2O$) C, H, N, Cl.

1,5-Bis(benzamidine-4-sulfonyl)pentane Dihydrochloride (63). Compound **63** was prepared from 1,5-bis[(4-cyano)phenylsulfonyl]pentane (**91**). The crude product was purified by preparative HPLC to yield compound **63** as a white solid (0.48 g, 37%); mp 225 °C (dec). ^{1}H NMR (DMSO-d_6), δ 9.64 (s, 4 H), 9.42 (s, 4H), 8.12 (d, $J = 8.5$ Hz, 4H), (8.06, d, $J = 8.5$ Hz, 4H), 3.42 (m, 4H), 1.54 (m, 4 H), 1.44 (m, 2H). HPLC (method A) $t_R = 3.37$ min (100 area %); m/z 437.0 (MH$^+$ of free base). Anal. ($C_{19}H_{24}N_4S_2O_4 \cdot 2HCl \cdot 0.4H_2O$) C, H, N, Cl.

1,5-Bis(benzamidine-4-amino)pentane Tetrahydrochloride (64).[50,66] Compound **64**was prepared from 1,5-bis(1-cyanobenzene-4-amino)pentane.[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **64** (0.43 g, 49%); mp 215 °C (dec) (lit.[66] 295 °C). ^{1}H NMR (DMSO-d_6), δ 8.89 (s, 4H), 8.70 (s, 4H), 8.19 (br s, 4H), 7.69 (d, $J = 8.8$ Hz, 4H), 6.69 (d, $J = 8.8$ Hz, 4H), 3.10 (br s, 4H), 1.58 (br s, 4H), 1.46 (br s, 2H). HPLC (method A) $t_R = 5.69$ min (100 area %). Anal. ($C_{19}H_{26}N_6 \cdot 4HCl \cdot 0.2H_2O$) C, H, N, Cl.

1,5-Bis[(*N*-isopropyl)benzamidine-4-amino]pentane Tetrahydrochloride (65). Compound **65** was prepared from 1,5-bis(1-cyanobenzene-4-amino)pentane.[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **65** (0.47 g, 49%); mp 199−202 °C. ^{1}H NMR (DMSO-d_6), δ 9.03 (br s, 4H), 8.65 (s, 2H), 7.56 (d, $J = 8.8$ Hz, 4H), 7.29 (br s, 4H), 6.70 (d, $J = 8.8$ Hz, 4H), 4.03 (m, 2H), 3.11 (br s, 4H), 1.58 (br s, 4H), 1.46 (br s, 2H), 1.25 (d, $J = 6.0$ Hz, 12H). HPLC (method A) $t_R = 6.94$ min (100 area %). Anal. ($C_{25}H_{38}N_6 \cdot 4HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis[4-(2-imidazolinyl)benzene-1-amino]pentane Dihydrochloride (66). Compound **66** was prepared from 1,5-bis(1-cyanobenzene-4-amino)pentane.[66] A crude product was purified by flash chromatography on C_{18} reversed phase silica gel to yield compound **66** (0.57 g, 58%); mp 182−185 °C. ^{1}H NMR (DMSO-d_6), δ 10.06 (s, 4H), 7.78 (d, $J = 8.8$ Hz, 4H), 7.06 (br s, 2H), 6.69 (d, $J = 8.8$ Hz, 4H), 3.88 (s, 8H), 3.11 (br s, 4H), 1.58 (m, 4H), 1.46 (m, 2H). HPLC (method A) $t_R = 5.86$ min (100 area %). Anal. ($C_{23}H_{30}N_6 \cdot 2HCl \cdot 1.5H_2O$) C, H, N, Cl.

6-Bis(3-cyanophenoxy)hexane (74). Potassium carbonate (5.00 g, 36.0 mmol) was added to a solution of 3-cyanophenol (3.00 g, 25.0 mmol) in dry DMF (50 mL) at 130 °C. The suspension was stirred at 130 °C for 20 min, and a solution of 1,6-dibromohexane (3.20 g, 13.0 mmol) in dry DMF (20 mL) was added dropwise. The reaction mixture was kept at 130−140 °C for 3 h, cooled to ambient temperature, and poured into ice−water mixture. A formed precipitate was filtered off, washed with water, dried, and recrystallized from 70% aqueous EtOH to yield compound **74** (3.60 g, 89%); mp 91−93 °C. ^{1}H NMR (DMSO-d_6), δ 7.48 (dd, $J_1 = 8.2$ Hz, $J_2 = 7.6$ Hz, 2H), 7.39 (d, $J = 1.6$ Hz, 2H), 7.37 (d, $J = 7.6$ Hz, 2H), 7.27 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.6$ Hz, 2H), 4.04 (t, $J = 6.6$ Hz, 4H), 1.74 (m, 4H), 1.47 (br s, 4H). HPLC (method B) $t_R = 9.15$ min (100 area %). Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

1-(4-Cyanophenoxy)-3-[(2-imidazolinyl)benzene-4-oxy]propane (81). A mixture of 4-(4,5-dihydro-1*H*-imidazol-2-yl)-phenol[100] (1.35 g, 8.30 mmol) and sodium bicarbonate (1.43 g, 17.0 mmol) in absolute ethanol (50 mL) was refluxed for 2 h. A solution of 1-bromo-3-(4-cyanophenoxy)propane[44,96,101] (2.00 g, 8.30 mmol) in absolute ethanol (50 mL) was added and the resulting mixture was refluxed for 7 days. A formed precipitate was collected, recrystallized twice from 50% EtOH, and was dried under high vacuum to yield compound **81** (0.70 g, 22%); mp 194−197 °C. ^{1}H NMR (DMSO-d_6), δ 8.05 (d, $J = 8.8$ Hz, 2H), 7.80 (d, $J = 8.8$ Hz, 2H), 7.25 (d, $J = 8.8$ Hz, 2H), 7.17 (d, $J = 8.8$ Hz, 2H), 4.20 (m, 4H), 4.05 (s, 4H), 2.25 (m, 2H). HPLC (method B) $t_R = 3.67$ min (98.0 area %).

1-(4-Cyanophenoxy)-3-(4-ethoxycarbonylphenoxy)propane (82). A mixture of ethyl 4-hydroxybenzoate (0.70 g, 4.20 mmol) and KOH (0.24 g, 4.30 mmol) in dry ethanol (20 mL) was refluxed for 1 h. A solution of 4-(3-bromopropoxy)-benzonitrile[44] (1.00 g, 4.20 mmol) in dry ethanol (10 mL) was added dropwise. The resulting mixture was refluxed for 1 day and a solid residue was filtered off. A filtrate was cooled to 0 °C and a formed precipitate was filtered off and recrystallized from EtOH to yield compound **82** (0.75 g, 54%); mp 109−110 °C. ^{1}H NMR ($CDCl_3$), δ 8.13 (d, $J = 8.8$ Hz, 2H), 7.65 (d, $J = 8.8$ Hz, 2H), 7.01 (d, $J = 8.8$ Hz, 2H), 6.96 (d, $J = 8.8$ Hz, 2H), 4.40 (q, $J = 6.5$ Hz, 2H), 4.27 (t, $J = 6.0$ Hz, 4H), 2.32 (m, 2H), 1.39 (t, $J = 6.5$ Hz, 3H). HPLC (method B) $t_R = 8.99$ min (98.0 area %).

1,5-Bis(4-bromo-2-ethoxycarbonylphenoxy)pentane (83). To a stirred solution of ethyl 5-bromosalicylate (16.0 g, 65.0 mmol) in dry DMF (100 mL) at 125−130 °C was added dried K_2CO_3 (16.0 g, 65.3 mmol). The mixture was stirred under argon at this temperature for 25 min, and a solution of 1,5-dibromopentane (7.59 g, 33.0 mmol) in DMF (50 mL) was added dropwise over 1.5 h. A progress of the reaction was monitored by HPLC. After about 1.5 h, the reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (400 mL), and washed with water (3 × 100 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a crude oily product (19.3 g). The crude material was purified by column chromatography eluting with EtOAc/hexanes (gradient 5 to 20%) to yield compound **83** (8.90 g, 48%); mp 107−108 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6), δ 7.73 (m, 2H), 7.68 (m, 2H), 7.12 (m, 2H), 4.23 (m, 4H), 4.05 (m, 4H), 1.78 (br s, 4H), 1.61 (br s, 2H), 1.25 (m, 6H). HPLC (method B) $t_R = 10.98$ min (100 area %). Anal. ($C_{23}H_{26}Br_2O_6$) C, H, Br.

1,5-Bis(4-cyano-2-ethoxycarbonylphenoxy)pentane (84). To a suspension of compound **83** (7.40 g, 13.0 mmol) in dry DMF was added dried CuCN (3.70 g, 41.0 mmol). The mixture was refluxed for 22 h, cooled to ambient temperature, diluted with EtOAc (500 mL), and washed with concentrated aqueous ammonia (300 mL) and water (2 × 300 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to give a crude brown product (5.40 g, 90%). The crude material was purified by column chromatography eluting with EtOAc/hexanes (1/1) to yield compound **84** (3.70 g, 62%); mp 114−115 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6), δ 8.04 (d, $J = 2.2$ Hz, 2H), 7.99 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 2H), 7.33 (d, $J = 8.8$ Hz, 2H), 4.25 (q, $J = 7.1$ Hz, 4H), 4.16 (t, $J = 6.0$ Hz, 4H), 1.81 (m, 4H), 1.61 (m, 2H), 1.26 (t, $J = 7.1$ Hz, 6H). HPLC (method B) $t_R = 8.57$ min (98.7 area %). Anal. ($C_{25}H_{26}N_2O_6$) C, H, N.

2-(Benzyloxy)-4-hydroxybenzonitrile (85). To a suspension of 4-amino-2-(benzyloxy)benzonitrile[102] (1.00 g, 4.50 mmol) in ice water (20 mL) was slowly added cold 30% H_2SO_4 (15 mL) to maintain temperature below 5−7 °C. A solution of $NaNO_2$ (1.00 g, 15.0 mmol) in water (15 mL) was added dropwise, and the resulting mixture was stirred for 30 min at temperature below 5−7 °C. After that, the reaction mixture was warmed to ambient temperature and then kept at 80 °C for 1 h. The mixture was cooled, diluted with EtOAc (200 mL), washed with brine (2 × 100 mL), dried over $MgSO_4$, and concentrated to give crude material (1.10 g). The crude product was purified by column chromatography eluting with EtOAc/hexanes (3/7) and recrystallized from a mixture of chloroform/hexane (3/1) to yield compound **85** (0.52 g, 52%); mp 108−110 °C. ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 6H), 6.48 (s, 1H), 6.45 (dd, $J_1 = 2.2$ Hz, $J_2 = 1.0$ Hz, 1H), 5.73 (s, 1H), 5.16 (s, 2H). HPLC (method B) $t_R = 5.20$ min (100 area %); m/z 225.9 (MH^+). Anal. ($C_{14}H_{11}NO_2 \cdot 0.2H_2O$) C, H, N.

1,5-Bis[4-cyano-3-(benzyloxy)-phenoxy]pentane (87). Following the procedure described above for synthesis of the compound **83**, **87** was prepared from 2-(benzyloxy)-4-hydroxybenzonitrile (**85**) and 1,5-dibromopentane. A crude product (3.70 g) was recrystallized from diethyl ether to yield compound **87** (1.90 g, 67%); mp 122−124 °C. ^{1}H NMR (DMSO-d_6), δ 7.65 (d, $J = 8.6$ Hz, 2H), 7.42 (m, 10H), 6.84 (d, $J = 1.9$ Hz, 2H), 6.67 (dd, $J_1 = 8.6$ Hz, $J_2 = 1.9$ Hz, 2H), 5.27 (s, 4H), 4.08 (t, $J = 6.6$ Hz, 4H), 1.78 (m, 4H), 1.52 (m, 2H). HPLC (method B) $t_R = 10.59$ min (100 area %). Anal. ($C_{33}H_{30}N_2O_4 \cdot 0.7H_2O$) C, H, N.

1,5-Bis(4-cyano-3-hydroxyphenoxy)pentane (88). A suspension of 1,5-bis[4-cyano-3-(benzyloxy)-phenoxy]pentane (**87**) (1.50 g, 2.90 mmol) and 10% Pd/C (0.4 g) in EtOH (200 mL) was hydrogenated at 50 psi of H_2 for 14 h. The mixture was filtered through a pad of celite, and the filtrate was concentrated to give a yellow crude solid, which was washed with diethyl ether to yield compound **88** (0.95 g, 97%); mp 208−212 °C. ^{1}H NMR (DMSO-d_6), δ 11.00 (br s, 2H), 7.49 (d, $J = 8.2$ Hz, 2H), 6.51 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.8$ Hz, 2H), 6.49 (d, $J = 1.8$ Hz, 2H), 4.00 (t, $J = 6.0$ Hz, 4H), 1.77 (m, 4H), 1.54 (m, 2H). HPLC (method B) $t_R = 5.79$ min (100 area %). Anal. ($C_{19}H_{18}N_2O_4 \cdot 0.2H_2O$) C, H, N.

1,5-Bis(4-cyano-3-fluorophenoxy)pentane (89). Following the procedure described above for the synthesis of compound **69**, dinitrile **89** was prepared from 2-fluoro-4-hydroxybenzonitrile and 1,5-dibromopentane (0.64 g, 51%); mp 97−98 °C. ^{1}H NMR (DMSO-d_6), δ 7.81 (t, $J = 8.2$ Hz, 2H), 7.15 (dd, $J_1 = 12$ Hz, $J_2 = 2.4$ Hz, 2H), 6.96 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz, 2H), 4.11 (t, $J = 6.3$ Hz, 4H), 1.80 (m, 4H), 1.56 (m, 2H). HPLC (method B) $t_R = 8.76$ min (100 area %). Anal. ($C_{19}H_{16}F_2N_2O_2$) C, H, N.

1,5-Bis[(4-cyano)phenylthio]pentane (90). Following the procedure described above for the synthesis of dinitrile **74**, compound **90** was prepared from 4-mercaptobenzonitrile[104] and 1,5-dibromobenzene. A crude product was recrystallized from acetonitrile to give **90** as white crystals (9.56 g, 94%); mp 121 °C. ^{1}H NMR (DMSO-d_6), δ 7.73 (d, $J = 8.4$ Hz, 4H), 7.44 (d, $J = 8.4$ Hz, 4H), 3.07 (t, $J = 7.0$ Hz, 4H), 1.65 (m, 4H), 1.58 (m, 2H). HPLC (method B) $t_R = 9.38$ min (100 area %). Anal. ($C_{19}H_{18}N_2S_2$) C, H, N.

1,5-Bis[(4-cyano)phenylsulfonyl]pentane (91). A solution of dinitrile (**90**) (3.39 g, 10.0 mmol) in acetonitrile (150 mL) and ethanol (150 mL) was added dropwise to a mixture of periodic acid (10.0 g, 43.9 mmol) and chromium trioxide (0.25 mg, 2.50 mmol) in acetonitrile (200 mL). After the mixture was stirred overnight, more periodic acid (5.00 g) and chromium oxide (0.27 g) were added. After 2 h, the mixture was filtered through a sintered funnel. The filtrate was washed with saturated solution of $Na_2S_2O_3$ and then with brine. The organic solution was dried over $MgSO_4$ and concentrated. The product was recrystallized from ethanol with a little acetonitrile to give **91** as white crystals (2.97 g, 74%); mp 217 °C. ^{1}H NMR (DMSO-d_6), δ 8.16 (d, $J = 8.3$ Hz, 4H), 8.07 (d, $J = 8.3$ Hz, 4H), 3.38 (m, 4H), 1.51 (m, 4H), 1.39 (m, 2H). HPLC (method B) $t_R = 5.22$ min (100 area %). Anal. ($C_{19}H_{18}N_2O_4S_2$) C, H, N.

Acknowledgment. This work was supported by the Bill and Melinda Gates Foundation and the Medicines for Malaria Venture Foundation.

Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Greenwood, B.; Mutabingwa, T. Malaria in 2002. *Nature* **2002**, *415*, 670–672.

(2) Guerin, P. J.; Olliaro, P.; Nosten, F.; Druilhe, P.; Laxminarayan, R.; Binka, F.; Kilama, W. L.; Ford, N.; White, N. J. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *Lancet Infect. Dis.* **2002**, *2*, 564–573.

(3) Biagini, G. A.; O'Neill, P. M.; Bray, P. G.; Ward, S. A. Current drug development portfolio for antimalarial therapies. *Curr. Opin. Pharmacol.* **2005**, *5*, 473–478.

(4) Tripathi, R. P.; Mishra, R. C.; Dwivedi, N.; Tewari, N.; Verma, S. S. Current status of malaria control. *Curr. Med. Chem.* **2005**, *12*, 2643–2659.

(5) Linares, G. E.; Rodriguez, J. B. Current status and progresses made in malaria chemotherapy. *Curr. Med. Chem.* **2007**, *14*, 289–314.

(6) Miller, L. H.; Baruch, D. I.; Marsh, K.; Doumbo, O. K. The pathogenic basis of malaria. *Nature* **2002**, *415*, 673–679.

(7) Apted, F. I. Present status of chemotherapy and chemopropylaxis of human trypanosomiasis in the Eastern Hemisphere. *Pharmacol. Ther.* **1980**, *11*, 391–413.

(8) Burri, C.; Brun, R. Human African Trypanosomiasis. In *Manson's Tropical Diseases*; Cook, G. C., Zumla, A., Eds.;W. B. Saunders: London, 2008; pp 1307−1325.

(9) Maudlin, I. African trypanosomiasis. *Ann. Trop. Med. Parasitol.* **2006**, *100*, 679–701.

(10) Simarro, P. P.; Jannin, J.; Cattand, P. Eliminating human African trypanosomiasis: Where do we stand and what comes next? *PLoS Med.* **2008**, *5*, 174–180.

(11) Barrett, M. P.; Boykin, D. W.; Brun, R.; Tidwell, R. R. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br. J. Pharmacol.* **2007**, *152*, 1155–1171.

(12) Herwaldt, B. L. Leishmaniasis. *Lancet* **1999**, *354*, 1191–1199.

(13) Guerin, P. J.; Olliaro, P.; Sundar, S.; Boelaert, M.; Croft, S. L.; Desjeux, P.; Wasunna, M. K.; Bryceson, A. D. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed

research and development agenda. *Lancet Infect. Dis.* **2002**, *2*, 494–501.

(14) Croft, S. L.; Yardley, V. Chemotherapy of leishmaniasis. *Curr. Pharm. Des.* **2002**, *8*, 319–342.

(15) Singh, S.; Sivakumar, R. Challenges and new discoveries in the treatment of leishmaniasis. *J. Infect. Chemother.* **2004**, *10*, 307–315.

(16) Mishra, J.; Saxena, A.; Singh, S. Chemotherapy of leishmaniasis: past, present and future. *Curr. Med. Chem.* **2007**, *14*, 1153–1169.

(17) Weina, P. J.; Neafie, R. C.; Wortmann, G.; Polhemus, M.; Aronson, N. E. Old world leishmaniasis: an emerging infection among deployed US military and civilian workers. *Clin. Infect. Dis.* **2004**, *39*, 1674–1680.

(18) Peters, B. S.; Fish, D.; Golden, R.; Evans, D. A.; Bryceson, A. D.; Pinching, A. J. Visceral leishmaniasis in HIV infection and AIDS: clinical features and response to therapy. *Q. J. Med.* **1990**, *77*, 1101–1111.

(19) Cruz, I.; Nieto, J.; Moreno, J.; Canavate, C.; Desjeux, P.; Alvar, J. Leishmania/HIV co-infections in the second decade. *Indian J. Med. Res.* **2006**, *123*, 357–388.

(20) Walker, M.; Kublin, J. G.; Zunt, J. R. Parasitic central nervous system infections in immunocompromised hosts: malaria, microsporidiosis, leishmaniasis, and African trypanosomiasis. *Clin. Infect. Dis.* **2006**, *42*, 115–125.

(21) Karp, C. L.; Auwaerter, P. G. Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clin. Infect. Dis.* **2007**, *45*, 1208–1213.

(22) Lloyd-Smith, J. O.; Poss, M.; Grenfell, B. T. HIV-1/parasite co-infection and the emergence of new parasite strains. *Parasitology* **2008**, *135*, 795–806.

(23) Pintado, V.; Martin-Rabadan, P.; Rivera, M. L.; Moreno, S.; Bouza, E. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients. A comparative study. *Medicine (Baltimore)* **2001**, *80*, 54–73.

(24) Wolday, D.; Berhe, N.; Akuffo, H.; Desjeux, P.; Britton, S. Emerging Leishmania/HIV co-infection in Africa. *Med. Microbiol. Immunol.* **2001**, *190*, 65–67.

(25) Desjeux, P.; Alvar, J. Leishmania/HIV co-infections: epidemiology in Europe. *Ann. Trop. Med. Parasitol.* **2003**, *97* (Suppl 1), 3–15.

(26) Rabello, A.; Orsini, M.; Disch, J. Leishmania/HIV co-infection in Brazil: an appraisal. *Ann. Trop. Med. Parasitol.* **2003**, *97* (Suppl 1), 17–28.

(27) Bronzan, R. N.; Taylor, T. E.; Mwenechanya, J.; Tembo, M.; Kayira, K.; Bwanaisa, L.; Njobvu, A.; Kondowe, W.; Chalira, C.; Walsh, A. L.; Phiri, A.; Wilson, L. K.; Molyneux, M. E.; Graham, S. M. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. *J. Infect. Dis.* **2007**, *195*, 895–904.

(28) Sartori, A. M.; Ibrahim, K. Y.; Nunes Westphalen, E. V.; Braz, L. M.; Oliveira, O. C., Jr.; Gakiya, E.; Lopes, M. H.; Shikanai-Yasuda, M. A. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. *Ann. Trop. Med. Parasitol.* **2007**, *101*, 31–50.

(29) Wongsrichanalai, C.; Pickard, A. L.; Wernsdorfer, W. H.; Meshnick, S. R. Epidemiology of drug-resistant malaria. *Lancet Infect. Dis.* **2002**, *2*, 209–218.

(30) Le Bras, J.; Durand, R. The mechanisms of resistance to antimalarial drugs in *Plasmodium falciparum*. *Fundam. Clin. Pharmacol.* **2003**, *17*, 147–153.

(31) Arav-Boger, R.; Shapiro, T. A. Molecular mechanisms of resistance in antimalarial chemotherapy: the unmet challenge. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 565–585.

(32) Hyde, J. E. Drug-resistant malaria. *Trends Parasitol.* **2005**, *21*, 494–498.

(33) Platteeuw, J. J. Resistance to sulphadrug-based antifolate therapy in malaria: are we looking in the right place? *Trop. Med. Int. Health* **2006**, *11*, 804–808.

(34) Woodrow, C. J.; Krishna, S. Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance. *Cell Mol. Life. Sci.* **2006**, *63*, 1586–1596.

(35) Brun, R.; Schumacher, R.; Schmid, C.; Kunz, C.; Burri, C. The phenomenon of treatment failures in human African trypanosomiasis. *Trop. Med. Int. Health* **2001**, *6*, 906–914.

(36) Fairlamb, A. H. Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends Parasitol.* **2003**, *19*, 488–494.

(37) Sundar, S. Drug resistance in Indian visceral leishmaniasis. *Trop. Med. Int. Health* **2001**, *6*, 849–854.

(38) Sundar, S.; Chatterjee, M. Visceral leishmaniasis—current therapeutic modalities. *Indian J. Med. Res.* **2006**, *123*, 345–352.

(39) Jha, T. K. Drug unresponsiveness and combination therapy for kala-azar. *Indian J. Med. Res.* **2006**, *123*, 389–398.

(40) Barrett, M. P.; Gilbert, I. H. Perspectives for new drugs against trypanosomiasis and leishmaniasis. *Curr. Top. Med. Chem.* **2002**, *2*, 471–482.

(41) Loiseau, P. M.; Bories, C. Mechanisms of drug action and drug resistance in *Leishmania* as basis for therapeutic target identification and design of antileishmanial modulators. *Curr. Top. Med. Chem.* **2006**, *6*, 539–550.

(42) Murray, H. W.; Berman, J. D.; Davies, C. R.; Saravia, N. G. Advances in leishmaniasis. *Lancet* **2005**, *366*, 1561–1577.

(43) Sundar, S.; Jha, T. K.; Thakur, C. P.; Sinha, P. K.; Bhattacharya, S. K. Injectable paromomycin for visceral leishmaniasis in India. *N. Engl. J. Med.* **2007**, *356*, 2571–2581.

(44) Ashley, J. N.; Barber, H. J.; Ewins, A. J.; Newbery, G.; Self, A. D. H. Chemotherapeutic comparison of the trypanocidal action of some aromatic diamidines. *J. Chem. Soc.* **1942**, 103–116.

(45) Bouteille, B.; Oukem, O.; Bisser, S.; Dumas, M. Treatment perspectives for human African trypanosomiasis. *Fundam. Clin. Pharmacol.* **2003**, *17*, 171–181.

(46) Pearson, R. D.; Hewlett, E. L. Pentamidine for the treatment of *Pneumocystis carinii* pneumonia and other protozoal diseases. *Ann. Intern. Med.* **1985**, *103*, 782–786.

(47) Goa, K. L.; Campoli-Richards, D. M. Pentamidine isethionate. A review of its antiprotozoal activity, pharmacokinetic properties and therapeutic use in *Pneumocystis carinii* pneumonia. *Drugs* **1987**, *33*, 242–258.

(48) Monk, J. P.; Benfield, P. Inhaled pentamidine. An overview of its pharmacological properties and a review of its therapeutic use in *Pneumocystis carinii* pneumonia. *Drugs* **1990**, *39*, 741–756.

(49) Bray, P. G.; Barrett, M. P.; Ward, S. A.; de Koning, H. P. Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. *Trends Parasitol.* **2003**, *19*, 232–239.

(50) Bell, C. A.; Hall, J. E.; Kyle, D. E.; Grogl, M.; Ohemeng, K. A.; Allen, M. A.; Tidwell, R. R. Structure−activity relationships of analogs of pentamidine against *Plasmodium falciparum* and *Leishmania mexicana amazonensis*. *Antimicrob. Agents Chemother.* **1990**, *34*, 1381–1386.

(51) Stead, A. M.; Bray, P. G.; Edwards, I. G.; DeKoning, H. P.; Elford, B. C.; Stocks, P. A.; Ward, S. A. Diamidine compounds: selective uptake and targeting in *Plasmodium falciparum*. *Mol. Pharmacol.* **2001**, *59*, 1298–1306.

(52) Docampo, R.; Moreno, S. N. Current chemotherapy of human African trypanosomiasis. *Parasitol. Res.* **2003**, *90* (Supp 1), S10−13.

(53) Burchmore, R. J.; Ogbunude, P. O.; Enanga, B.; Barrett, M. P. Chemotherapy of human African trypanosomiasis. *Curr. Pharm. Des.* **2002**, *8*, 256–267.

(54) Legros, D.; Ollivier, G.; Gastellu-Etchegorry, M.; Paquet, C.; Burri, C.; Jannin, J.; Buscher, P. Treatment of human African trypanosomiasis—present situation and needs for research and development. *Lancet Infect. Dis.* **2002**, *2*, 437–440.

(55) Das, B. P.; Boykin, D. W. Synthesis and antiprotozoal activity of 2,5-bis(4-guanylphenyl)furans. *J. Med. Chem.* **1977**, *20*, 531–536.

(56) Steck, E. A.; Kinnamon, K. E.; Davidson, D. E., Jr.; Duxbury, R. E.; Johnson, A. J.; Masters, R. E. *Trypanosoma rhodesiense*: evaluation of the antitrypanosomal action of 2,5-bis(4-guanylphenyl)furan dihydrochloride. *Exp. Parasitol.* **1982**, *53*, 133–144.

(57) Ulrich, P.; Cerami, A. Trypanocidal 1,3-arylene diketone bis(guanylhydrazone)s. Structure−activity relationships among substituted and heterocyclic analogues. *J. Med. Chem.* **1984**, *27*, 35–40.

(58) Bell, C. A.; Cory, M.; Fairley, T. A.; Hall, J. E.; Tidwell, R. R. Structure−activity relationships of pentamidine analogs against *Giardia lamblia* and correlation of antigiardial activity with DNA-binding affinity. *Antimicrob. Agents Chemother.* **1991**, *35*, 1099–1107.

(59) Blagburn, B. L.; Sundermann, C. A.; Lindsay, D. S.; Hall, J. E.; Tidwell, R. R. Inhibition of *Cryptosporidium parvum* in neonatal Hsd:(ICR)BR Swiss miceby polyether ionophores and aromatic amidines. *Antimicrob. Agents Chemother.* **1991**, *35*, 1520–1523.

(60) Lindsay, D. S.; Blagburn, B. L.; Hall, J. E.; Tidwell, R. R. Activity of pentamidine and pentamidine analogs against *Toxoplasma gondii* in cell cultures. *Antimicrob. Agents Chemother.* **1991**, *35*, 1914–1916.

(61) Boykin, D. W. Antimicrobalactivity of the DNA minor groove binders furamidine and analogs. *J. Braz. Chem. Soc.* **2002**, *13*, 763–771.

(62) Brendle, J. J.; Outlaw, A.; Kumar, A.; Boykin, D. W.; Patrick, D. A.; Tidwell, R. R.; Werbovetz, K. A. Antileishmanial activities of several classes of aromatic dications. *Antimicrob. Agents Chemother.* **2002**, *46*, 797–807.

(63) Soeiro, M. N.; De Souza, E. M.; Stephens, C. E.; Boykin, D. W. Aromatic diamidines as antiparasitic agents. *Expert Opin. Investig. Drugs* **2005**, *14*, 957–972.

(64) Werbovetz, K. Diamidines as antitrypanosomal, antileishmanial and antimalarial agents. *Curr. Opin. Investig. Drugs* **2006**, *7*, 147–157.

(65) Walzer, P. D.; Kim, C. K.; Foy, J.; Linke, M. J.; Cushion, M. T. Cationic antitrypanosomal and other antimicrobial agents in the therapy of experimental *Pneumocystis carinii* pneumonia. *Antimicrob. Agents Chemother.* **1988**, *32*, 896–905.

(66) Tidwell, R. R.; Jones, S. K.; Geratz, J. D.; Ohemeng, K. A.; Cory, M.; Hall, J. E. Analogues of 1,5-bis(4-amidinophenoxy)pentane (pentamidine) in the treatment of experimental *Pneumocystis carinii* pneumonia. *J. Med. Chem.* **1990**, *33*, 1252–1257.

(67) Jones, S. K.; Hall, J. E.; Allen, M. A.; Morrison, S. D.; Ohemeng, K. A.; Reddy, V. V.; Geratz, J. D.; Tidwell, R. R. Novel pentamidine analogs in the treatment of experimental *Pneumocystis carinii* pneumonia. *Antimicrob. Agents Chemother.* **1990**, *34*, 1026–1030.

(68) Donkor, I. O.; Jones, S. K.; Tidwell, R. R. Pentamidine congeners 1: Synthesis of *cis*- and *trans*-Butamidine analogues as anti-*Pneumocystis carinii* pneumonia agents. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1137–1140.

(69) Donkor, I. O.; Tidwell, R. R.; Jones, S. K. Pentamidine congeners. 2. 2-Butene-bridged aromatic diamidines and diimidazolines as potential anti-*Pneumocystis carinii* pneumonia agents. *J. Med. Chem.* **1994**, *37*, 4554–4557.

(70) Boykin, D. W.; Kumar, A.; Spychala, J.; Zhou, M.; Lombardy, R. J.; Wilson, W. D.; Dykstra, C. C.; Jones, S. K.; Hall, J. E.; Tidwell, R. R.; Laughton, C.; Nunn, C. M.; Neidle, S. Dicationic diarylfurans as anti-*Pneumocystis carinii* agents. *J. Med. Chem.* **1995**, *38*, 912–916.

(71) Boykin, D. W.; Kumar, A.; Hall, J. E.; Bender, B. C.; Tidwell, R. R. Anti-pneumocystis activity of bis-amidoximes and bis-*O*-alkylamidoximes prodrugs. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3017–3020.

(72) Huang, T. L.; Zhang, Q.; White, A. T.; Queener, S. F.; Bartlett, M. S.; Smith, J. W.; Donkor, I. O. Synthesis and anti-*Pneumocystis carinii* activity of piperidine-linked aromatic diimidazolines. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2087–2090.

(73) Delia, T. J.; Nagarajan, A.; Queener, S. F.; Bartlett, M. S. Ring-based analogs of pentamidine versus *P. carinii* pneumonia in culture. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2367–2370.

(74) Boykin, D.; Kumar, A.; Xiao, G.; D., W.; Bender, B.; McCurdy, D.; Hall, J.; Tidwell, R. 2,5-Bis[4-(*N*-alkylamidino)phenyl]furans as anti-*Pneumocystis carinii* agents. *J. Med. Chem.* **1998**, *41*, 124–129.

(75) Rahmathullah, S. M.; Hall, J. E.; Bender, B. C.; McCurdy, D. R.; Tidwell, R. R.; Boykin, D. W. Prodrugs for amidines: synthesis and anti-*Pneumocystis carinii* activity of carbamates of 2,5-bis(4-amidinophenyl)furan. *J. Med. Chem.* **1999**, *42*, 3994–4000.

(76) Tao, B.; Huang, T. L.; Zhang, Q.; Jackson, L.; Queener, S. F.; Donkor, I. O. Synthesis and anti-*Pneumocystis carinii* activity of conformationally restricted analogues of pentamidine. *Eur. J. Med. Chem.* **1999**, *34*, 531–538.

(77) Vanden Eynde, J. J.; Mayence, A.; Huang, T. L.; Collins, M. S.; Rebholz, S.; Walzer, P. D.; Cushion, M. T. Novel bisbenzamidines as potential drug candidates for the treatment of *Pneumocystis carinii* pneumonia. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4545–4548.

(78) Anne, J.; De Clercq, E.; Eyssen, H.; Dann, O. Antifungal and antibacterial activities of diarylamidine derivatives. *Antimicrob. Agents Chemother.* **1980**, *18*, 231–239.

(79) Del Poeta, M.; Schell, W. A.; Dykstra, C. C.; Jones, S.; Tidwell, R. R.; Czarny, A.; Bajic, M.; Kumar, A.; Boykin, D.; Perfect, J. R. Structure−in vitro activity relationships of pentamidine analogues and dication-substituted bis-benzimidazoles as new antifungal agents. *Antimicrob. Agents Chemother.* **1998**, *42*, 2495–2502.

(80) Geratz, J. D.; Whitmore, A. C. Diamidino-α,*ω*-diphenoxyalkanes. Structure−activity relationships for the inhibition of thrombin, pancreatic kallikrein, and trypsin. *J. Med. Chem.* **1973**, *16*, 970–975.

(81) Geratz, J. D.; Cheng, M. C.; Tidwell, R. R. New aromatic diamidines with central α-oxyalkane or α,*ω*-dioxyalkane chains. Structure−activity relationships for the inhibition of trypsin, pancreatic kallikrein, and thrombin and for the inhibition of the overall coagulation process. *J. Med. Chem.* **1975**, *18*, 477–481.

(82) Geratz, J. D.; Cheng, M. C.; Tidwell, R. R. Novel bis(benzamidino) compounds with an aromatic central link. Inhibitors of thrombin, pancreatic kallikrein, trypsin, and complement. *J. Med. Chem.* **1976**, *19*, 634–639.

(83) Parrish, R. F.; Straus, J. W.; Paulson, J. D.; Polakoski, K. L.; Tidwell, R. R.; Geratz, J. D.; Stevens, F. M. Structure−activity relationships for the inhibition of acrosin by benzamidine derivatives. *J. Med. Chem.* **1978**, *21*, 1132–1136.

(84) Tidwell, R. R.; Geratz, J. D.; Dann, O.; Volz, G.; Zeh, D.; Loewe, H. Diarylamidine derivatives with one or both of the aryl moieties consisting of an indole or indole-like ring. Inhibitors of arginine-specific esteroproteases. *J. Med. Chem.* **1978**, *21*, 613–623.

(85) Clercq, E. D.; Dann, O. Diaryl amidine derivatives as oncornaviral DNA polymerase inhibitors. *J. Med. Chem.* **1980**, *23*, 787–795.

(86) Lansiaux, A.; Dassonneville, L.; Facompre, M.; Kumar, A.; Stephens, C. E.; Bajic, M.; Tanious, F.; Wilson, W. D.; Boykin, D. W.; Bailly, C. Distribution of furamidine analogues in tumor cells: influence of the number of positive charges. *J. Med. Chem.* **2002**, *45*, 1994–2002.

(87) Lansiaux, A.; Tanious, F.; Mishal, Z.; Dassonneville, L.; Kumar, A.; Stephens, C. E.; Hu, Q.; Wilson, W. D.; Boykin, D. W.; Bailly, C. Distribution of furamidine analogues in tumor cells: targeting of the nucleus or mitochondria depending on the amidine substitution. *Cancer Res.* **2002**, *62*, 7219–7229.

(88) Pathak, M. K.; Dhawan, D.; Lindner, D. J.; Borden, E. C.; Farver, C.; Yi, T. Pentamidine is an inhibitor of PRL phosphatases with anticancer activity. *Mol. Cancer Ther.* **2002**, *1*, 1255–1264.

(89) Baraldi, P. G.; Bovero, A.; Fruttarolo, F.; Preti, D.; Tabrizi, M. A.; Pavani, M. G.; Romagnoli, R. DNA minor groove binders as potential antitumor and antimicrobial agents. *Med. Res. Rev.* **2004**, *24*, 475–528.

(90) Maciejewska, D.; Kazmierczak, P.; Zabinski, J.; Wolska, I.; Popis, S. Pentamidine analogs: syntheses, structures in solid state by ^{13}C CP/MAS NMR spectroscopy, and X-ray crystallography and their preliminary biological screening against human cancer. *Monatsh. Chem.* **2006**, *137*, 1225–1240.

(91) Steck, E. A.; Kinnamon, K. E.; Rane, D. S.; Hanson, W. L. *Leishmania donovani*, *Plasmodium berghei*, *Trypanosoma rhodesiense*: antiprotozoal effects of some amidine types. *Exp. Parasitol.* **1981**, *52*, 404–413.

(92) Clement, B.; Raether, W. Amidoximes of pentamidine: synthesis, trypanocidal and leishmanicidal activity. *Arzneim. Forsch.* **1985**, *35*, 1009–1014.

(93) Berger, B. J.; Lombardy, R. J.; Marbury, G. D.; Bell, C. A.; Dykstra, C. C.; Hall, J. E.; Tidwell, R. R. Metabolic N-hydroxylation of pentamidine in vitro. *Antimicrob. Agents Chemother.* **1990**, *34*, 1678–1684.

(94) Kratzl, K.; Meisert, E. Chemistry of vanillin and its derivatives. X. Amidines, imidazolines, and tetrahydropyrimidinediones with guaiacol substituents. *Monatsh. Chem.* **1957**, *88*, 1056–1063.

(95) Dox, A. W.; Whitmore, F. C. Acetamidine Hydrochloride. In *Organic Synthesis*, 2nd ed.; Blatt, A. H., Ed.; John Wiley & Sons: New York, 1941; Vol. 1, pp 5−7.

(96) Berg, S. S.; Newbery, G. The Search for Chemotherapeutic Amidines Part X. Substituted 4,4′-Diamidino-α,*ω*-diphenoxyalkanes and -diphenyl ethers. *J. Chem. Soc.* **1949**, 642–648.

(97) Willson, M.; Perie, J. J.; Malecaze, F.; Opperdoes, F.; Callens, M. Biological properties of amidinium sulfinic and sulfonic acid derivatives: inhibition of glycolytic enzymes of *Trypanosoma brucei* and protective effect on cell growth. *Eur. J. Med. Chem.* **1992**, *27*, 799–808.

(98) Stephens, C. E.; Tanious, F.; Kim, S.; Wilson, W. D.; Schell, W. A.; Perfect, J. R.; Franzblau, S. G.; Boykin, D. W. Diguanidino and “reversed” diamidino 2,5-diarylfurans as antimicrobial agents. *J. Med. Chem.* **2001**, *44*, 1741–1748.

(99) Shearer, B. G.; Oplinger, J. A.; Lee, S. *S*-2-Naphthylmethyl thioacetimidate hydrobromide: a new odorless reagent for the mild synthesis of substituted acetamidines. *Tetrahedron Lett.* **1997**, *38*, 179–182.

(100) Spychala, J. 4-(Cyclic-amidino)phenols. Preparation and use in a diamidine synthesis. *Synth. Commun.* **2000**, *30*, 1083–1094.

(101) Kubota, H.; Kakefuda, A.; Watanabe, T.; Ishii, N.; Wada, K.; Masuda, N.; Sakamoto, S.; Tsukamoto, S. Synthesis and pharmacological evaluation of 1-*oxo*-2-(3-Piperidyl)-1,2,3,4- tetrahydroisoquinolines and related analogues as a new class of specific bradycardic agents possessing I_f channel inhibitory activity. *J. Med. Chem.* **2003**, *46*, 4728–4740.

(102) Mackman, R. L.; Katz, B. A.; Breitenbucher, J. G.; Hui, H. C.; Verner, E.; Luong, C.; Liu, L.; Sprengeler, P. A. Exploiting subsite S1 of trypsin-like serine proteases for selectivity: potent and selective inhibitors of urokinase-type plasminogen activator. *J. Med. Chem.* **2001**, *44*, 3856–3871.

(103) Peters, W.; Davies, E. E.; Robinson, B. L. Chemotherapy of rodent malaria. XXIII. Causal prophylaxis. II. Practical experience with *Plasmodium yoelii nigeriensis* in drug screening. *Ann. Trop. Med. Parasitol* **1975**, *69*, 311–328.

(104) Krishnamurthy, S.; Aimino, D. Rapid and selective reduction of functionalized aromatic disulfides with lithium tri-*tert*-butoxyaluminohydride. A remarkable steric and electronic control. Comparison of various hydride reagents. *J. Org. Chem.* **1989**, *54*, 4458–4462.

(105) Liang, X.; Jie, C.; Trudell, M. L. Chromium(VI) oxide catalyzed oxidation of sulfides to sulfones with periodic acid. *J. Org. Chem.* **2003**, *68*, 5388–5391.

(106) Bacchi, C. J.; Brun, R.; Croft, S. L.; Alicea, K.; Buhler, Y. In vivo trypanocidal activities of new *S*-adenosylmethionine decarboxylase inhibitors. *Antimicrob. Agents Chemother.* **1996**, *40*, 1448–1453.

(107) Donkor, I. O.; Assefa, H.; Rattendi, D.; Lane, S.; Vargas, M.; Goldberg, B.; Bacchi, C. Trypanocidal activity of dicationic compounds related to pentamidine. *Eur. J. Med. Chem.* **2001**, *36*, 31–538.

(108) Donkor, I. O.; Huang, T. L.; Tao, B.; Rattendi, D.; Lane, S.; Vargas, M.; Goldberg, B.; Bacchi, C. Trypanocidal activity of conforma-

tionally restricted pentamidine congeners. *J. Med. Chem.* **2003**, *46*, 1041–1048.

(109) Borges, M. N.; Messeder, J. C.; Figueroa-Villar, J. D. Synthesis, anti-*Trypanosoma cruzi* activity and micelle interaction studies of bisguanylhydrazones analogous to pentamidine. *Eur. J. Med. Chem.* **2004**, *39*, 925–929.

(110) Dardonville, C.; Brun, R. Bisguanidine, bis(2-aminoimidazoline), and polyamine derivatives as potent and selective chemotherapeutic agents against *Trypanosoma brucei rhodesiense*. Synthesis and in vitro evaluation. *J. Med. Chem.* **2004**, *47,* 2296–2307.

(111) Huang, T. L.; Vanden Eynde, J. J.; Mayence, A.; Donkor, I. O.; Khan, S. I.; Tekwani, B. L. Anti-plasmodial and anti-leishmanial activity of conformationally restricted pentamidine congeners. *J. Pharm. Pharmacol.* **2006**, *58*, 1033–1042.

(112) Bakunova, S. M.; Bakunov, S. A.; Wenzler, T.; Barszcz, T.; Werbovetz, K. A.; Brun, R.; Hall, J. E.; Tidwell, R. R. Synthesis and in vitro antiprotozoal activity of bisbenzofuran cations. *J. Med. Chem.* **2007**, *50*, 5807–5823.

(113) Kaminsky, R.; Schmid, C.; Brun, R. An "in vitro selectivity index" for evaluation of cytotoxicity of antitrypanosomal compounds. *In Vitro Toxicol.* **1996**, *9*, 315–324.

(114) Patrick, D. A.; Bakunov, S. A.; Bakunova, S. M.; Kumar, E. V.; Lombardy, R. J.; Jones, S. K.; Bridges, A. S.; Zhirnov, O.; Hall, J. E.; Wenzler, T.; Brun, R.; Tidwell, R. R. Synthesis and in vitro antiprotozoal activities of dicationic 3,5-diphenylisoxazoles. *J. Med. Chem.* **2007**, *50*, 2468–2485.

(115) Stephens, C. E.; Brun, R.; Salem, M. M.; Werbovetz, K. A.; Tanious, F.; Wilson, W. D.; Boykin, D. W. The activity of diguanidino and "reversed" diamidino 2,5-diarylfurans versus *Trypanosoma cruzi* and *Leishmania donovani*. . *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2065–2069.

(116) Cory, M.; Tidwell, R. R.; Fairley, T. A. Structure and DNA binding activity of analogues of 1,5-bis(4-amidinophenoxy)pentane (pentamidine). *J. Med. Chem.* **1992**, *35*, 431–438.

(117) Bailly, C.; Perrine, D.; Lancelot, J. C.; Saturnino, C.; Robba, M.; Waring, M. J. Sequence-selective binding to DNA of bis(amidinophenoxy)alkanes related to propamidine and pentamidine. *Biochem. J.* **1997**, *323* (Pt 1), 23–31.

(118) Ismail, M. A.; Brun, R.; Easterbrook, J. D.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. Synthesis and antiprotozoal activity of aza-analogues of furamidine. *J. Med. Chem.* **2003**, *46*, 4761–4769.

(119) Ismail, M.; Brun, R.; Wenzler, T.; Tanious, F.; Wilson, W.; Boykin, D. Dicationic biphenyl benzimidazole derivatives as antiprotozoal agents. *Bioorg. Med. Chem.* **2004**, *12*, 5405–5413.

(120) Arafa, R. K.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Stephens, C. E.; Boykin, D. W. Synthesis, DNA affinity, and antiprotozoal activity of fused ring dicationic compounds and their prodrugs. *J. Med. Chem.* **2005**, *48*, 5480–5488.

(121) Ismail, M. A.; Arafa, R. K.; Brun, R.; Wenzler, T.; Miao, Y.; Wilson, W. D.; Generaux, C.; Bridges, A.; Hall, J. E.; Boykin, D. W. Synthesis, DNA affinity, and antiprotozoal activity of linear dications: terphenyl diamidines and analogues. *J. Med. Chem.* **2006**, *49*, 5324–5332.

(122) Clement, B.; Immel, M.; Terlinden, R.; Wingen, F. J. Reduction of amidoxime derivatives to pentamidine in vivo. *Arch. Pharm. (Weinheim)* **1992**, *325*, 61–62.

(123) Kamm, W.; Hauptmann, J.; Behrens, I.; Sturzebecher, J.; Dullweber, F.; Gohlke, H.; Stubbs, M.; Klebe, G.; Kissel, T. Transport of peptidomimetic thrombin inhibitors with a 3-amidino-phenylalanine structure: permeability and efflux mechanism in monolayers of a human intestinal cell line (Caco-2). *Pharm. Res.* **2001**, *18*, 1110–1118.

(124) Clement, B. Reduction of N-hydroxylated compounds: amidoximes (*N*-hydroxyamidines) as prodrugs of amidines. *Drug Metab. Rev.* **2002**, *34*, 565–579.

(125) Ansede, J. H.; Anbazhagan, M.; Brun, R.; Easterbrook, J. D.; Hall, J. E.; Boykin, D. W. *O*-Alkoxyamidine prodrugs of furamidine: in vitro transport and microsomal metabolism as indicators of in vivo efficacy in a mouse model of *Trypanosoma brucei rhodesiense* infection. *J. Med. Chem.* **2004**, *47*, 4335–4338.

(126) de Koning, H. P. Uptake of pentamidine in *Trypanosoma brucei brucei* is mediated by three distinct transporters: implications for cross-resistance with arsenicals. *Mol. Pharmacol.* **2001**, *59*, 586–592.

(127) Zhang, X.; Berger, B. J.; Ulrich, P. Synthesis and trypanocidal activity of the bis-carba analogue of pentamidine. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1035–1036.

(128) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.

(129) Baltz, T.; Baltz, D.; Giroud, C.; Crockett, J. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense*, and *T. gambiense*. *EMBO J.* **1985**, *4*, 1273–1277.

(130) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(131) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, F. C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Santo Tomas, J.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N. Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature* **2004**, *430*, 900–904.

(132) Werbovetz, K. A.; Sackett, D. L.; Delfin, D.; Bhattacharya, G.; Salem, M.; Obrzut, T.; Rattendi, D.; Bacchi, C. Selective antimicrotubule activity of *N*1-phenyl-3,5-dinitro-*N*4,*N*4-di-*n*-propylsulfanilamide (GB-II-5) against kinetoplastid parasites. *Mol. Pharmacol.* **2003**, *64*, 1325–1333.

(133) Werbovetz, K. A.; Brendle, J. J.; Sackett, D. L. Purification, characterization, and drug susceptibility of tubulin from *Leishmania*. *Mol. Biochem. Parasitol.* **1999**, *98*, 53–65.

(134) Thuita, J. K.; Karanja, S. M.; Wenzler, T.; Mdachi, R. E.; Ngotho, J. M.; Kagira, J. M.; Tidwell, R.; Brun, R. Efficacy of the diamidine DB75 and its prodrug DB289, against murine models of human African trypanosomiasis. *Acta Trop.* **2008**, *108*, 6–10.

JM801547T