Synthesis and Antiprotozoal Activity of Cationic 2-Phenylbenzofurans

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A series of cationically substituted 2-phenylbenzofurans **1**−**49** have been synthesized, and their in vitro antiprotozoal properties against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani,* as well as cytotoxicity against mammalian cells, have been evaluated. Eight dications exhibited antitrypanosomal activities comparable to that of pentamidine and melarsoprol. Twenty-six compounds were more active than pentamidine, and seven dications demonstrated increased activities against *P. falciparum* than artemisinin. Five congeners were more active against *L. donovani* than pentamidine. Introduction of methoxy or hydroxy groups in the 7- and/or 2′-position afforded derivatives that were highly selective against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. Fourteen 2-phenylbenzofurans displayed excellent in vivo efficacies in the acute mouse model of trypanosomiasis, curing 3/4 or 4/4 animals at 4 × 5 mg/kg. Diamidine **1** and di(*N*-isopropyl)amidine **45**, administered at 4 × 1 mg/kg, exhibited potency comparable to that of melarsoprol, providing 3/4 and 2/4 cures, respectively.

Introduction

Although many aromatic diamidines have been reported to possess a wide range of antifungal and antiprotozoal properties,[1−14] only 1,5-bis(4-amidinophenoxy)pentane (pentamidine) has been widely employed as a drug in humans. Pentamidine has been used for more than 50 years against early stage *Trypanosoma brucei gambiense* related human African trypanosomiasis (HAT[a] or sleeping sickness)[15−18] as well as against antimony-resistant leishmaniasis[19,20] and pneumonia caused by the opportunistic pathogen *Pneumocystis jiroveci* (formerly *P. carinii*).[21−23] The drug demonstrated some activity against selected strains of malaria,[24−26] even though it was never approved to treat the disease. Lack of oral bioavailability[17] of pentamidine due to the ionization of its two amidine groups at physiological pH necessitates the parenteral administration, which is not practical in remote areas where most cases of sleeping sickness and leishmaniasis take place. Although several adverse effects, such as hypotension, abdominal pain, vertigo, hypersalivation, hypoglycemia, nausea, and mild nephrotoxocity, have been reported upon pentamidine administration,[27−29] the drug is fairly well tolerated by patients.

In recent years, the number of treatment failures of malaria, HAT, and leishmaniasis has increased because of development of resistant strains of parasites. Reduced efficiency of the most commonly used antimalarial remedies, such as chloroquine and sulfadoxine-pyrimethamine, requires the use of drug combinations or more expensive artemisinin-containing therapies to slow the development of resistance.[30−33] Also, many current antimalarial drugs are structurally related or have similar modes of action, which explains the appearance of the shared mechanisms of resistance. This enhances the risk of development of cross-resistance and clinical failure of newly introduced medications.[34−36] Treatments for HAT and leishmaniasis require parenteral administration and suffer from toxicity, poor efficacy, and increasing number of treatment failures.[18,29,37,38] This is currently the case for visceral leishmaniasis, although new options for its treatment have recently become available.[39,40] In addition, the overlap of parasitic infections and HIV in different parts of the world results in growing number of cases of dually infected patients.[41−46] The need for safe and affordable antiprotozoal therapies capable of overcoming parasite resistance makes the identification of new drug candidates an urgent priority.

In our continuing search for novel dicationic compounds with enhanced antiprotozoal potency and lowered toxicity, we investigated a series of dicationic 2-phenylbenzofurans **1**−**49**, many of which are structurally similar to the well-known DNA fluorescent stain 4′,6-diamidino-2-phenylindole (DAPI). In the early 1970s, syntheses of DAPI as well as three 2-phenylbenzofurans **1**, **3**, and **11** were reported[47,48] and the antitrypanosomal properties of 2-(4-amidinophenyl)-5-amidinobenzofuran (**1**), 2-(4-amidinophenyl)-6-amidinobenzofuran (**11**), and DAPI were published.[47] Here, we report the lead optimization of these dications. All 2-phenylbenzofurans **1**−**49** were tested in vitro against *T. b. 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 screening in the STIB900 acute mouse model of trypanosomiasis.

Chemistry

Syntheses of compounds bearing cyano groups in the 5-position of the benzofuran moiety and 4′-position of the phenyl ring are based on the previously reported procedure by Dann,[47] as depicted in Scheme 1. Commercially available 5-bromosalicylaldehyde was reacted with α-bromo-*p*-tolunitrile followed by base-catalyzed intramolecular ring closure to form 5-bromo-2-(4-cyanophenyl)benzofuran (**50**). Compound **50** was treated with

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[a] Abbreviations: HAT, human African trypanosomiasis; HIV, human immunodeficiency virus; DAPI, 4′,6-diamidino-2-phenylindole; IBX, *o*-iodoxylbenzoic acid; PMD, pentamidine; MLSP, melarsoprol; CQ, chloroquine; ATMS, artemisinin; PPT, podophyllotoxin.

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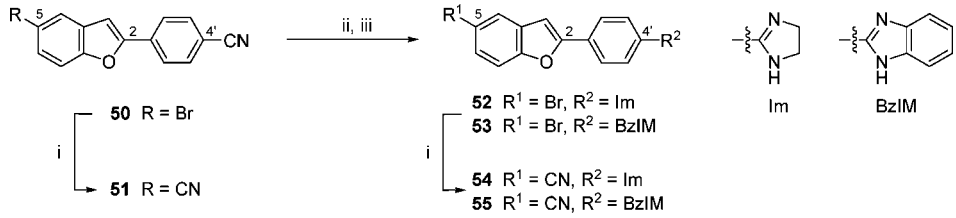
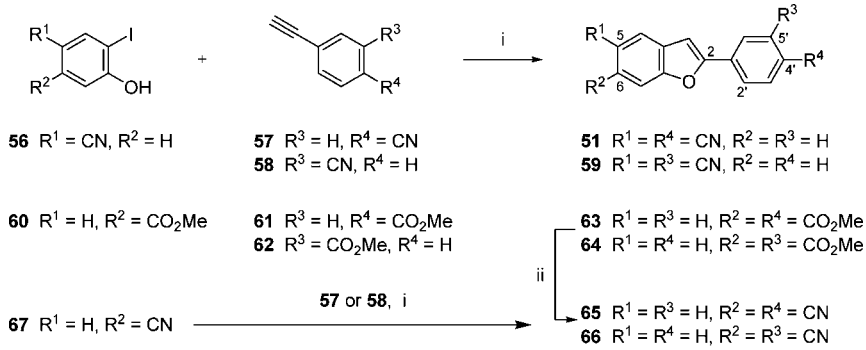
Scheme 1[a]



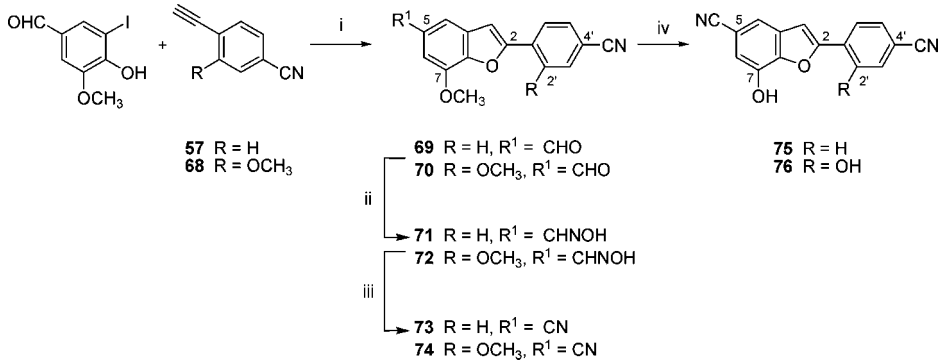
[a] Reagents and conditions: (i) CuCN, DMF, reflux, 15 h; (ii) 1,4-dioxane, EtOH, HCl, 1−3 days; (iii) ethylenediamine, EtOH, 1−2 days or 1,2-diaminobenzene, MeOH, reflux, 15 h.

Scheme 2[a]



[a] Reagents and conditions: (i) Cu_2O, pyridine, 100 °C, 15 h; (ii) Me_2AlNH_2, *o*-xylene, 80−100 °C, 3 h.

Scheme 3[a]



[a] Reagents and conditions: (i) Cu_2O, pyridine, 100 °C, 15 h; (ii) hydroxylamine hydrochloride, pyridine; (iii) Ac_2O, reflux, 15 h; (iv) pyridine hydrochloride, 120−140 °C, 3 h.

copper(I) cyanide in DMF at elevated temperature to yield dinitrile **51**.[47] Conversion of nitrile **50** to an imidate ester under modified Pinner conditions[49] followed by reactions with ethylenediamine or 1,2-diaminobenzene afforded 2-phenylbenzofurans **52** and **53**, bearing imidazoline and benzimidazole cationic groups in the 4′-positions. Reaction of bromobenzofurans **52** and **53** with copper(I) cyanide in DMF at elevated temperature yielded the corresponding nitriles **54** and **55**.

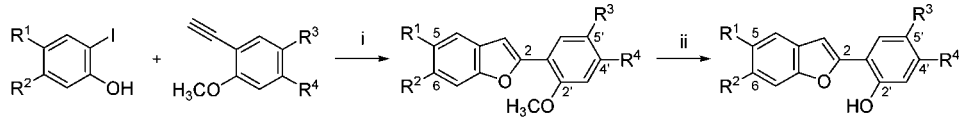
Unfortunately, extensive application of this synthetic strategy is constrained by limited availability of 6-bromosalicylaldehyde as a starting material. Also, the aforementioned approach is only suitable for preparation of compounds bearing an electron-withdrawing group in the *p*-position of the phenyl ring. Consequently, our attempts to use it for synthesis of *m*-substituted 2-phenylbenzofurans were unsuccessful.

An alternative method of synthesis of benzofurans involves the palladium-catalyzed (Sonogashira reaction)[50−54] or copper-catalyzed (Castro reaction)[55,56] interaction of *o*-halogenated phenols with acetylenes. This approach allows greater structural diversity and was therefore utilized to prepare 2-phenylbenzofuran congeners bearing cyano groups in the various positions of the aromatic rings. The use of copper-catalyzed heteroannulation (modified[57,58] Castro reaction) to synthesize substituted 2-phenylbenzofurans is depicted in Schemes 2−4.

4-Hydroxy-3-iodobenzonitrile (**56**)[59] was prepared by iodination of 4-cyanophenol following the procedure of Edgar and Falling.[60] Syntheses of 4-ethynylbenzonitrile (**57**) and 3-ethynylbenzonitrile (**58**) were previously reported.[61] Copper-catalyzed heteroannulation of iodophenol **56** with phenylacetylenes **57** and **58** under the conditions of the modified Castro reaction afforded 5,4′- and 5,5′-substituted dinitriles **51** and **59** in moderate yields (Scheme 2).

A similar approach was employed to synthesize 6-substituted derivatives. The commercially available 3-hydroxybenzoic acid underwent iodination[60] to 3-hydroxy-4-iodobenzoic acid, followed by esterification to methyl benzoate **60** under acidic conditions.[62] Methyl 4-ethynylbenzoate (**61**) and methyl 3-ethynylbenzoate (**62**) were synthesized following the established protocol.[59] Iodophenol **60** was reacted with phenylacetylenes **61** and **62** under modified Castro reaction conditions to provide benzofuran diesters **63** and **64**, which were directly converted to dinitriles **65** and **66** by treatment with dimethylaluminum

Scheme 4[a]



56 R^1 = CN, R^2 = H **68** R^3 = H, R^4 = CN
67 R^1 = H, R^2 = CN **77** R^3 = CN, R^4 = H

78 $R^1 = R^4$ = CN, $R^2 = R^3$ = H
79 $R^1 = R^3$ = CN, $R^2 = R^4$ = H
80 $R^2 = R^4$ = CN, $R^1 = R^3$ = H
81 $R^2 = R^3$ = CN, $R^1 = R^4$ = H

82 $R^1 = R^4$ = CN, $R^2 = R^3$ = H
83 $R^1 = R^3$ = CN, $R^2 = R^4$ = H
84 $R^2 = R^4$ = CN, $R^1 = R^3$ = H
85 $R^2 = R^3$ = CN, $R^1 = R^4$ = H

[a] Reagents and conditions: (i) Cu_2O, pyridine, 100 °C, 15 h; (ii) pyridine hydrochloride, 120−140 °C, 3 h.

amide.[63] A more expedient, one-step preparation of **65** and **66** involved modified Castro reactions between 3-hydroxy-4-iodobenzonitrile (**67**)[64] and phenylacetylenes **57** and **58**, thus circumventing the low-yield transformations of **63** and **64** to **65** and **66**.

The synthesis of congeners bearing 7-methoxy and hydroxy groups is depicted in Scheme 3. Commercially available 5-iodovanillin was reacted with the phenylacetylenes **57** and **68**[61] under modified Castro reaction conditions to form formylbenzofurans **69** and **70** in high yields. Reaction of compounds **69** and **70** with hydroxylamine hydrochloride in pyridine afforded oximes **71** and **72**, which were refluxed in Ac_2O to form dinitriles **73** and **74**. Compounds **73** and **74** underwent O-demethylation by treatment with molten pyridine hydrochloride to yield 7-hydroxydinitriles **75** and **76**.

o-Iodophenols **56** and **67** were reacted with the methoxyphenylacetylenes **68** and **77** to give the 2′-methoxy substituted dinitriles **78**−**81** (Scheme 4). O-Demethylation using molten pyridine hydrochloride afforded the corresponding 2′-hydroxy derivatives **82**−**85**.

Dicationic 2-phenylbenzofurans **1**−**4** and **6**−**49** (Table 1) were synthesized by the modified Pinner method[49] (Scheme 5). The dinitriles **51**, **54**, **55**, **59**, **65**, **66**, **73**, **75**, **76**, **78**−**85** were converted to imidate esters, which reacted with ethanolic solutions of ammonia, isopropylamine, or ethylenediamine at ambient temperature or with 1,2-diaminobenzene in refluxing methanol. 5-(1*H*-Imidazol-2-yl)-2-[4-(1*H*-imidazol-2-yl)phenyl]-benzofuran (**5**) was prepared from the corresponding 5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran (**3**) by oxidation with *o*-iodoxylbenzoic acid (IBX),[65] following a published protocol.[66]

Results and Discussion

In the case of pentamidine analogues and cationic diphenylisoxazoles, the introduction of the methoxy groups resulted in enhanced antiprotozoal activities[24] and decreased cytotoxicities of select compounds, therefore affording greater selectivity toward specific parasites.[61] We designed several cationic substituted 2-phenylbenzofurans with the methoxy and hydroxy groups attached to the aromatic rings to evaluate how this structural variation would affect antiprotozoal properties of compounds **1**−**49**. Dications **4**−**7**, bearing aromatic cationic groups, were synthesized to investigate the effect of the reduced basicity and increased lipophilicity of these congeners on their activities against different parasites. The results of the in vitro testing against bloodstream form trypomastigotes of *T. b. rhodesiense* (STIB900), chloroquine resistant *P. falciparum* (K1), and axenic amastigotes of *L. donovani* (MHOM/SD/62/1S-$CL2_D$) as well as the assessment of cytotoxicity of the compounds **1**−**49** against rat myoblast cells (L6) are summarized in Table 1 and compared to the results of pentamidine and DAPI. Other controls employed were melarsoprol (*T. b. rhodesiense*), chloroquine and artemisinin (*P. falciparum*), and podophyllotoxin (L6). In addition to activities, three selectivity indexes SI_T, SI_P, and SI_L, 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*)].

Cytotoxicity Study. 2-Phenylbenzofurans **1**−**49** displayed a range of in vitro cytotoxicities with IC_{50} values varying from 0.8 μM to >221 μM. Di(*N*-isopropyl)amidines were less cytotoxic than diamidines and diimidazolines.[59,61] Replacement of amidine groups in the 2-phenylbenzofuran **1** with benzimidazole or imidazole moieties in dications **4** and **5** afforded derivatives exhibiting cytotoxicities comparable to that of diamidine **1**. Compound **7**, bearing a benzimidazole substituent in the 4′-position of the phenyl ring, was 30-fold less cytotoxic than dication **1**.

The position of the cationic substituents mainly affected the cytotoxicity of diamidines and diimidazolines but had lesser effect on di(*N*-isopropyl)amidines. 5-Substituted diamidines and diimidazolines exhibited lower cytotoxicity than the 6-substituted derivatives in all cases except diamidines **8** and **32** and diimidazoline **34**, which were more toxic than corresponding 6-substituted isomers **14**, **44**, and **46**, respectively. Likewise, diamidines and diimidazolines bearing one cationic group in the 5′-position of the phenyl ring were less toxic than analogous 4′-substituted isomers except diamidine **44** and diimidazolines **3** and **46**, which displayed higher cytotoxic IC_{50} values than similar 5′-substituted derivatives **47**, **10**, and **49**. Thus, 5,5′-substituted diamidines **8** and **35** and 6,5′-substituted isomer **14** were 10-, 6-, and 160-fold less toxic than the corresponding 4′-substituted 2-phenylbenzofurans **1**, **32**, and **11**. The position of the cationic groups influenced the cytotoxicity of the compounds **6** and **7**. Attachment of the imidazoline moiety to the 4′-position of the phenyl ring in the dication **6** afforded a derivative nearly 10-fold more cytotoxic than compound **7**, bearing the imidazoline moiety in the 5-position of the benzofuran scaffold. Di(*N*-isopropyl)amidines exhibited comparable cytotoxic IC_{50} values regardless of the position of cationic fragments.

The effect of the methoxy and hydroxy groups on the cytotoxicity of the 2-phenylbenzofurans **1**−**49** depended on the type and the position of the cationic substituents. For instance, introduction of the methoxy and hydroxy substituents in either the 7-position of the benzofuran or 2′-position of the phenyl ring of the 5,4′-substituted diamidine **1** resulted in a 15- to 100-fold reduction in cytotoxicity (derivatives **17**, **20**, **26**, and **32**). However, diamidine **23**, bearing hydroxy groups in both the 7- and 2′-positions, was equally cytotoxic as compound **1**. Among 5,5′- and 6,4′-substituted diamidines, dications bearing methoxy

Table 1. Structures, Cytotoxicity, and in Vitro Antiprotozoal Activity of 2-Phenylbenzofurans **1**−**49**

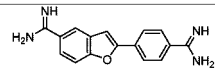
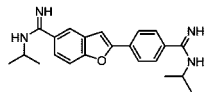
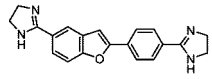
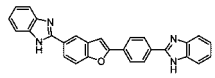
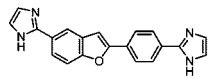
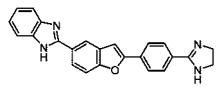
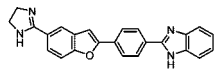
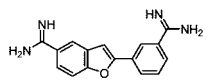
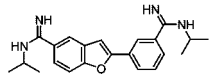
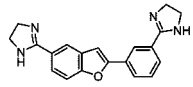
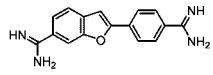
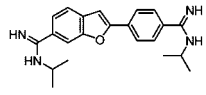
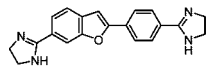
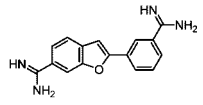
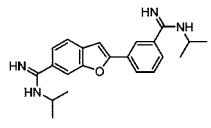
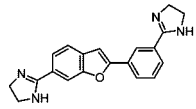
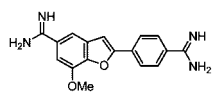
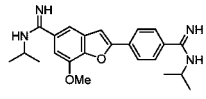
Compd	Structure	Cytotox.[g]	*T. b. rhodesiense*[h]		*P. falciparum*[j]		*L. donovani*[l]	
		IC_{50} (μM)	IC_{50} (μM)	SI_T[i]	IC_{50} (μM)	SI_P[k]	IC_{50} (μM)	SI_L[m]
1		1.90	0.003	633	0.047	40	0.99	2
2		>198	0.125	>1584	0.073	>2712	1.6	125
3		39.7	0.189	210	0.001	39700	21	2
4		6.7	0.249	27	0.031	216	>100	0.1
5		3.3	0.606	5	0.033	100	>100	0.03
6		6.1	0.122	50	0.040	153	5.7	1
7		59.3	0.228	260	0.061	972	>100	0.6
8		21.2	0.024	883	0.019	1116	5.8	4
9		>199	0.473	>421	0.331	>601	13	15
10		33.9	0.078	435	0.076	446	24	1.5
11		0.80	0.007	114	0.003	267	3.1	0.3
12		>199	0.148	>1345	0.015	>13267	12	17
13		4.50	0.319	14	0.109	41	>50	0.1
14		131	0.021	6238	0.004	32750	14	9
15		>200	0.534	>375	0.059	>3390	24	8
16		12.8	0.333	38	0.205	62	>100	0.1
17		30.7	0.006	5117	0.006	5117	3.0	10
18		>190	0.090	>2111	0.048	>3958	15	12

Table 1. Continued

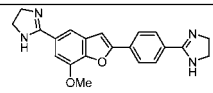
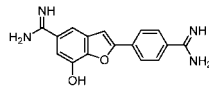
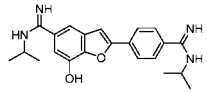
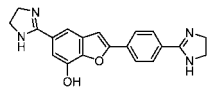
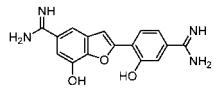
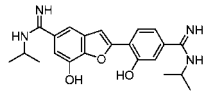
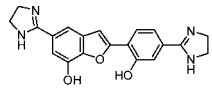
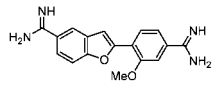
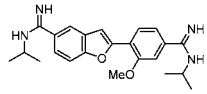
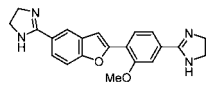
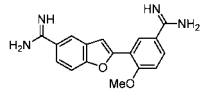
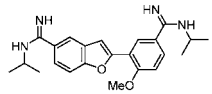
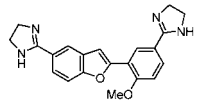
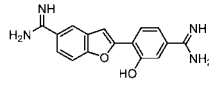
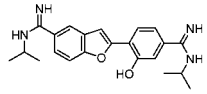
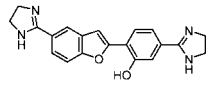
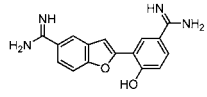
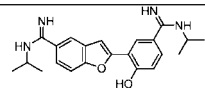
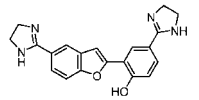
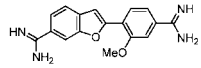
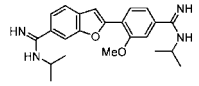
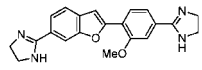
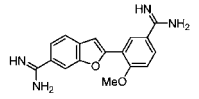
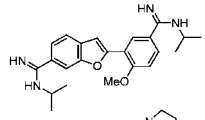
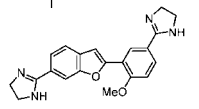
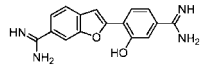
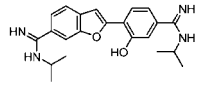
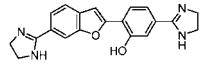
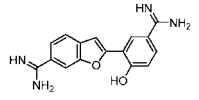
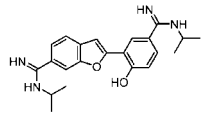
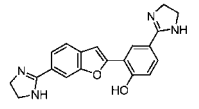
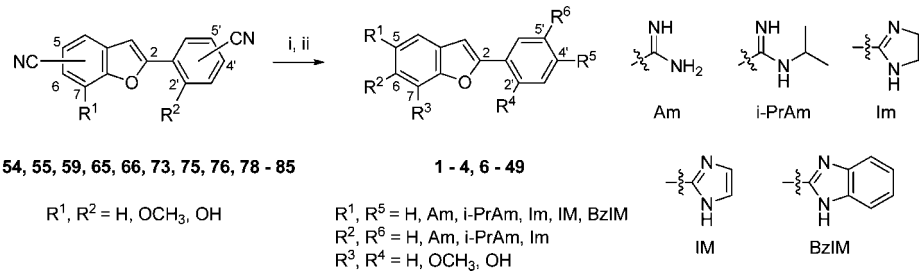
Compd	Structure	Cytotox.[g]	*T. b. rhodesiense*[h]		*P. falciparum*[j]		*L. donovani*[l]	
		IC_{50} (μM)	IC_{50} (μM)	SI_T[i]	IC_{50} (μM)	SI_P[k]	IC_{50} (μM)	SI_L[m]
19		19.9	0.124	160	0.032	622	4.5	5
20		184	0.003	61333	0.047	3915	5.3	35
21		>185	0.028	>6607	0.056	>3304	20	9
22		>203	0.004	>50750	0.022	>9227	7.4	27
23		2.10	0.018	117	0.061	34	14	0.2
24		>179	0.023	>7783	0.310	>577	46	4
25		>193	0.010	>19300	0.030	>6433	16	12
26		157	0.003	52333	0.003	52333	2.0	80
27		98.1	0.074	1326	0.020	4905	1.5	67
28		7.40	0.247	30	0.031	239	60	0.1
29		179	0.002	89500	0.040	4475	5.7	31
30		>179	0.155	>1155	0.250	>716	25	7
31		30.8	0.027	1141	0.065	474	>50	0.6
32		33.3	0.002	16650	0.004	8325	1.8	19
33		>183	0.021	>8714	0.028	>6536	1.8	101
34		150	0.008	18750	0.006	25000	5.8	26
35		>217	0.005	>43400	0.793	>274	20	11

Table 1. Continued

Compd	Structure	Cytotox.[g]	*T. b. rhodesiense*[h]		*P. falciparum*[j]		*L. donovani*[l]	
		IC_{50} (μM)	IC_{50} (μM)	SI_T[i]	IC_{50} (μM)	SI_P[k]	IC_{50} (μM)	SI_L[m]
36		>180	1.005	>179	0.516	>349	40	5
37		>199	0.227	>877	0.023	>8652	57	4
38		108	0.003	36000	0.020	5400	3.7	29
39		>181	0.054	>3352	0.206	>879	32	6
40		4.40	0.049	90	0.077	57	>100	0.04
41		128	0.011	11636	0.018	7111	3.6	36
42		>175	0.929	>188	0.293	>597	54	3
43		11.1	0.017	653	0.074	150	34	0.3
44		>221	0.004	>55250	0.070	>3157	29	8
45		>183	0.009	>20333	0.597	>307	39	5
46		>193	0.011	>17545	0.098	>1969	13	15
47		164	0.029	5655	0.378	434	41	4
48		>187	5.101	>37	0.636	>294	>100	2
49		>189	0.083	>2277	0.103	>1835	35	6
PMD[a]		46.6	0.003	15533	0.058	803	1.8	25
DAPI[b]		0.86	0.017	51	0.003	287		
MLSP[c]		7.78	0.004	1945				
CQ[d]		117			0.124	944		
ATMS[e]		450			0.006	75000		
PPT[f]		0.019						

[a] PMD, pentamidine. [b] DAPI, 4′,6-diamidino-2-phenylindole. [c] MLSP, melarsoprol. [d] CQ, chloroquine. [e] ATMS, artemisinin. [f] PPT, podophyllotoxin. [g] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[76] [h] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[7,71,72] [i] Selectivity index[70] for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50}(L6)/IC_{50}(*T. b. rhodesiense*)]. [j] Chloroquine resistant *Plasmodium falciparum* (K1). Average of duplicate determinations.[7,73] [k] Selectivity index[70] for *P. falciparum* (SI_P), expressed as the ratio [IC_{50}(L6)/IC_{50}(*P. falciparum*)]. [l] *Leishmania donovani* (MHOM/SD/62/1S-CL2$_D$) axenic amastigotes. Average of duplicate determinations.[74,75] [m] Selectivity index[70] for *L. donovani* (SI_L), expressed as the ratio [IC_{50}(L6)/IC_{50}(*L. donovani*)].

Scheme 5[a]



[a] Reagents and conditions: (i) 1,4-dioxane, EtOH, HCl, 1−3 days; (ii) appropriate amine, EtOH, 1−2 days.

(**29** and **38**) or hydroxy groups (**35** and **44**) in the 2′-position of the phenyl ring exhibited lower cytotoxicity than compounds **8** and **11**, respectively. Thus, 2′-hydroxy substituted dication **44** was 270-fold less toxic than 2-phenylbenzofuran **11**. In the case of 6,5′-substituted diamidines, addition of the methoxy (**41**) or hydroxy (**47**) groups in the 2′-position of the molecule had little effect on toxicity. Attachment of the methoxy or hydroxy substituents to the 7- or/and 2′-positions of the aromatic rings resulted in slightly increased cytotoxicity of di(*N*-isopropyl)amidines relative to 2-phenylbenzofurans **2**, **9**, **12**, and **15**. Diimidazolines **19**, **28**, **31**, **40**, and **43** bearing methoxy substituents were more cytotoxic than the parent compounds **3**, **10**, **13**, and **16** regardless of the position of the cationic fragments, while the introduction of hydroxy groups afforded dications with lower cytotoxicity. For example, 2′-hydroxy substituted diimidazoline **46** was nearly 40-fold less toxic than the 2-phenylbenzofuran **13**.

Antitrypanosomal Activity. All compounds **1**−**49** exhibited in vitro antitrypanosomal potencies ranging from 2 nM to 5.1 μM with only two dications in the group showing IC_{50} values exceeding 1 μM. Ten diamidines (**1**, **11**, **17**, **20**, **26**, **29**, **32**, **35**, **38**, and **44**), one di(*N*-isopropyl)amidine (**45**), and three diimidazolines (**22**, **25**, and **34**) displayed antitrypanosomal IC_{50} values equal to or less than 10 nM. Eight of these dications (**1**, **20**, **22**, **26**, **29**, **32**, **38**, and **44**), all diamidines except diimidazoline **22**, demonstrated antitrypanosomal potencies comparable to or lower than that of melarsoprol (IC_{50} = 4 nM). Six 2-phenylbenzofurans (**1**, **20**, **26**, **29**, **32**, and **38**) exhibited antitrypanosomal activities equal or superior to that of pentamidine (IC_{50} = 3 nM). 5-Substituted diamidines **29** and **32**, bearing methoxy and hydroxy groups in the 2′-position of the phenyl ring, respectively, were the most potent compounds in the series, with antitrypanosomal IC_{50} values of 2 nM.

Generally, diamidines were more active in vitro against *T. b. rhodesiense* than di(*N*-isopropyl)amidines and diimidazolines.[59,61,67−69] Antitrypanosomal potency of 5′-substituted dications decreased in the order Am > Im > *i*-PrAm, whereas for compounds bearing one cationic group in the 4′-position of the phenyl ring there was no apparent correlation between the type of cationic substituents and activities against *T. b. rhodesiense*. Replacement of the amidine groups in 2-phenylbenzofuran **1** with aromatic cationic moieties resulted in a 40- to 200-fold decrease of antitrypanosomal activities (compounds **4**−**7**).

The position of the cationic groups affected the antitrypanosomal properties of 2-phenylbenzofurans **1**−**49**. Thus, 5-substituted diamidines and di(*N*-isopropyl)amidines were more active against *T. b. rhodesiense* than 6-substituted derivatives except diamidine **8** and di(*N*-isopropyl)amidines **27** and **33**, which displayed lower antitrypanosomal potencies than the corresponding 6-substituted isomers **14**, **39**, and **45**. In the case of diimidazolines, 6-substituted derivatives **40**, **43**, and **49** revealed higher antitrypanosomal activities relative to the 5-substituted derivatives **28**, **31**, and **37**, respectively. Compounds bearing one cationic group in the 4′-position of the phenyl ring exhibited higher activities against *T. b. rhodesiense* relative to 5′-substituted 2-phenylbenzofurans in all cases except for dications **3**, **26**, **28**, and **40**, which were less active against trypanosomes than the corresponding isomers **10**, **29**, **31**, and **43**, respectively.

In many cases, the attachment of the methoxy and hydroxy groups resulted in enhanced antitrypanosomal activity of 2-phenylbenzofurans. 5,4′-Substituted dications **17**, **20**, **26**, and **32** with the methoxy or hydroxy groups in the 7-position of the benzofuran moiety or 2′-position of the phenyl ring exhibited antitrypanosomal activity comparable to that of the diamidine **1**. Among the 5,5′-, 6,4′-, and 6,5′-substituted diamidines, derivatives bearing methoxy groups in the 2′-position of the phenyl ring were the most potent against *T. b. rhodesiense*. For example, 2′-methoxy substituted diamidine **29** exhibited 12-fold increase in the antitrypanosomal activity compared to the diamidine **8**. However, diamidine **23** bearing two hydroxy groups in the 7- and 2′-positions was 6-fold less as potent against *T. b. rhodesiense* as 2-phenylbenzofuran **1**. 4′-Substituted di(*N*-isopropyl)amidines and diimidazolines, bearing hydroxy groups in the 7- or/and 2′-positions, displayed higher antitrypanosomal activities than those with methoxy groups and the parent dications **2** and **3**. Thus, 2′-hydroxy substituted di(*N*-isopropyl)-amidines **33** and **45** revealed antitrypanosomal potencies 6- and 16-fold greater than 2-phenylbenzofurans **2** and **12**. Likewise, attachment of methoxy groups to the 7-position of the benzofuran ring in the diimidazoline **22** or to the 2′-position of the phenyl ring in the diimidazoline **34** resulted in increases activity against *T. b. rhodesiense* by 47- and 24-fold, respectively, relative to the parent dication **3**.

All compounds **1**−**49** were selective against the *Trypanosoma* parasite (selectivity index[70] SI_T, defined as a ratio of cytotoxic IC_{50} to antitrypanosomal IC_{50} values, varied from 5 to 89 500). Twelve dications (**20**, **22**, **25**, **26**, **29**, **32**, **34**, **35**, **38**, **44**−**46**) displayed antitrypanosomal selectivity indexes SI_T greater than that of pentamidine (SI_P = 15533).

Diamidines usually exhibited higher antitrypanosomal selectivity indexes SI_T compared to di(*N*-isopropyl)amidines and diimidazolines. However, in the series of 5,4′- and 6,4′-substituted dications, di(*N*-isopropyl)amidines **2** and **12** were the most selective against *T. b. rhodesiense*. Also, among 5,4′-substituted derivatives bearing hydroxy groups in the 2′- or/and the 7-positions of the aromatic moieties, diimidazolines **22** and **34** displayed the highest antitrypanosomal selectivity. 2-Phenylbenzofurans **4**−**7** bearing aromatic cationic moieties were 2- to 126-fold less selective against *T. b. rhodesiense* than dication **1**.

The relative antitrypanosomal selectivity of 5- and 6-substituted dications as well as 4′- and 5′-substituted isomers depended

on the type of the cationic substituents and the presence or absence of the methoxy and hydroxy groups in the molecules. 5-Substituted 2-phenylbenzofurans were more selective against *T. b. rhodesiense* compared to the 6-substituted congeners in all cases except diamidines **8** and **32**, di(*N*-isopropyl)amidines **27** and **33**, and diimidazolines **28** and **37**, which displayed lower antitrypanosomal selectivity indexes SI_T than the corresponding 6-substituted isomers **14**, **44**, **39**, **45**, **40**, and **39**, respectively. In the case of diamidines and diimidazolines, relative antitrypanosomal selectivities of 4′- and 5′-substituted isomers were affected by the presence or absence of methoxy or hydroxy substituents in the molecules. For instance, 5,5′-substituted diamidines **8**, **29**, and **35** as well as the 6,5′-substituted diamidine **14** were more selective against *T. b. rhodesiense* than the corresponding 4′-substituted isomers **1**, **26**, **32**, and **11**. Similarly, 5,5′- and 6,5′-substituted diimidazolines **10**, **16**, **31**, and **43** exhibited higher selectivity indexes SI_T than 4′-substituted derivatives **3**, **13**, **28**, and **40**, respectively. All other 4′-substituted 2-phenylbenzofurans exhibited higher antitrypanosomal selectivity indexes SI_T than corresponding 5′-substituted dications.

Addition of the methoxy or hydroxy groups to the 2′- or/and 7-position of the aromatic rings in many cases improved selectivity of 2-phenylbenzofurans **1**−**49** against *T. b. rhodesiense*. 5,4′-Substituted dications bearing the methoxy or hydroxy groups in the 7-position of the benzofuran ring displayed increased antitrypanosomal selectivity compared to dications **1**−**3** in all cases except diimidazoline **19** and diamidine **23**. Thus, 7-hydroxy substituted diamidine **20** and diimidazoline **22** exhibited antitrypanosomal selectivity indexes SI_T that were 97- and 242-fold greater than those of the compounds **1** and **3**, respectively. In the cases of 5,4′- and 5,5′-substituted diamidines bearing methoxy and hydroxy groups in the 2′-position of the phenyl ring, the antitrypanosomal selectivity increased in the order H < OH < OMe. Thus, 2′-methoxy substituted dications **26** and **29** were approximately 80- and 100-fold more selective against *T. b. rhodesiense* than corresponding diamidines **1** and **8**. The diamidine **29** not only exhibited the highest selectivity index SI_T of 89 500, which was nearly 6-fold greater than that of pentamidine (SI_T = 15 533), but also was one of two the most potent compounds in the series, displaying an antitrypanosomal IC_{50} value of 2 nM. In the case of all 6,4′-substituted dications, antitrypanosomal selectivity indexes SI_T increased in the order H < OMe < OH. For example, the antitrypanosomal selectivities of 2-hydroxy substituted diamidine **44** and diimidazoline **46** were nearly 500 times and more than 1200 times greater than that of the corresponding dehydroxy compounds **11** and **13**, respectively. The introduction of the hydroxy group in the 7- and/or 2′-position of the aromatic moieties of the 4′-substituted di(*N*-isopropyl)amidines improved their antitrypanosomal selectivity indexes SI_T. In the case of 5′-substituted isomers, however, such transformation reduced the selectivity of dications against the parasite compared to 2-phenylbenzofurans lacking substituents in the 2′-position.

Antiplasmodial Activity. Dications **1**−**49** exhibited potencies against *P. falciparum* with in vitro IC_{50} values ranging from 1 to 793 nM (Table 1). Only 13 compounds in the series displayed antimalarial IC_{50} values exceeding 100 nM. Twenty-six compounds (**1**, **3**−**6**, **8**, **11**, **12**, **14**, **17**−**22**, **25**−**29**, **32**−**34**, **37**, **38**, and **41**) exhibited antiplasmodial activities higher than pentamidine (IC_{50} = 58 nM). Twelve compounds (**3**, **8**, **11**, **12**, **14**, **17**, **26**, **27**, **32**, **34**, **38**, and **41**) demonstrated in vitro antiplasmodial IC_{50} values equal to or less than 20 nM. This group included eight diamidines (**8**, **11**, **14**, **17**, **26**, **32**, **38**, and **41**), two di(*N*-isopropyl)amidines (**12** and **27**), and two diimidazolines (**3** and **34**). Seven of these dications (**3**, **11**, **14**, **17**, **26**, **33**, and **34**), all diamidines except diimidazolines **3** and **34**, exhibited antiplasmodial IC_{50} values superior to that of artemisinin (IC_{50} = 6 nM). Diimidazoline **3**, bearing cationic substituents in the 5-position of the benzofuran moiety and the 4′-position of the phenyl ring, was the most potent compound in the series, with an antiplasmodial IC_{50} value of 1 nM.

Diamidines and diimidazolines usually exhibited higher antimalarial potency in vitro than di(*N*-isopropyl)amidines. Replacement of the amidine groups in the 2-phenylbenzofuran **1** with aromatic cationic substituents reduced antiplasmodial activities of the compounds **4**−**7**.

The position of cationic groups influenced the antiplasmodial properties of compounds **1**−**49**. 6-Substituted diamidines and di(*N*-isopropyl)amidines without the methoxy or hydroxy groups in the 2′-position of the phenyl ring were more active against *P. falciparum* than corresponding 5-substituted derivatives. However, among the 2′-methoxy and 2′-hydroxy substituted diamidines and di(*N*-isopropyl)amidines, compounds bearing cationic substituents in the 5-position of the benzofuran ring displayed higher antiplasmodial activities relative to the 6-substituted congeners in all cases except diamidines **29** and **35**, which were less active against *P. falciparum* than the corresponding 6-substituted dications **41** and **47**. All 5-substituted diimidazolines exhibited antiplasmodial activities greater than that of the 6-substituted derivatives. 2-Phenylbenzofurans bearing one cationic group in the 4′-position of the phenyl ring generally demonstrated higher antiplasmodial activities compared to their 5′-substituted counterparts apart from diamidines **1** and **38** as well as diimidazoline **40**, which were less effective against *P. falciparum* than the corresponding dications **8**, **41**, and **43**.

Addition of methoxy and hydroxy substituents improved antiplasmodial activities of some dications. For 5,4′-substituted diamidines and di(*N*-isopropyl)amidines antiplasmodial potencies increased in the order H < OH < OMe regardless of the position of the methoxy and hydroxy groups. For example, 7-methoxy substituted diamidine **17** displayed antiplasmodial activity nearly 8-fold greater than the dication **1**. Similarly, the diamidines **26** and **32** bearing methoxy and hydroxy groups in the 2′-position were 16- and 12-fold more active against *P. falciparum* than compound **1**. However, addition of two hydroxy groups to the 7- and 2′-position of the aromatic moieties in 2-phenylbenzofurans resulted in decreased antiplasmodial activities regardless of the nature of the cationic groups.

While the introduction of the methoxy and hydroxy groups increased antiplasmodial activities of selected 2-phenylbenzofurans, such as 5,4′-substituted diamidines and di(*N*-isopropyl)-amidines as well as 5,5′-, 6,4′-, and 6,5′-substituted diimidazolines, in some cases this structural modification afforded congeners with lower potencies against *P. falciparum* compared to the dications without methoxy or hydroxy substituents. For example, antiplasmodial activities of 5,5′-, 6,4′-, and 6,5′-substituted diamidines, 6,4′-, and 6,5′-substituted di(*N*-isopropyl)amidines, as well as 5,4′-substituted diimidazolines decreased in the order H > OMe > OH relative to the corresponding demethoxy and dehydroxy analogues.

All dications **1**−**49** were selective against *P. falciparum* (selectivity index[70] SI_P, defined as a ratio of cytotoxic IC_{50} to antiplasmodial IC_{50} values, varied from 34 to 52 333). Twenty-six dications (**2**, **3**, **7**, **8**, **12**, **14**, **15**, **17**, **18**, **20**−**22**, **25**−**27**, **29**,

32−**34**, **37**−**39**, **41**, **44**, **46**, **49**) displayed antiplasmodial selectivity indexes SI_P greater than that of pentamidine (SI_P = 803).

Diamidines and diimidazolines usually exhibited higher antimalarial selectivity indexes SI_P than di(*N*-isopropyl)amidines. Compounds **4**−**7** bearing aromatic cationic fragments displayed reduced antiplasmodial selectivity, except compound **7**, which was slightly more selective against *P. falciparum* than pentamidine.

The relative antiplasmodial selectivity of 5- and 6-substituted dications as well as 4′- and 5′-substituted congeners depended on the type of the cationic scaffolds and the presence of methoxy and hydroxy groups in the molecules. For instance, 6-substituted diamidines and di(*N*-isopropyl)amidines lacking methoxy and hydroxy groups in the 2′-position exhibited higher antiplasmodial selectivity indexes SI_P than their 5-substituted counterparts, while 5-substituted diimidazolines were more selective against *P. falciparum* compared to the 6-substituted analogues. Among the 2-phenylbenzofurans containing 2′-methoxy and hydroxy groups, compounds bearing cationic substituents in the 5-position of the benzofuran ring displayed higher antimalarial selectivity in all cases except diamidines **29** and **35**, which were less selective against *P. falciparum* than corresponding 6-substituted isomers **41** and **47**. In the case of diamidines and diimidazolines, antiplasmodial selectivities of 4′- and 5′-substituted isomers depended on the presence or absence of methoxy or hydroxy groups in the molecules. For instance, 6,5′-substituted diamidines **14** and **41** and diimidazolines **16** and **43** were more selective against *P. falciparum* than the corresponding 6,4′-substituted isomers **11**, **38**, **13**, and **40**. Similarly, 5,5′-substituted diamidine **8** and diimidazole **31** exhibited higher selectivity indexes SI_P than 5,4′-substituted derivatives **1** and **28**, respectively. In all other cases diamidines and diimidazolines bearing one cationic group in the 4′-position of the phenyl ring were more selective against the parasite than the 5′-substituted isomers. All 4′-substituted di(*N*-isopropyl)amidines exhibited higher antiplasmodial selectivity indexes SI_P than corresponding 5′-substituted dications.

In general, the position of the methoxy or hydroxy group in the 7- or 2′-position of the aromatic rings increased selectivity of some 2-phenylbenzofurans **1**−**49** against *P. falciparum*. Among 5,4′-substituted diamidines and di(*N*-isopropyl)amidines the selectivity against *P. falciparum* increased in the order H < OH < OMe. Thus, 7-methoxy and hydroxy substituted diamidines **17** and **20** exhibited antiplasmodial selectivity indexes SI_P that were almost 130- and 100-fold greater than that of dication **1**. Similarly, 2′- methoxy and hydroxy substituted dications **26** and **32** were 1300- and 200-fold more selective against *P. falciparum* than the diamidine **1**. Diamidine **26** not only was among the most potent compounds in the series, with an antiplasmodial IC_{50} value of 3 nM, but also was the most selective dication in the group, exhibiting an antiplasmodial selectivity index 65 times greater than that of pentamidine. However, introduction of two hydroxy groups in the 7- and 2′-positions of the aromatic moieties reduced the selectivity of diamidine **23** and di(*N*-isopropyl)amidine **24** compared to dications **1** and **2**. In the case of diimidazolines, antiplasmodial selectivity indexes SI_P of 5,5′-, 6,4′-, 6,5′-substituted derivatives increased in the order H < OMe < OH. For example, the introduction of hydroxy groups in the 2′-position of the phenyl ring afforded diimidazolines **46** and **49**, which were 48- and 30-fold as selective against *P. falciparum* as dications **13** and **16**, respectively. However, in several cases the introduction of methoxy or hydroxy groups in the 2′-position of the phenyl ring reduced antiplasmodial selectivity indexes SI_P of selected 2-phenylbenzofurans. Thus, 5,5′-substituted diamidines and di(*N*-isopropyl)amidines with the 2′-hydroxy group in the molecules were less selective against the parasite compared to the corresponding analogues lacking substitution in the 2′-position. In the case of 6,5′-substituted diamidines as well as 6,4′- and 6,5′-substituted di(*N*-isopropyl)amidines, antiplasmodial selectivity of the dications decreased in the order H > OMe > OH.

Antileishmanial Activity. All 2-phenylbenzofurans except dications **4**, **5**, **7**, **16**, **40**, and **48** were active in the *L. donovani* axenic amastigote assay, demonstrating activities ranging from 0.99 to 60 μM. Diamidines **1**, **26**, and **32** as well as di(*N*-isopropyl)amidines **2**, **27**, and **33** displayed antileishmanial IC_{50} values less than 2 μM. All these six compounds were 5,4′-substituted derivatives with or without methoxy or hydroxy groups in the 2′-position of the phenyl ring. Five 2-phenylbenzofurans (**1**, **2**, **27**, **32**, and **33**) exhibited antileishmanial IC_{50} values equal or greater than that of pentamidine (IC_{50} = 1.8 μM). Diamidine **1** revealed the highest antileishmanial potency in the series, exhibiting an IC_{50} value of 0.99 μM.

In general, substitution on the amidine groups resulted in decreased activity of the compounds **1**−**49** against *L. donovani* axenic amastigotes, although exceptions did occur. 2-Phenylbenzofurans **4**−**7** bearing aromatic cationic substituents possessed no antileishmanial activity except compound **6**, with an IC_{50} value of 5.7 μM.

The position of the cationic moieties had significant effect on antileishmanial properties of 2-phenylbenzofurans **1**−**49**. Compounds bearing one of the cationic groups in the 5-position of the benzofuran ring were more potent against *L. donovani* than 6-substituted derivatives in all cases except diamidine **29** and diimidazolines **31** and **37**, which displayed lower antileishmanial activity than the corresponding 6-substituted isomers **41**, **43**, and **49**, respectively. Similarly, 4′-substituted 2-phenylbenzofurans exhibited higher activities against *L. donovani* compared to compounds having one cationic group attached to the 5′-position of the phenyl ring on all occasions except for diamidine **38** and diimidazolines **28** and **40**, which displayed lower antileishmanial activities than corresponding dications **41**, **31**, and **43**.

Introduction of methoxy and hydroxy substituents in the 2′-position of the phenyl ring and in the 7-position of the benzofuran moiety resulted in decreased antileishmanial activities of the 5,4′-substituted diamidines.[24] For example, diamidine **23** containing two hydroxy groups attached to the 7- and the 2′-position of the molecule was 14 times less active against *L. donovani* than dication **1**. In the series of 5,5′- and 6,5′-substituted diamidines and 5,4′-substituted di(*N*-isopropyl)amidines, compounds bearing hydroxy groups in the 2′-positions of the phenyl ring displayed lower antileishmanial activities than parent dications **8**, **14**, and **2**. However, the introduction of the 2′-methoxy substituent in the molecules of the aforementioned dications afforded congeners with improved potency against *L. donovani*. Thus, 2′-methoxy substituted diamidine **41** revealed an antileishmanial activity nearly 4 times greater than dication **14**. Antileishmanial activities of 6,4′-substituted diamidines, 5,5′-, 6,4′-, and 6,5′- substituted di(*N*-isopropyl)amidines as well as 5,5′-substituted diimidazolines decreased in the order H > OMe > OH.

All 2-phenylbenzofurans, except dications **4**−**7**, **10**, **11**, **13**, **16**, **23**, **28**, **31**, **40**, and **43**, were selective against *L. donovani* and displayed selectivity indexes[70] (SI_L, defined as a ratio of cytotoxic IC_{50} to antileishmanial IC_{50} values) ranging from 2

to 125. Ten dications (**2**, **20**, **22**, **26**, **27**, **29**, **33**, **34**, **38**, and **41**) revealed antileishmanial selectivity indexes SI_L greater than that of pentamidine ($SI_L = 25$).

Diamidines and di(*N*-isopropyl)amidines generally exhibited higher antileishmanial selectivity indexes SI_L than diimidazolines. Dications **4**−**7** bearing aromatic cationic motifs did not show any selectivity against *L. donovani*.

Antileishmanial selectivity of 5- and 6-substituted dications as well as 4′- and 5′-substituted isomers depended on the type of the cationic substituents and the presence or absence of methoxy and hydroxy groups in the molecules. Compounds bearing cationic groups at the 5-position were more selective against *L. donovani* in all cases except diamidines **8** and **29** and diimidazoline **37**, which displayed lower antileishmanial selectivity indexes SI_L than corresponding 6-substituted isomers **14**, **41**, and **49**. Likewise, 4′-substituted 2-phenylbenzofurans usually exhibited higher antileishmanial selectivity indexes SI_L than corresponding 5′-substituted isomers apart from 5,4′-substituted diamidine **1** and 6,4′-substituted diamidines **11** and **38**, which were less selective against *L. donovani* than corresponding 5′-substituted derivatives **8**, **14**, and **41**.

Introduction of the methoxy and hydroxy substituents affected the antileishmanial selectivity of the 2-phenylbenzofurans **1**−**49**. 5,4′-Substituted diamidines and diimidazolines bearing methoxy or hydroxy groups in the 7-position of the benzofuran ring were more selective against *L. donovani* than dications **1** and **3** in all cases except diamidine **23**, which contained hydroxy groups both in 7- and in 2′-positions of the aromatic moieties. For example, 7-hydroxy substituted diamidine **20** and diimidazoline **22** revealed antileishmanial selectivity indexes SI_L that were 18- and 14-fold greater than those of compounds **1** and **3**, respectively. Conversely, di(*N*-isopropyl)amidines with substituents in the 7-position of the benzofuran ring exhibited reduced antileishmanial selectivity indexes compared to the compound **2**. Thus, di(*N*-isopropyl)amidine **24**, bearing the hydroxy groups in the 7- and 2′-position of the molecule, was 31-fold less selective against *L. donovani* than dication **2**. Introduction of the methoxy and hydroxy groups at the 2′-position of the phenyl ring also improved selectivity indexes SI_L of some cationic 2-phenylbenzofurans. Antileishmanial selectivity of 5,4′-, 5,5′-, and 6,4′-substituted diamidines bearing methoxy and hydroxy groups in the 2′-position of the phenyl ring increased in the order H < OH < OMe. For example, 2′-methoxy substituted dications **26** and **29** were 42- and 9-fold more selective against *L. donovani* than corresponding diamidines **1** and **8**. The attachment of the hydroxy group to the 2′-position of the phenyl ring of diimidazolines increased their antileishmanial selectivity compared to unsubstituted dications, while the addition of the 2′-methoxy substituent resulted in less selective compounds. Thus, 2′-hydroxy substituted diimidazoline **34** exhibited the antileishmanial selectivity index SI_L that was nearly 14-fold higher than that of dication **3**. In the case of di(*N*-isopropyl)amidines, the introduction of the 2′-methoxy or hydroxy groups in the molecules reduced selectivity of these dications against *L. donovani*. Thus, di(*N*-isopropyl)amidine **2** not only was more active than pentamidine against *L. donovani* but also displayed the highest selectivity index in the series ($SI_L = 125$), which was 5-fold greater than that of pentamidine.

In Vivo Antitrypanosomal Activity. Select 2-phenylbenzofurans exhibiting promising in vitro activities against *T. b. rhodesiense* were evaluated in the STIB900 animal model of African trypanosomiasis (Table 2). Initial screening was conducted using intraperitoneal dosing at 20 mg/kg daily for 4 days. However, because of the high curative rate and a significant increase in the survival time of the infected animals, the administered amount was reduced to 5 mg/kg daily for 4 days.

Table 2. Activity of Select 2-Phenylbenzofurans 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
pentamidine	3	4 × 20	2/4	>57.5
		4 × 5	2/4	>45
DAPI	17	4 × 20	4/4	>60
		4 × 5	0/4	>40.8
1	3	4 × 5	3/4	>53
		4 × 1	3/4	>56
		1 × 10	2/4	>51.8
2	125	4 × 5	3/4	>53
		4 × 1	0/4	15.3
		1 × 10	0/4	18.3
3	189	4 × 20	0/4	11
4	249	4 × 5	0/4	5.5
6	122	4 × 5	0/4	11
11	7	4 × 5	4/4	>60
		4 × 1	0/4	26.5
		1 × 10	1/4	>39.5
12	148	4 × 5	1/4	>38.5
14	21	4 × 5	0/4	33
17	6	4 × 5	4/4	>60
		4 × 1	0/4	27.3
		1 × 10	2/4	>44
19	124	4 × 20	0/4	11.5
20	3	4 × 5	4/4	>60
		4 × 1	1/4	>30.8
		1 × 10	1/4	>43
21	28	4 × 5	4/4	>60
		4 × 1	0/4	19.8
		1 × 10	2/4	>36.3
22	4	4 × 5	3/4	>49.8
		4 × 1	1/4	>39
		1 × 10	3/4	>51.3
23	18	4 × 5	3/4	>56.8
		4 × 1	1/4	>33.5
		1 × 10	2/4	>49.5
24	23	4 × 5	4/4	>60
		4 × 1	0/4	26.3
		1 × 10	2/4	>50.7
25	10	4 × 5	0/4	>21
26	3	4 × 5	2/4	>47
27	74	4 × 5	0/4	>39
29	2	4 × 5	2/4	>57
31	27	4 × 5	0/4	8.5
32	2	4 × 5	3/4	>54.5
		4 × 1	0/4	34.8
		1 × 10	2/4	>55.3
33	21	4 × 5	3/4	>50.5
		4 × 1	0/4	18.5
		1 × 10	0/4	26.8
34	8	4 × 5	3/4	>60
		4 × 1	0/4	20
		1 × 10	0/4	23.5
35	5	4 × 5	4/4	>60
		4 × 1	0/4	26
		1 × 10	2/4	>51.5
38	3	4 × 5	1/4	>34.5
39	54	4 × 5	1/4	>38
40	49	4 × 5	0/4	7
41	11	4 × 5	1/4	>48
43	17	4 × 5	0/4	25
44	4	4 × 5	2/4	>39
45	9	4 × 5	3/4	>55.5
		4 × 1	2/4	>46.5
		1 × 10	3/4	>51.8
46	11	4 × 5	0/4	23
47	29	4 × 5	1/4	>36
49	83	4 × 5	0/4	14

[a] Average of duplicate determinations. [b] STIB900 acute mouse model.[7] [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 days 7 and 8 after infection.

Even at this very low dosage 17 2-phenylbenzofurans (**1**, **2**, **11**, **17**, **20**−**24**, **26**, **29**, **32**−**35**, **44**, and **45**) displayed excellent in vivo efficacies, curing 2/4 animals or better. In addition, dications **12** and **14** provided 3/3 and 3/4 cures, respectively, when administered intraperitoneally at 20 mg/kg for 4 days (data not shown).

Diamidines and di(*N*-isopropyl)amidines usually exhibited higher in vivo efficacies than diimidazolines. For example, diamidine **1** and di(*N*-isopropyl)amidine **2** provided 3/4 cures when administered intraperitoneally at 5 mg/kg for 4 days, while diimidazole **3** was inactive even at the dose of 20 mg/kg.

The position of attachment of cationic substituents influenced the efficacies of 2-phenylbenzofurans in the acute mouse model of trypanosomiasis. Thus, 5-substituted dications were more effective in vivo than 6-substituted analogues and 4′-substituted derivatives exhibited higher potencies than 5′-substituted isomers. Overall, 12 out of 17 compounds active in the acute mouse model had their cationic moieties attached to the 5- and 4′-position of the aromatic rings.

Introduction of methoxy and hydroxy groups improved in vivo potencies of 2-phenylbenzofurans. For example, diamidine **20** and di(*N*-isopropyl)amidine **21** bearing the hydroxy group in the 7-position of the benzofuran moiety displayed improved antitrypanosomal efficacy relative to that of dications **1** and **2**, curing 4/4 animals. Likewise, 7-hydroxy substituted and 2′-hydroxy substituted diimidazolines **22** and **34** cured 3/4 animals, while the aforementioned 2-phenylbenzofuran **3** was inactive in the STIB900 model.

Fourteen dications providing 3/4 or 4/4 cures when administered at 5 mg/kg daily for 4 days were tested at an even lower intraperitoneal dosage of 1 mg/kg daily for 4 days as well as at a single-dose administration of 10 mg/kg. In the single-dose regimen, seven dications (**1**, **17**, **21**, **23**, **24**, **32**, and **35**) provided 2/4 cures, while 7-hydroxy substituted diimidazoline **22** and 2′-hydroxy substituted di(*N*-isopropyl)amidine **45** cured 3/4 mice. Among the five 2-phenylbenzofurans (**1**, **20**, **22**, **23**, and **45**) retaining curative ability in vivo at the regiment of 1 mg/kg daily for 4 days, diamidine **1** and di(*N*-isopropyl)amidine **45** exhibited in vivo activity comparable to that of melarsoprol, providing 3/4 and 2/4 cures, respectively.

Conclusions

We have reported the synthesis and the in vitro evaluation of the antiprotozoal properties of cationic substituted 2-phenylbenzofurans **1**−**49** against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani* as well as the cytotoxicity study of these compounds against mammalian cells. In our screens, diamidines usually exhibited higher antitrypanosomal, antimalarial, and antileishmanial activities compared to di(*N*-isopropyl)amidines and diimidazolines. Replacement of the amidine groups with aromatic cationic moieties afforded more cytotoxic congeners with decreased antiparasitic activity. The position of attachment of cationic groups influenced the antiprotozoal properties of 2-phenylbenzofurans. Generally, 5-substituted dications were more active against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* than the 6-substituted congeners, and 4′-substituted derivatives exhibited higher activities than 5′-substituted isomers. In our cytotoxicity study, however, 5,5′-substituted dications displayed higher antiparasitic selectivity than 5,4′-substituted congeners. Introduction of the methoxy or hydroxy substituents in the 7- or 2′-position of select 2-phenylbenzofurans reduced their cytotoxicity, enhanced antiprotozoal activity, or achieved both, providing derivatives with higher selectivity against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. Promising antiprotozoal activity of cationic substituted 2-phenylbenzofurans in vitro, excellent potency in the acute mouse model of trypanosomiasis and the reduced cytotoxicity compared to pentamidine warrant further investigation of this class of compounds.

Experimental Section

General Experimental Information. In vitro antitrypanosomal, antiplasmodial, and antileishmanial activities and cytotoxicities were determined following established protocols.[7,71−76] In vivo antitrypanosomal testing was conducted as previously described.[7] 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 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 and 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 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 that consisted of water containing formic acid (40 mM) and ammonium formate (10 mM). A concentrated reaction mixture was dissolved in the initial mobile phase. In the case of low solubility, heating of the mixture and/or addition of a small amount of methanol as a cosolvent was 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 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.

Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ±0.4% of calculated values.

General Procedure for Syntheses of Diamidines (1−3, 8−49). 2-(4-Amidinophenyl)-5-amidinobenzofuran Dihydrochloride (1).[47] A mixture of dry 1,4-dioxane (100 mL) and dry EtOH (40 mL) in a three-neck 500 mL flask equipped with a gas inlet tube, a thermometer, and a drying tube was saturated with gaseous HCl at 0 °C. 2-(4-Cyanophenyl)benzofuran-5-carbonitrile (**51**) (5.00 g, 20.5 mmol) was added in one portion, the flask was sealed, and the mixture was stirred at ambient temperature until the starting material was no longer detectable by HPLC. The reaction mixture was diluted with dry ether and a solid product was filtered off under argon, washed with diethyl ether, and dried under high vacuum to afford a diimidate ester (8.37 g, 100%), which was reacted immediately with appropriate amines.

To a suspension of the diimidate ester (2.00 g, 4.89 mmol) in dry EtOH (30 mL) was added dry EtOH saturated with gaseous ammonia (20 mL), and the reaction mixture was stirred at ambient temperature. The progress of the reaction was monitored by HPLC. Upon completion, the reaction mixture was concentrated to give crude product, which was purified by preparative HPLC to afford **1** as a white solid (1.14 g, 66%): mp >350 °C (lit.[47] 355−358 °C). ^{1}H NMR (DMSO-d_6) δ 9.55 (br s, 4H), 9.37 (br s, 4H), 8.30 (d, J = 1.6 Hz, 1H), 8.21 (d, J = 8.8 Hz, 2H), 8.05 (d, J = 8.8 Hz, 2H), 7.94 (s, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.87 (dd, J = 8.8 and 1.6 Hz, 1H). HPLC (method A) t_R = 4.04 min (100 area %). Anal. ($C_{16}H_{14}N_4O\cdot 2HCl\cdot 0.4H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (2). White solid (0.67 g, 42%): mp 355−357 °C. ^{1}H NMR (DMSO-d_6) δ 9.77 (br s, 1H), 9.70 (d, J = 7.5 Hz, 1H), 9.63 (br s, 1H), 9.57 (br s, 1H), 9.30 (br s, 1H), 9.24 (br s, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.18 (s, 1H), 8.17 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 4H), 7.74 (dd, J = 8.2 and 1.6 Hz, 1H), 4.16 (m, 2H), 1.31 (d, J = 6.3 Hz, 6H), 1.30 (d, J = 6.3 Hz, 6H). HPLC (method A) t_R = 6.20 min (100 area %). Anal. ($C_{22}H_{26}N_4O\cdot 2HCl\cdot 2.3H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (3).[48] White solid (1.12 g, 76%): mp >350 °C (lit.[48] no melting point). ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 4H), 8.55 (d, J = 1.6 Hz, 1H), 8.25 (s, 4H), 8.10 (dd, J = 8.2 and 1.6 Hz, 1H), 7.99 (s, 1H), 7.97 (d, J = 8.2 Hz, 1H), 4.03 (s, 8H). HPLC (method A) t_R = 5.19 min (100 area %). Anal. ($C_{20}H_{18}N_4O\cdot 2HCl\cdot 1H_2O$) C, H, N, Cl.

2-(3-Amidinophenyl)-5-amidinobenzofuran Dihydrochloride (8). White solid (0.17 g, 33%): mp 294−296 °C. ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 2H), 9.53 (s, 2H), 9.46 (s, 2H), 9.33 (s, 2H), 8.52 (dd, J = 1.6 and 1.6 Hz, 1H), 8.32 (d, J = 7.7 Hz, 1H), 8.31 (s, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.88 (s, 1H), 7.85 (dd, J = 8.8 and 1.6 Hz, 1H), 7.79 (dd, J = 7.7 and 7.7 Hz, 1H). HPLC (method A) t_R = 4.23 min (100 area %). Anal. ($C_{16}H_{14}N_4O\cdot 2HCl\cdot 1.2H_2O\cdot 0.1EtOH$) C, H, N, Cl.

2-(3-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (9). White solid (0.34 g, 53%): mp 265−268 °C. ^{1}H NMR (DMSO-d_6) δ 9.87 (d, J = 7.5 Hz, 1H), 9.71 (s, 1H), 9.70 (d, J = 7.5 Hz, 1H), 9.57 (s, 1H), 9.39 (s, 1H), 9.25 (s, 1H), 8.37 (s, 1H), 8.29 (dd, J = 7.7 and 1.6 Hz, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.87 (s, 1H), 7.82 (dd, J = 7.7 and 1.6 Hz, 1H), 7.77 (dd, J = 7.7 and 7.7 Hz, 1H), 7.73 (dd, J = 8.8 and 1.6 Hz, 1H), 4.16 (m, 2H), 1.32 (d, J = 6.3 Hz, 6H), 1.31 (d, J = 6.3 Hz, 6H). HPLC (method A) t_R = 6.14 min (98.0 area %). Anal. ($C_{22}H_{26}N_4O\cdot 2HCl\cdot 0.9H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (10). White solid (0.28 g, 56%): mp 356−358 °C. ^{1}H NMR (DMSO-d_6) δ 11.04 (br s, 4H), 8.90 (s, 1H), 8.54 (d, J = 1.6 Hz, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 8.08 (dd, J = 8.2 and 1.6 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.94 (s, 1H), 7.82 (dd, J = 7.7 and 7.7 Hz, 1H), 4.06 (s, 4H), 4.04 (s, 4H). HPLC (method A) t_R = 5.27 min (100 area %). Anal. ($C_{20}H_{18}N_4O\cdot 2HCl\cdot 1H_2O$) C, H, N, Cl.

2-(4-Amidinophenyl)-6-amidinobenzofuran Dihydrochloride (11).[47] White solid (1.07 g, 62%): mp >355 °C (lit.[47] 365−370 °C). ^{1}H NMR (DMSO-d_6) δ 9.56 (br s, 4H), 9.34 (br s, 4H), 8.28 (s, 1H), 8.22 (d, J = 8.2 Hz, 2H), 8.04 (d, J = 8.2 Hz, 2H), 7.96 (d, J = 8.2 Hz, 1H), 7.90 (s, 1H), 7.82 (dd, J = 8.2 and 1.6 Hz, 1H). HPLC (method A) t_R = 4.02 min (100 area %). Anal. ($C_{16}H_{14}N_4O\cdot 2HCl\cdot 1.2H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidinophenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (12). White solid (0.27 g, 24%): mp 338−340 °C. ^{1}H NMR (DMSO-d_6) δ 9.71 (br s, 6H), 8.19 (d, J = 8.2 Hz, 2H), 8.17 (s, 1H), 7.93 (d, J = 8.2 Hz, 3H), 7.88 (s, 1H), 7.69 (dd, J = 8.2 and 1.6 Hz, 1H), 4.15 (m, 2H), 1.32 (d, J = 6.3 Hz, 6H), 1.31 (d, J = 6.3 Hz, 6H). HPLC (method A) t_R = 6.06 min (100 area %). Anal. ($C_{22}H_{26}N_4O\cdot 2HCl\cdot 0.9H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (13). White solid (0.51 g, 47%): mp >330 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 4H), 8.52 (s, 1H), 8.28 (d, J = 8.2 Hz, 2H), 8.23 (d, J = 8.2 Hz, 2H), 8.01 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.93 (s, 1H), 4.04 (s, 8H). HPLC (method A) t_R = 5.15 min (100 area %). Anal. ($C_{20}H_{18}N_4O\cdot 2HCl\cdot 1.8H_2O$) C, H, N, Cl.

2-(3-Amidinophenyl)-6-amidinobenzofuran Dihydrochloride (14). White solid (1.07 g, 62%): mp >355 °C. ^{1}H NMR (DMSO-d_6) δ 9.51 (br s, 8H), 8.51 (br s, 1H), 8.32 (d, J = 8.2 Hz, 1H), 8.27 (s, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.84 (s, 1H), 7.81 (dd, J = 8.2 and 8.2 Hz, 1H), 7.80 (dd, J = 8.2 and 1.6 Hz, 1H). HPLC (method A) t_R = 4.08 min (100 area %). Anal. ($C_{16}H_{14}N_4O\cdot 2HCl\cdot 0.3H_2O$) C, H, N, Cl.

2-(3-*N*-Isopropylamidinophenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (15). White solid (0.35 g, 41%): mp 325−327 °C. ^{1}H NMR (DMSO-d_6) δ 9.91 (d, J = 7.5 Hz, 1H), 9.75 (d, 1H), 9.74 (s, 1H), 9.64 (s, 1H), 9.40 (s, 1H), 9.29 (s, 1H), 8.37 (dd, J = 1.6 and 1.6 Hz, 1H), 8.28 (dd, J = 7.7 and 1.6 Hz, 1H), 8.17 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.84 (s, 1H), 7.83 (dd, J = 7.7 and 1.6 Hz, 1H), 7.78 (dd, J = 7.7 and 7.7 Hz, 1H), 7.69 (dd, J = 8.2 and 1.6 Hz, 1H), 4.13 (m, 2H), 1.31 (d, J = 6.3 Hz, 6H), 1.30 (d, J = 6.3 Hz, 6H). HPLC (method A) t_R = 6.01 min (100 area %). Anal. ($C_{22}H_{26}N_4O\cdot 2HCl\cdot 0.8H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (16). White solid (0.81 g, 74%): mp >330 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.11 (br s, 4H), 8.86 (s, 1H), 8.53 (s, 1H), 8.32 (d, J = 7.7 Hz, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.82 (dd, J = 7.7 and 7.7 Hz, 1H), 4.05 (s, 4H), 4.03 (s, 4H). HPLC (method A) t_R = 5.16 min (100 area %). Anal. ($C_{20}H_{18}N_4O\cdot 2HCl\cdot 1.4H_2O$) C, H, N, Cl.

2-(4-Amidinophenyl)-5-amidino-7-methoxybenzofuran Dihydrochloride (17). White solid (0.40 g, 54%): mp 303−305 °C. ^{1}H NMR (DMSO-d_6) δ 9.57 (br s, 4H), 9.34 (br s, 2H), 9.29 (br s, 2H), 8.19 (d, J = 8.2 Hz, 2H), 8.02 (d, J = 8.2 Hz, 2H), 7.91 (s, 1H), 7.89 (s, 1H), 7.52 (s, 1H), 4.11 (s, 3H). HPLC (method A) t_R = 4.59 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2\cdot 1.9HCl\cdot 1.5H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidino-7-methoxybenzofuran Dihydrochloride (18). White solid (0.48 g, 54%): mp 330−331 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.73 (br s, 2H), 9.59 (br s, 2H), 9.21 (br s, 2H), 8.17 (d, J = 8.2 Hz, 2H), 7.91 (d, J = 8.2 Hz, 2H), 7.88 (s, 1H), 7.74 (s, 1H), 7.39 (s, 1H), 4.11 (m, 2H), 4.09 (s, 3H), 1.31 (d, J = 6.0 Hz, 6H), 1.30 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.55 min (100 area %). Anal. ($C_{23}H_{28}N_4O_2\cdot 2HCl\cdot 0.4H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-7-methoxybenzofuran Dihydrochloride (19).

White solid (0.68 g, 80%): mp >350 °C. ^{1}H NMR (DMSO-d_6) δ 10.84 (br s, 4H), 8.24 (d, J = 8.2 Hz, 2H), 8.18 (d, J = 8.2 Hz, 2H), 8.10 (s, 1H), 7.97 (s, 1H), 7.78 (s, 1H), 4.10 (s, 3H), 4.04 (s, 4H), 4.03 (s, 4H). HPLC (method A) t_R = 5.66 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2 \cdot 2HCl \cdot 1.7H_2O$) C, H, N, Cl.

2-(4-Amidinophenyl)-5-amidinobenzofuran-7-ol Dihydrochloride (20). White solid (0.23 g, 40%): mp >350 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.12 (br s, 1H), 9.55 (br s, 2H), 9.38 (br s, 4H), 9.20 (br s, 2H), 8.20 (d, J = 8.2 Hz, 2H), 8.04 (d, J = 8.2 Hz, 2H), 7.85 (s, 1H), 7.66 (s, 1H), 7.26 (s, 1H). HPLC (method A) t_R = 3.90 min (100 area %). Anal. ($C_{16}H_{14}N_4O_2 \cdot 2.1HCl \cdot 0.6H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran-7-ol Dihydrochloride (21). White solid (0.20 g, 28%): mp 321−323 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.14 (br s, 1H), 9.74 (d, J = 7.7 Hz, 1H), 9.59 (d, J = 7.0 Hz, 2H), 9.43 (br s, 1H), 9.24 (br s, 1H), 9.09 (br s, 1H), 8.17 (d, J = 8.2 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.83 (s, 1H), 7.54 (d, J = 1.6 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 4.10 (m, 2H), 1.30 (d, J = 6.0 Hz, 6H), 1.29 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 5.77 min (100 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran-7-ol Dihydrochloride (22). White solid (0.30 g, 47%): mp 329−330 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.13 (br s, 1H), 10.77 (br s, 4H), 8.23 (br s, 4H), 7.88 (s, 2H), 7.39 (s, 1H), 4.02 (s, 4H), 3.99 (s, 4H). HPLC (method A) t_R = 5.03 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2 \cdot 2HCl \cdot 1.4H_2O$) C, H, N, Cl.

2-(4-Amidino-2-hydroxyphenyl)-5-amidinobenzofuran-7-ol Dihydrochloride (23). White solid (0.46 g, 44%): mp 333−335 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.60−8.60 (br s, 8H), 8.11 (d, J = 8.2 Hz, 1H), 7.68 (s, 1H), 7.67 (d, J = 1.6 Hz, 1H), 7.50 (d, J = 1.6 Hz, 1H), 7.40 (dd, J = 8.2 and 1.6 Hz, 1H), 7.21 (d, J = 1.6 Hz, 1H). HPLC (method A) t_R = 4.08 min (100 area %). Anal. ($C_{16}H_{14}N_4O_3 \cdot 2HCl \cdot 1.6H_2O$) C, H, N, Cl.

2-(2-Hydroxy-4-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran-7-ol Dihydrochloride (24). Yellow solid (0.52 g, 41%): mp 319−320 °C. ^{1}H NMR (DMSO-d_6) δ 11.61 (s, 1H), 11.04 (s, 1H), 9.70 (d, J = 7.7 Hz, 1H), 9.57 (d, J = 8.0 Hz, 1H), 9.52 (s, 1H), 9.41 (s, 1H), 9.19 (s, 1H), 9.08 (s, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.65 (s, 1H), 7.55 (d, J = 1.6 Hz, 1H), 7.46 (d, J = 1.6 Hz, 1H), 7.32 (dd, J = 8.2 and 1.6 Hz, 1H), 7.14 (d, J = 1.6 Hz, 1H), 4.08 (m, 2H), 1.28 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R = 5.92 min (100 area %). Anal. ($C_{22}H_{26}N_4O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]benzofuran-7-ol Dihydrochloride (25). Yellow solid (0.65 g, 55%): mp >360 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 1.6 Hz, 1H), 7.68 (s, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.61 (dd, J = 8.2 and 1.6 Hz, 1H), 7.40 (d, J = 1.6 Hz, 1H), 4.00 (s, 8H). HPLC (method A) t_R = 5.19 min (100 area %). Anal. ($C_{20}H_{18}N_4O_3 \cdot 2HCl \cdot 1.7H_2O$) C, H, N, Cl.

2-(4-Amidino-2-methoxyphenyl)-5-amidinobenzofuran Dihydrochloride (26). White solid (0.50 g, 65%): mp 337−338 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.70−9.15 (br s, 8H), 8.26 (d, J = 1.6 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.83 (dd, J = 8.2 and 1.6 Hz, 1H), 7.74 (s, 1H), 7.73 (d, J = 1.1 Hz, 1H), 7.64 (dd, J = 8.2 and 1.1 Hz, 1H), 4.16 (s, 3H). HPLC (method A) t_R = 4.57 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 2HCl \cdot 0.2H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidino-2-methoxyphenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (27). White solid (0.80 g, 87%): mp 325−326 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.90−9.12 (br s, 6H), 8.16 (d, J = 1.6 Hz, 1H), 8.14 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.73 (dd, J = 8.2 and 1.6 Hz, 1H), 7.72 (s, 1H), 7.65 (d, J = 1.1 Hz, 1H), 7.53 (dd, J = 8.2 and 1.1 Hz, 1H), 4.17 (s, 3H), 4.16 (m, 2H), 1.32 (d, J = 6.0 Hz, 6H), 1.31 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.79 min (100 area %). Anal. ($C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 1.1H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]benzofuran Dihydrochloride (28). White solid (0.60 g, 70%): mp >350 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.86 (br s, 4H), 8.48 (s, 1H), 8.18 (d, J = 8.2 Hz, 1H), 8.03 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.73 (s, 1H), 4.15 (s, 3H), 4.04 (s, 8H). HPLC (method A) t_R = 5.74 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

2-(5-Amidino-2-methoxyphenyl)-5-amidinobenzofuran Dihydrochloride (29). White solid (0.57 g, 78%): mp 252−254 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.49 (br s, 2H), 9.48 (br s, 2H), 9.27 (br s, 2H), 9.24 (br s, 2H), 8.47 (d, J = 2.2 Hz, 1H), 8.27 (d, J = 1.6 Hz, 1H), 7.98 (dd, J = 8.8 and 2.2 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.83 (dd, J = 8.2 and 1.6 Hz, 1H), 7.66 (s, 1H), 7.49 (d, J = 8.8 Hz, 1H), 4.14 (s, 3H). HPLC (method A) t_R = 4.52 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 2HCl \cdot 1.3H_2O$) C, H, N, Cl.

2-(5-*N*-Isopropylamidino-2-methoxyphenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (30). White solid (0.48 g, 54%): mp 240−242 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.68 (d, J = 8.0 Hz, 2H), 9.54 (d, J = 8.2 Hz, 2H), 9.20 (d, J = 8.5 Hz, 2H), 8.34 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.86 (dd, J = 8.8 and 2.2 Hz, 1H), 7.72 (dd, J = 8.2 and 1.6 Hz, 1H), 7.67 (s, 1H), 7.47 (d, J = 8.8 Hz, 1H), 4.13 (s, 3H), 4.12 (m, 2H), 1.31 (d, J = 6.0 Hz, 6H), 1.30 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.65 min (100 area %). Anal. ($C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 2.1H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]benzofuran Dihydrochloride (31). White solid (0.55 g, 67%): mp 329−330 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.68 (br s, 4H), 8.63 (d, J = 2.2 Hz, 1H), 8.43 (d, J = 1.6 Hz, 1H), 8.12 (dd, J = 8.8 and 2.2 Hz, 1H), 7.99 (dd, J = 8.8 and 1.6 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.68 (s, 1H), 7.56 (d, J = 8.8 Hz, 1H), 4.16 (s, 3H), 4.04 (s, 4H), 4.02 (s, 4H). HPLC (method A) t_R = 5.66 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2 \cdot 2HCl \cdot 0.9H_2O$) C, H, N, Cl.

2-(4-Amidino-2-hydroxyphenyl)-5-amidinobenzofuran Dihydrochloride (32). White solid (0.41 g, 56%): mp 346−347 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.67 (br s, 1H), 9.47 (br s, 4H), 9.26 (br s, 4H), 8.28 (d, J = 1.6 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.81 (dd, J = 8.8 and 1.6 Hz, 1H), 7.74 (s, 1H), 7.53 (d, J = 1.1 Hz, 1H), 7.39 (dd, J = 8.2 and 1.1 Hz, 1H). HPLC (method A) t_R = 4.22 min (100 area %). Anal. ($C_{16}H_{14}N_4O_2 \cdot 2HCl \cdot 1.9H_2O$) C, H, N, Cl.

2-(2-Hydroxy-4-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (33). White solid (0.65 g, 74%): mp 317−318 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.67 (br s, 1H), 9.70 (d, J = 8.2 Hz, 1H), 9.67 (d, J = 7.7 Hz, 1H), 9.51 (br s, 2H), 9.16 (br s, 2H), 8.15 (d, J = 1.6 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.72 (s, 1H), 7.70 (dd, J = 8.8 and 1.6 Hz, 1H), 7.48 (d, J = 1.1 Hz, 1H), 7.30 (dd, J = 8.2 and 1.1 Hz, 1H), 4.10 (m, 2H), 1.30 (d, J = 6.0 Hz, 6H), 1.29 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.40 min (100 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot 2.2H_2O$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]benzofuran Dihydrochloride (34). White solid (0.57 g, 68%): mp >360 °C. ^{1}H NMR (DMSO-d_6) δ 11.78 (br s, 1H), 10.83 (br s, 4H), 8.52 (d, J = 1.6 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 8.05 (dd, J = 8.8 and 1.6 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.74 (s, 1H), 7.64 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 4.03 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R = 5.36 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

2-(5-Amidino-2-hydroxyphenyl)-5-amidinobenzofuran Dihydrochloride (35). White solid (0.30 g, 44%): mp 323−325 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.19 (br s, 1H), 9.46 (br s, 2H), 9.35 (br s, 2H), 9.22 (br s, 2H), 9.08 (br s, 2H), 8.42 (d, J = 2.2 Hz, 1H), 8.26 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.68 (s, 1H), 7.38 (d, J = 8.2 Hz, 1H). HPLC (method A) t_R = 3.85 min (100 area %). Anal. ($C_{16}H_{14}N_4O_2 \cdot 2.1HCl \cdot 2.4H_2O$) C, H, N, Cl.

2-(2-Hydroxy-5-*N*-Isopropylamidinophenyl)-5-*N*-isopropylamidinobenzofuran Dihydrochloride (36). White solid (0.39 g, 45%): mp 273−275 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.11 (br s, 1H), 9.65 (d, J = 8.2 Hz, 1H), 9.55 (d, J = 8.2 Hz, 1H), 9.53 (br s, 1H), 9.42 (br s, 1H), 9.21 (br s, 1H), 9.08 (br s, 1H), 8.27 (d, J = 2.2 Hz, 1H), 8.15 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.72−7.65 (m, 2H), 7.67 (s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 4.13 (m, 2H), 1.30 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R = 6.00 min (100 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot 1.2H_2O \cdot 0.6EtOH$) C, H, N, Cl.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]benzofuran Dihydrochloride (37). White solid (0.52 g, 67%): mp 278−280 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H), 11.00−10.50 (br s, 4H), 8.58 (d, J = 2.2 Hz, 1H), 8.47 (d, J = 1.6 Hz, 1H), 8.03 (dd, J = 8.8 and 1.6 Hz, 1H), 8.00 (dd, J = 8.2 and 2.2 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.66 (s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 4.03 (s, 4H), 4.00 (s, 4H). HPLC (method A) t_R = 5.13 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2 \cdot 2HCl \cdot 1.8H_2O$) C, H, N, Cl.

2-(4-Amidino-2-methoxyphenyl)-6-amidinobenzofuran Dihydrochloride (38). Light-yellow solid (0.39 g, 56%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.66 (s, 2H), 9.52 (s, 2H), 9.38 (s, 2H), 9.30 (s, 2H), 8.24 (s, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.74 (s, 1H), 7.66 (d, J = 8.2 Hz, 1H), 4.16 (s, 3H). HPLC (method A) t_R = 4.76 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 2HCl \cdot 1.7H_2O$) C, H, N, Cl.

2-(4-*N*-Isopropylamidino-2-methoxyphenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (39). White solid (0.46 g, 57%): mp >250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.84 (d, J = 7.7 Hz, 1H), 9.71 (s, 1H), 9.67 (d, J = 8.2 Hz, 1H), 9.56 (s, 1H), 9.31 (s, 1H), 9.21 (s, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.13 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.73 (s, 1H), 7.67 (d, J = 8.2 Hz, 1H), 7.65 (s, 1H), 7.54 (d, J = 8.2 Hz, 1H), 4.16 (s, 3H), 4.13 (m, 2H), 1.32 (d, J = 6.0 Hz, 6H), 1.30 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.82 min (98.7 area %). Anal. ($C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 1.7H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]benzofuran Dihydrochloride (40). Light-yellow solid (0.51 g, 65%): mp >255 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.08 (s, 2H), 10.90 (s, 2H), 8.46 (s, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.03 (d, J = 1.1 Hz, 1H), 7.99 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.83 (dd, J = 8.2 and 1.1 Hz, 1H), 7.77 (s, 1H), 4.14 (s, 3H), 4.05 (s, 8H). HPLC (method A) t_R = 5.79 min (99.1 area %). Anal. ($C_{21}H_{20}N_4O_2 \cdot 2.4HCl \cdot 3.1H_2O$) C, H, N, Cl.

2-(5-Amidino-2-methoxyphenyl)-6-amidinobenzofuran Dihydrochloride (41). Purple solid (0.41 g, 59%): mp >230 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.55 (s, 2H), 9.53 (s, 2H), 9.32 (s, 2H), 9.26 (s, 2H), 8.46 (s, 1H), 8.24 (s, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.68 (s, 1H), 7.50 (d, J = 8.8 Hz, 1H), 4.14 (s, 3H). HPLC (method A) t_R = 5.03 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 2.1HCl \cdot 1.7H_2O$) C, H, N, Cl.

2-(5-*N*-Isopropylamidino-2-methoxyphenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (42). White solid (0.35 g, 41%): mp >240 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.71 (br s, 2H), 9.57 (br s, 2H), 9.21 (s, 1H), 9.16 (s, 1H), 8.31 (d, J = 1.6 Hz, 1H), 8.14 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.87 (dd, J = 8.8 and 1.6 Hz, 1H), 7.68 (s, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 4.13 (m, 2H), 4.12 (s, 3H), 1.31 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R = 6.66 min (100 area %). Anal. ($C_{23}H_{28}N_4O_2 \cdot 2.1HCl \cdot 2.6H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]benzofuran Dihydrochloride (43). Light-yellow solid (0.61 g, 77%): mp 252−255 °C. ^{1}H NMR (DMSO-d_6) δ 11.08 (s, 2H), 10.88 (s, 2H), 8.64 (s, 1H), 8.50 (s, 1H), 8.21 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.66 (s, 1H), 7.53 (d, J = 8.8 Hz, 1H), 4.14 (s, 3H), 4.03 (s, 8H). HPLC (method A) t_R = 5.95 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2 \cdot 2HCl \cdot 2.5H_2O$) C, H, N, Cl.

2-(4-Amidino-2-hydroxyphenyl)-6-amidinobenzofuran Dihydrochloride (44). Light-yellow solid (0.31 g, 45%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.70 (s, 1H), 9.49 (s, 4H), 9.27 (s, 4H), 8.23 (s, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.56 (s, 1H), 7.52 (d, J = 1.6 Hz, 1H), 7.39 (dd, J = 8.2 and 1.6 Hz, 1H). HPLC (method A) t_R = 4.08 min (100 area %). Anal. ($C_{16}H_{14}N_4O_2 \cdot 2HCl \cdot 2.2H_2O$) C, H, N, Cl.

2-(2-Hydroxy-4-*N*-Isopropylamidinophenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (45). White solid (0.41 g, 48%): mp 258−261 °C. ^{1}H NMR (DMSO-d_6) δ 11.75 (s, 1H), 9.73 (d, J = 8.2 Hz, 1H), 9.68 (d, J = 8.2 Hz, 1H), 9.57 (s, 1H), 9.55 (s, 1H), 9.25 (s, 2H), 8.13 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.73 (s, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.50 (s, 1H), 7.31 (d, J = 8.2 Hz, 1H), 4.17 (m, 2H), 1.31 (d, J = 6.0 Hz, 6H), 1.29 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 6.08 min (100 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2.1HCl \cdot 2.1H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]benzofuran Dihydrochloride (46). Yellow solid (0.45 g, 57%): mp 255−257 °C. ^{1}H NMR (DMSO-d_6) δ 11.76 (s, 1H), 10.76 (s, 2H), 10.70 (s, 2H), 8.41 (s, 1H), 8.15 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.92 (dd, J = 8.2 and 1.1 Hz, 1H), 7.80 (s, 1H), 7.56 (d, J = 1.1 Hz, 1H), 7.54 (dd, J = 8.2 and 1.1 Hz, 1H), 4.04 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R = 5.21 min (98.0 area %). Anal. ($C_{20}H_{18}N_4O_2 \cdot 2.3HCl \cdot 2H_2O$) C, H, N, Cl.

2-(5-Amidino-2-hydroxyphenyl)-6-amidinobenzofuran Dihydrochloride (47). White solid (0.31 g, 45%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.16 (s, 1H), 9.48 (s, 2H), 9.38 (s, 2H), 9.22 (s, 2H), 9.07 (s, 2H), 8.39 (s, 1H), 8.20 (s, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.69 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H). HPLC (method A) t_R = 4.09 min (100 area %). Anal. ($C_{16}H_{14}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

2-(2-Hydroxy-5-*N*-Isopropylamidinophenyl)-6-*N*-isopropylamidinobenzofuran Dihydrochloride (48). White solid (0.48 g, 57%): mp >275 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.12 (s, 1H), 9.69 (d, J = 8.2 Hz, 1H), 9.62 (d, J = 8.2 Hz, 1H), 9.59 (s, 1H), 9.46 (s, 1H), 9.23 (s, 1H), 9.10 (s, 1H), 8.25 (d, J = 2.2 Hz, 1H), 8.13 (s, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.69 (dd, J = 7.7 and 2.2 Hz, 1H), 7.68 (s, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 4.13 (m, 2H), 1.32 (d, J = 6.0 Hz, 6H), 1.30 (d, J = 6.0 Hz, 6H). HPLC (method A) t_R = 5.84 min (98.7 area %). Anal. ($C_{22}H_{26}N_4O_2 \cdot 2.1HCl \cdot 1.5H_2O$) C, H, N, Cl.

6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]benzofuran Dihydrochloride (49). Tan solid (0.45 g, 57%): mp 270−273 °C. ^{1}H NMR (DMSO-d_6) δ 12.41 (s, 1H), 11.06 (s, 2H), 10.73 (s, 2H), 8.58 (d, J = 1.6 Hz, 1H), 8.48 (s, 1H), 8.01 (dd, J = 8.8 and 1.6 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H), 7.69 (s, 1H), 7.42 (d, J = 8.8 Hz, 1H), 4.03 (s, 4H), 4.00 (s, 4H). HPLC (method A) t_R = 5.24 min (98.5 area %). Anal. ($C_{20}H_{18}N_4O_2 \cdot 2.3HCl \cdot 2.6H_2O$) C, H, N, Cl.

5-(1*H*-Benzimidazolyl)-2-[4-(1*H*-benzimidazolyl)phenyl]benzofuran Dihydrochloride (4). A mixture of an imidate ester (1.91 g, 4.66 mmol), prepared from **51** (1.20 g, 4.91 mmol) following the procedure described above for **1**, and 1,2-diaminobenzene (1.11 g, 10.3 mmol) in dry MeOH (100 mL) was refluxed overnight. A precipitate formed, was separated, washed with ether, and dried to give **4** as a yellow solid (1.75 g, 75%): mp >340 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.57 (d, J = 1.6 Hz, 1H), 8.36 (d, J = 8.8 Hz, 2H), 8.24 (dd, J = 8.8 and 1.6 Hz, 1H), 8.20 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 7.80−7.65 (m, 4H), 7.36−7.25 (m, 4H). HPLC (method A) t_R = 5.24 min (98.5 area %). Anal. ($C_{28}H_{18}N_4O \cdot 2HCl \cdot 1.8H_2O$) C, H, N, Cl.

5-(1*H*-Imidazol-2-yl)-2-[4-(1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (5). To a suspension of **3** (free base) (2.38 g, 7.20 mmol) in dry DMSO (60 mL) was added *o*-iodoxylbenzoic acid[65] (8.05 g, 28.8 mmol), and the mixture was stirred at 50−60 °C for 48 h. Saturated aqueous $Na_2S_2O_3$ (30 mL) was added upon

cooling, and the mixture was basified with 1 M KOH (50 mL), diluted with iced water (300 mL), and stirred for 1 h. A precipitate formed, was separated, washed with water, and dried. The crude product was recrystallized from DMF and converted to a hydrochloride salt by recrystallization from 1 M HCl/EtOH to give **5** as a tan solid (1.33 g, 46%): mp >340 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.61 (d, J = 1.6 Hz, 1H), 8.37 (d, J = 8.8 Hz, 2H), 8.25 (d, J = 8.8 Hz, 2H), 8.22 (dd, J = 8.8 and 1.6 Hz, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.91 (s, 1H), 7.85 (s, 2H), 7.82 (s, 2H). HPLC (method A) t_R = 5.82 min (98.2 area %). Anal. ($C_{20}H_{14}N_4O\cdot 2HCl\cdot 1.8H_2O$) C, H, N, Cl.

5-(1*H*-Benzimidazolyl)-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran Dihydrochloride (6). A mixture of **52** (2.60 g, 7.62 mmol) and copper(I) cyanide (2.05 g, 22.9 mmol) in dry DMF (100 mL) was refluxed for 48 h under an Ar atmosphere. The reaction mixture, after successive treatments with ethylenediamine and sodium cyanide solutions, was filtered, and a solid residue was washed with 15% aqueous NH_3 (3 × 150 mL) and water. The dry solid was recrystallized from DMF to give 5-cyano-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran (**54**), which was converted to an imidate ester following the procedure described above for **1**. A mixture of the imidate ester (1.15 g, 2.83 mmol) and 1,2-diaminobenzene (0.34 g, 3.11 mmol) in dry MeOH (60 mL) was refluxed overnight. A precipitate formed, was separated, washed with ether, and dried to give **6** as a tan solid (0.39 g, 23%): mp >350 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.84 (br s, 2H), 8.78 (d, J = 1.6 Hz, 1H), 8.40 (dd, J = 8.8 and 1.6 Hz, 1H), 8.25 (d, J = 8.8 Hz, 2H), 8.20 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.8 Hz, 1H), 8.01 (s, 1H), 7.90−7.80 (m, 2H), 7.60−7.45 (m, 2H), 4.03 (s, 4H). HPLC (method A) t_R = 7.78 min (98.5 area %). Anal. ($C_{24}H_{18}N_4O\cdot 2HCl\cdot 1.7H_2O$) C, H, N, Cl.

2-[4-(1*H*-Benzimidazolyl)phenyl]-5-(4,5-dihydro-1*H*-imidazol-2-yl)benzofuran Dihydrochloride (7). A mixture of **53** (2.51 g, 6.45 mmol) and copper(I) cyanide (1.73 g, 19.3 mmol) in dry DMF (120 mL) was refluxed for 48 h under an Ar atmosphere. The reaction mixture, after successive treatments with ethylenediamine and sodium cyanide solutions, was filtered, and a solid residue was washed with 15% aqueous NH_3 (3 × 150 mL) and water. The dry solid was recrystallized from DMF to give 5-cyano-2-[4-(1*H*-benzimidazolyl)phenyl]benzofuran (**55**), which was converted to an imidate ester following the procedure described above for **1**. A mixture of the imidate ester (1.41 g, 3.10 mmol) and ethylenediamine (2 mL) in dry EtOH (50 mL) was stirred at ambient temperature for 48 h. The reaction mixture was concentrated and the residue was purified by preparative HPLC to give **7** as a tan solid (0.12 g, 4%): mp >340 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.72 (s, 4H), 8.47 (d, J = 8.8 Hz, 2H), 8.46 (s, 1H), 8.29 (d, J = 8.8 Hz, 2H), 8.00 (s, 2H), 7.93 (s, 1H), 7.80 (m, 2H), 7.48 (m, 2H), 4.04 (s, 4H). HPLC (method A) t_R = 8.29 min (100 area %). Anal. ($C_{24}H_{18}N_4O\cdot 2HCl\cdot 3H_2O$) C, H, N, Cl.

5-Bromo-2-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]benzofuran (52). A mixture of an imidate ester (3.48 g, 9.14 mmol), prepared from 5-bromo-2-(4-cyanophenyl)benzofuran **50** following the procedure described above for **1**, and ethylenediamine (5 mL) in dry EtOH (100 mL) was stirred at ambient temperature overnight and then refluxed for 2 h. A precipitate formed, was separated, rinsed with ether, and dried. The residue was washed with 1 M NaOH (50 mL), water, EtOH, and dried to give crude product, which was recrystallized from EtOH/DMF to afford **52** as a white solid (2.93 g, 94%): mp 298−300 °C. ^{1}H NMR (DMSO-d_6) δ 7.98 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.90 (d, J = 1.9 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.52 (s, 1H), 7.49 (dd, J = 8.8 and 1.9 Hz, 1H), 7.05 (br s, 1H), 3.63 (s, 4H). HPLC (method B) t_R = 4.87 min (100 area %). Anal. ($C_{17}H_{13}BrN_2O$) C, H, N, Br.

5-Bromo-2-[4-(1*H*-benzimidazolyl)phenyl]benzofuran (53). A mixture of an imidate ester (3.48 g, 9.14 mmol), prepared from **50** following the procedure described above for **1**, and 1,2-diaminobenzene (1.10 g, 10.2 mmol) in dry MeOH (100 mL) was refluxed overnight. A precipitate formed, was separated, rinsed with ether, and dried. The residue was washed with 1 M NaOH (50 mL), water, EtOH and dried to give crude product, which was recrystallized from EtOH/DMF to afford **53** as a white solid (3.04 g, 85%): mp 300−301 °C. ^{1}H NMR (DMSO-d_6) δ 8.32 (d, J = 8.5 Hz, 2H), 8.13 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 2.0 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.60 (m, 2H), 7.57 (s, 1H), 7.50 (dd, J = 8.8 and 2.0 Hz, 1H), 7.23 (m, 2H). HPLC (method B) t_R = 8.47 min (100 area %). Anal. ($C_{21}H_{13}BrN_2O\cdot 0.4H_2O$) C, H, N, Br.

General Procedure for the Copper-Mediated Heteroannulation. 2-(4-Cyanophenyl)benzofuran-5-carbonitrile (51).[47,48] A mixture of methyl-4-hydroxy-3-iodobenzonitrile (**56**) (9.80 g, 40.0 mmol), 4-ethynylbenzonitrile (**57**) (6.00 g, 47.2 mmol), and copper(I) oxide (3.43 g, 24.0 mmol) in dry pyridine (180 mL) was refluxed overnight. The mixture was allowed to cool and concentrated. The residue was diluted with 1 M HCl (50 mL), and a brown precipitate was filtered off and washed with water, 10% aqueous ammonia, water, and EtOH. The product was purified by flash chromatography, eluting with $CHCl_3$ followed by recrystallization from MeCN to afford **51** as a white solid (4.24 g, 43%): mp 230−232 °C. (lit.[47] 243−245 °C, lit.[48] 235−240 °C). ^{1}H NMR (DMSO-d_6) δ 8.32 (d, J = 1.6 Hz, 1H), 8.14 (d, J = 8.2 Hz, 2H), 8.01 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.8 Hz, 1H), 7.84 (dd, J = 8.8 and 1.6 Hz, 1H), 7.83 (s, 1H). HPLC (method B) t_R = 8.31 min (100 area %). Anal. ($C_{16}H_8N_2O$) C, H, N.

2-(3-Cyanophenyl)benzofuran-5-carbonitrile (59). Purification was by flash chromatography, eluting with hexanes/$CHCl_3$, to give **59** as a white solid (1.50 g, 45%): mp 173−175 °C. ^{1}H NMR (DMSO-d_6) δ 8.46 (dd, J = 1.6 and 1.6 Hz, 1H), 8.31 (d, J = 1.1 Hz, 1H), 8.28 (dd, J = 8.2 and 1.6 Hz, 1H), 7.93 (dd, J = 8.2 and 1.1 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.83 (dd, J = 8.2 and 1.6 Hz, 1H), 7.78 (s, 1H), 7.76 (dd, J = 8.2 and 8.2 Hz, 1H). HPLC (method B) t_R = 8.38 min (100 area %). Anal. ($C_{16}H_8N_2O\cdot 0.1EtOH$) C, H, N.

Methyl-2-(4-(methoxycarbonyl)phenyl)benzofuran-6-carboxylate (63). Purification was by flash chromatography, eluting with CH_2Cl_2, to give **63** as a white solid (11.0 g, 71%): mp 200−202 °C. ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 8.13 (s, 2H), 8.12 (s, 2H), 7.92 (dd, J = 8.2 and 1.1 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 3.91 (s, 3H), 3.90 (s, 3H). HPLC (method B) t_R = 9.58 min (100 area %). Anal. ($C_{18}H_{14}O_5$) C, H.

Methyl-2-(3-(methoxycarbonyl)phenyl)benzofuran-6-carboxylate (64). Purification was by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **64** as a white solid (5.30 g, 69%): mp 159−161 °C. ^{1}H NMR (DMSO-d_6) δ 8.49 (dd, J = 1.6 and 1.6 Hz, 1H), 8.26 (dd, J = 8.2 and 1.6 Hz, 1H), 8.22 (s, 1H), 8.03 (dd, J = 8.2 and 1.6 Hz, 1H), 7.90 (dd, J = 8.2 and 1.1 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.73 (d, J = 1.1 Hz, 1H), 7.71 (dd, J = 8.2 and 8.2 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H). HPLC (method B) t_R = 9.59 min (100 area %). Anal. ($C_{18}H_{14}O_5$) C, H.

2-(4-Cyanophenyl)-5-formyl-7-methoxybenzofuran (69). Purification was by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **69** as a white solid (5.00 g, 69%): mp 198−200 °C ($CHCl_3$/EtOH). ^{1}H NMR (DMSO-d_6) δ 10.04 (s, 1H), 8.11 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 1.1 Hz, 1H), 7.88 (s, 1H), 7.46 (d, J = 1.1 Hz, 1H), 4.06 (s, 3H). HPLC (method B) t_R = 8.09 min (100 area %). Anal. ($C_{17}H_{11}NO_3\cdot 0.8H_2O$) C, H, N.

2-(4-Cyano-2-methoxyphenyl)-5-formyl-7-methoxybenzofuran (70). Purification was by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **70** as a white solid (6.63 g, 80%): mp 310−312 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 10.03 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.93 (s, 1H), 7.69 (s, 1H), 7.67 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.46 (s, 1H), 4.08 (s, 6H). HPLC (method B) t_R = 8.78 min (100 area %). Anal. ($C_{18}H_{13}NO_4$) C, H, N.

2-(4-Cyano-2-methoxyphenyl)benzofuran-5-carbonitrile (78). Yellow solid (8.72 g, 92%): mp 282−283 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 8.29 (d, J = 1.6 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.83 (dd, J = 8.2 and 1.6 Hz, 1H), 7.75 (d, J = 1.1 Hz, 1H), 7.68 (s, 1H), 7.61 (dd, J = 8.2 and 1.1 Hz, 1H), 4.08 (s, 3H). HPLC (method B) t_R = 8.86 min (100 area %). Anal. ($C_{17}H_{10}N_2O_2$) C, H, N.

2-(5-Cyano-2-methoxyphenyl)benzofuran-5-carbonitrile (79). Yellow solid (8.72 g, 92%): mp 220−222 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 8.29 (d, J = 1.6 Hz, 1H), 8.24 (s, 1H), 7.94 (dd, J = 8.2 and 1.6 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.80 (dd, J = 8.2 and 1.6 Hz, 1H), 7.56 (s, 1H), 7.43 (d, J = 8.8 Hz, 1H), 4.10 (s, 3H). HPLC (method B) t_R = 8.57 min (100 area %). Anal. ($C_{17}H_{10}N_2O_2$) C, H, N.

2-(4-Cyano-2-methoxyphenyl)benzofuran-6-carbonitrile (80). Purification was by flash chromatography, eluting with $CHCl_3$, to give **80** as a yellow solid (3.79 g, 41%): mp 256−258 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.26 (s, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.74 (s, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.69 (s, 1H), 7.60 (d, J = 8.2 Hz, 1H), 4.08 (s, 3H). HPLC (method B) t_R = 8.81 min (100 area %). Anal. ($C_{17}H_{10}N_2O_2$) C, H, N.

2-(5-Cyano-2-methoxyphenyl)benzofuran-6-carbonitrile (81). Purification was by flash chromatography, eluting with $CHCl_3$, to give **81** as a yellow solid (5.10 g, 57%): mp 197−198 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 8.24 (s, 1H), 7.96 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.62 (s, 1H), 7.44 (d, J = 8.8 Hz, 1H), 4.10 (s, 3H). HPLC (method B) t_R = 8.53 min (100 area %). Anal. ($C_{17}H_{10}N_2O_2$) C, H, N.

2-(4-Cyanophenyl)benzofuran-6-carbonitrile (65).[47] **Method 1. Conversion of Benzofuran Esters to Dinitriles with Dimethylaluminum Amide.** Anhydrous NH_3 was bubbled through dry *o*-xylene (280 mL) for 20 min at 0 °C. A 2.0 M solution of $AlMe_3$ in toluene (120 mL, 240 mmol) was added, and the NH_3 was passed through the mixture for 20 min. Then the solution was stirred at room temperature for 1 h. Methyl-2-(4-(methoxycarbonyl)phenyl)-benzofuran-6-carboxylate (**63**) (16.4 g 52.8 mmol) was added in one portion. The mixture was stirred at 100−110 °C for 3 h, allowed to cool to ambient temperature, and diluted with $CHCl_3$ (400 mL). Water (100 mL) was added dropwise with vigorous stirring, and a formed precipitate was filtered off. The filtrate was concentrated to give crude product, which was purified by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **65** as a white solid (3.70 g, 29%): mp 243−245 °C (acetone) (lit.[47] 221−223 °C). ^{1}H NMR (DMSO-d_6) δ 8.30 (br s, 1H), 8.14 (d, J = 8.2 Hz, 2H), 8.02 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.2 Hz, 1H), 7.85 (s, 1H), 7.72 (dd, J = 8.2 and 1.6 Hz, 1H). HPLC (method B) t_R = 8.38 min (100 area %). Anal. ($C_{16}H_8N_2O$) C, H, N.

Method 2. Copper-Mediated Heteroannulation. Following the procedure described above for **51**, **65** was prepared from 3-hydroxy-4-iodobenzonitrile (**67**) (6.00 g, 24.5 mmol), **57** (3.74 g, 29.4 mmol), and Cu_2O (2.10 g, 14.7 mmol) in pyridine (120 mL). Crude product was purified by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **65** as a white solid (4.57 g, 76%).

2-(3-Cyanophenyl)benzofuran-6-carbonitrile (66). Following the procedure described above for **65** in method 1, **66** was prepared from **64** (15.5 g, 50.0 mmol) as a white solid (3.65 g, 30%): mp 204−205 °C (acetone). ^{1}H NMR (DMSO-d_6) δ 8.45 (d, J = 1.6 Hz, 1H), 8.28 (s, 1H), 8.27 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.79 (s, 1H), 7.76 (dd, J = 8.2 and 8.2 Hz, 1H), 7.71 (dd, J = 8.2 and 1.6 Hz, 1H). HPLC (method B) t_R = 8.40 min (100 area %). Anal. ($C_{16}H_8N_2O$) C, H, N.

Following the procedure described above for **51**, **66** was prepared from **67** (6.00 g, 24.5 mmol), **58** (3.74 g, 29.4 mmol), and Cu_2O (2.10 g, 14.7 mmol) in pyridine (120 mL). Crude product was purified by flash chromatography, eluting with hexanes/CH_2Cl_2, to give **66** as a white solid (3.53 g, 59%).

General Procedure for Synthesis of Oximes (71, 72). 2-(4-Cyanophenyl)-5-formyl-7-methoxybenzofuran Oxime (71). A mixture of **69** (4.28 g, 15.4 mmol) and $NH_2OH\cdot HCl$ (1.18 g, 17.0 mmol) in dry Py (70 mL) was stirred at ambient temperature for 2 h. The mixture was diluted with iced water and stirred for 1 h. A precipitate formed, was separated, washed with water, and dried. Recrystallization was from $CHCl_3$ to yield **71** as a white solid (3.56 g, 79%): mp 205−206 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1H), 8.23 (s, 1H), 8.08 (d, J = 8.8 Hz, 2H), 7.97 (d, J = 8.8 Hz, 2H), 7.73 (s, 1H), 7.46 (d, J = 1.6 Hz, 1H), 7.29 (d, J = 1.6 Hz, 1H), 4.01 (s, 3H). HPLC (method B) t_R = 7.11 min (100 area %). Anal. ($C_{17}H_{12}N_2O_3$) C, H, N.

2-(4-Cyano-2-methoxyphenyl)-5-formyl-7-methoxybenzofuran Oxime (72). White solid (5.16 g, 80%): mp 255−257 °C. ^{1}H NMR (DMSO-d_6) δ 11.17 (s, 1H), 8.21 (s, 1H), 8.05 (d, J = 7.7 Hz, 1H), 7.69 (s, 1H), 7.59 (s, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.46 (s, 1H), 7.27 (s, 1H), 4.06 (s, 3H), 4.00 (s, 3H). HPLC (method B) t_R = 7.66 min (100 area %). Anal. ($C_{18}H_{14}N_2O_4\cdot 0.5H_2O$) C, H, N.

General Procedure for Synthesis of Dinitriles (73, 74). 2-(4-Cyanophenyl)-7-methoxybenzofuran-5-carbonitrile (73). A mixture of **71** (4.00 g, 13.7 mmol) in acetic anhydride (100 mL) was refluxed overnight. The reaction mixture was cooled, diluted with water (100 mL), and stirred for 1 h at ambient temperature. A precipitate formed, was separated, washed with water, dried, and recrystallized from $CHCl_3$ to yield **73** as a tan solid (3.35 g, 90%): mp 251−253 °C. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 1.1 Hz, 1H), 7.78 (s, 1H), 7.47 (d, J = 1.1 Hz, 1H), 4.04 (s, 3H). HPLC (method B) t_R = 8.48 min (100 area %). Anal. ($C_{17}H_{10}N_2O_2\cdot 0.1H_2O$) C, H, N.

2-(4-Cyano-2-methoxyphenyl)-7-methoxybenzofuran-5-carbonitrile (74). Tan solid (7.15 g, 84%): mp 325−326 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 8.07 (d, J = 8.2 Hz, 1H), 7.84 (s, 1H), 7.70 (s, 1H), 7.63 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.43 (s, 1H), 4.08 (s, 3H), 4.05 (s, 3H). HPLC (method B) t_R = 9.09 min (100 area %). Anal. ($C_{18}H_{12}N_2O_3$) C, H, N.

General Procedure for the Demethylation of 2-Phenyl Benzofurans 75, 76, 82−85. 2-(4-Cyanophenyl)-7-hydroxybenzofuran-5-carbonitrile (75). To molten pyridine hydrochloride (12 g) was added **73** (2.00 g, 7.29 mmol), and the reaction mixture was kept at 160−180 °C for 3 h, cooled to 80−90 °C, and diluted with water (100 mL). A precipitate formed, was separated, washed with water, and dried to give **75** as a gray solid (1.58 g, 83%): mp 330−332 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 11.09 (s, 1H), 8.14 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.8 Hz, 2H), 7.77 (s, 1H), 7.73 (d, J = 1.6 Hz, 1H), 7.12 (d, J = 1.6 Hz, 1H). HPLC (method B) t_R = 6.85 min (100 area %). Anal. ($C_{16}H_8N_2O_2\cdot 0.1H_2O$) C, H, N.

2-(4-Cyano-2-hydroxyphenyl)-7-hydroxybenzofuran-5-carbonitrile (76). Gray solid (2.83 g, 78%): mp >360 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 11.49 (s, 1H), 11.00 (br s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 1.1 Hz, 1H), 7.64 (s, 1H), 7.45 (dd, J = 8.2 and 1.1 Hz, 1H), 7.35 (d, J = 1.6 Hz, 1H), 7.08 (d, J = 1.6 Hz, 1H). HPLC (method B) t_R = 5.99 min (100 area %). Anal. ($C_{16}H_8N_2O_3\cdot 0.4H_2O$) C, H, N.

2-(4-Cyano-2-hydroxyphenyl)benzofuran-5-carbonitrile (82). Gray solid (3.19 g, 84%): mp 302−303 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 11.51 (s, 1H), 8.28 (d, J = 1.6 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.80 (dd, J = 8.2 and 1.6 Hz, 1H), 7.70 (s, 1H), 7.43 (dd, J = 8.2 and 1.1 Hz, 1H), 7.36 (d, J = 1.1 Hz, 1H). HPLC (method B) t_R = 7.54 min (100 area %). Anal. ($C_{16}H_8N_2O_2$) C, H, N.

2-(5-Cyano-2-hydroxyphenyl)benzofuran-5-carbonitrile (83). White solid (3.21 g, 86%): mp 257−260 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 11.93 (s, 1H), 8.26 (s, 1H), 8.24 (d, J = 1.6 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 8.2 and 1.6 Hz, 1H), 7.75 (dd, J = 8.2 and 1.1 Hz, 1H), 7.59 (s, 1H), 7.19 (d, J = 8.2 Hz, 1H). HPLC (method B) t_R = 7.13 min (100 area %). Anal. ($C_{16}H_8N_2O_2\cdot 0.2$ EtOAc) C, H, N.

2-(4-Cyano-2-hydroxyphenyl)benzofuran-6-carbonitrile (84). White solid (1.52 g, 80%): mp >300 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 11.57 (s, 1H), 8.26 (s, 1H), 8.05 (d, J = 8.2 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.74 (s, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.36 (s, 1H). HPLC (method B) t_R = 7.44 min (100 area %). Anal. ($C_{16}H_8N_2O_2\cdot 0.3$EtOH) C, H, N.

2-(5-Cyano-2-hydroxyphenyl)benzofuran-6-carbonitrile (85). White solid (1.56 g, 82%): mp >300 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 11.99 (s, 1H), 8.25 (d, J = 1.6 Hz, 1H), 8.24 (s, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.76 (dd, J = 8.6 and 1.6 Hz, 1H), 7.70 (dd, J = 8.2 and 1.1 Hz, 1H), 7.65 (d, J = 1.1 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H). HPLC (method B) t_R = 7.07 min (100 area %). Anal. ($C_{16}H_8N_2O_2\cdot 0.2\ H_2O$) C, H, N.

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

References

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

(2) Das, B. P.; Boykin, D. W. Synthesis and antiprotozoal activity of 2,5-bis(4-guanylphenyl)thiophenes and -pyrroles. *J. Med. Chem.* **1977**, *20*, 1219–1221.

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

(4) 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.

(5) Bell, C. A.; Dykstra, C. C.; Naiman, N. A.; Cory, M.; Fairley, T. A.; Tidwell, R. R. Structure−activity studies of dicationically substituted bis-benzimidazoles against *Giardia lamblia*: correlation of antigiardial activity with DNA binding affinity and giardial topoisomerase II inhibition. *Antimicrob. Agents Chemother.* **1993**, *37*, 2668–2673.

(6) 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.

(7) 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.

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

(9) 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.

(10) 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.

(11) Salom-Roig, X. J.; Hamze, A.; Calas, M.; Vial, H. J. Dual molecules as new antimalarials. *Comb. Chem. High Throughput Screening* **2005**, *8*, 49–62.

(12) 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.

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

(14) Rodriguez, F.; Rozas, I.; Kaiser, M.; Brun, R.; Nguyen, B.; Wilson, W. D.; Garcia, R. N.; Dardonville, C. New bis(2-aminoimidazoline) and bisguanidine DNA minor groove binders with potent in vivo antitrypanosomal and antiplasmodial activity. *J. Med. Chem.* **2008**, *51*, 909–923.

(15) Doua, F.; Miezan, T. W.; Sanon Singaro, J. R.; Boa Yapo, F.; Baltz, T. The efficacy of pentamidine in the treatment of early-late stage *Trypanosoma brucei gambiense* trypanosomiasis. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 586–588.

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

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

(18) 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.

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

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

(21) 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.

(22) 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.

(23) O'Brien, J. G.; Dong, B. J.; Coleman, R. L.; Gee, L.; Balano, K. B. A 5-year retrospective review of adverse drug reactions and their risk factors in human immunodeficiency virus-infected patients who were receiving intravenous pentamidine therapy for *Pneumocystis carinii* pneumonia. *Clin. Infect. Dis.* **1997**, *24*, 854–859.

(24) 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.

(25) 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.

(26) 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.

(27) Burchmore, R. J.; Ogbunude, P. O.; Enanga, B.; Barrett, M. P. Chemotherapy of human African trypanosomiasis. *Curr. Pharm. Des.* **2002**, *8*, 257–267.

(28) 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.

(29) Burri, C.; Brun, R. Human African Trypanosomiasis. In *Manson's Tropical Diseases*; Cook, G. C., Zumla, A., Eds.; W. B. Saunders: London, 2003; pp 1303−1323.

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

(31) May, J.; Meyer, C. G. Chemoresistance in falciparum malaria. *Trends Parasitol* **2003**, *19*, 432–435 (discussion 435−436).

(32) 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.

(33) 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.

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

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

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

(37) 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.

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

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

(40) 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.

(41) 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.

(42) 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.

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

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

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

(46) 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.

(47) Dann, O.; Bergen, G.; Demant, E.; Volz, G. Trypanocide diamidine des 2-phenyl-benzofurans, 2-phenyl-indens und 2-phenyl-indols. *Liebigs Ann. Chem.* **1971**, *749*, 68–89.

(48) Dann, O.; Char, H.; Griessmeier, H. Synthesen biskationisher, trypanocider 1-benzofuran-verbindungen. *Liebigs Ann. Chem.* **1982**, 1836–1869.

(49) 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.
(50) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, *16*, 4467–4470.
(51) Lutjens, H.; Scammells, P. J. Synthesis of natural products possessing a benzo[β]furan skeleton. *Tetrahedron Lett.* **1998**, *39*, 6581–6584.
(52) Verner, E.; Katz, B. A.; Spencer, J. R.; Allen, D.; Hataye, J.; Hruzewicz, W.; Hui, H. C.; Kolesnikov, A.; Li, Y.; Luong, C.; Martelli, A.; Radika, K.; Rai, R.; She, M.; Shrader, W.; Sprengeler, P. A.; Trapp, S.; Wang, J.; Young, W. B.; Mackman, R. L. Development of serine protease inhibitors displaying a multicentered short (< 2.3 Å) hydrogen bond binding mode: inhibitors of urokinase-type plasminogen activator and factor Xa. *J. Med. Chem.* **2001**, *44*, 2753–2771.
(53) Sonogashira, K. Development of Pd−Cu catalyzed cross-coupling of terminal acetylenes with sp^2-carbon halides. *J. Organomet. Chem.* **2002**, *653*, 46–49.
(54) Bates, C. G.; Saejueng, P.; Murphy, J. M.; Venkataraman, D. Synthesis of 2-arylbenzo[β]furans via copper(I)-catalyzed coupling of *o*-iodophenols and aryl acetylenes. *Org. Lett.* **2002**, *4*, 4727–4729.
(55) Castro, C. E.; Gaughan, E. J.; Owsley, D. C. Indoles, benzofurans, phthalides, and tolanes via copper(I) acetylides. *J. Org. Chem.* **1966**, *31*, 4071–4078.
(56) Castro, C. E.; Havlin, R.; Honwad, V. K.; Malte, A. M.; Moje, S. W. Copper(I) substitutions. Scope and mechanism of cuprous acetylide substitutions. *J. Am. Chem. Soc.* **1969**, *91*, 6464–6470.
(57) Doad, G. J. S.; Barltrop, J. A.; Petty, C. M.; Owen, T. C. A versatile and convenient synthesis of benzofurans. *Tetrahedron Lett.* **1989**, *30*, 1597–1598.
(58) Pilling, R. J.; Whiting, D. A. FIQ and FIQ2, new Q-site inhibitors for photosynthetic electron transport: synthesis and the relationship between stereochemistry and biological activity. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2077–2086.
(59) 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.
(60) Edgar, K. J.; Falling, S. N. An efficient and selective method for the preparation of iodophenols. *J. Org. Chem.* **1990**, *55*, 5287–5291.
(61) 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.
(62) Diaz, P.; Gendre, F.; Stella, L.; Charpentier, B. New synthetic retinoids obtained by palladium-catalized tandem cyclisation−hydride capture process. *Tetrahedron* **1998**, *54*, 4579–4590.
(63) Wood, J. L.; Khatri, N. A.; Weinreb, S. M. A direct conversion of esters to nitriles. *Tetrahedron Lett.* **1979**, *20*, 4907–4910.
(64) Sagi, K.; Nakagawa, T.; Yamanashi, M.; Makino, S.; Takahashi, M.; Takayanagi, M.; Takenaka, K.; Suzuki, N.; Oono, S.; Kataoka, N.; Ishikawa, K.; Shima, S.; Fukuda, Y.; Kayahara, T.; Takehana, S.; Shima, Y.; Tashiro, K.; Yamamoto, H.; Yoshimoto, R.; Iwata, S.; Tsuji, T.; Sakurai, K.; Shoji, M. Rational design, synthesis, and structure−activity relationships of novel factor Xa inhibitors: (2-substituted-4-amidinophenyl)pyruvic and -propionic acids. *J. Med. Chem.* **2003**, *46*, 1845–1857.
(65) Frigerio, M.; Santagostino, M.; Sputore, S. A user-friendly entry to 2-iodoxybenzoic acid (IBX). *J. Org. Chem.* **1999**, *64*, 4537–4538.
(66) Nicolaou, K. C.; Mathison, C. J. N.; Montagnon, T. *o*-Iodoxybenzoic acid (IBX) as a viable reagent in the manipulation of nitrogen- and sulfur-containing substrates: scope, generality, and mechanism of IBX-mediated amine oxidations and dithiane deprotections. *J. Am. Chem. Soc.* **2004**, *126*, 5192–5201.
(67) 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.
(68) 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*, 531–538.
(69) Donkor, I. O.; Huang, T. L.; Tao, B.; Rattendi, D.; Lane, S.; Vargas, M.; Goldberg, B.; Bacchi, C. Trypanocidal activity of conformationally restricted pentamidine congeners. *J. Med. Chem.* **2003**, *46*, 1041–1048.
(70) 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.
(71) 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.
(72) Raz, B.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) in vitro. *Acta Trop.* **1997**, *68*, 139–147.
(73) Matile, H.; Pink, J. R. L. *Plasmodium falciparum* Malaria Parasite Culture and Their Use in Immunology. In *Immunological Methods*; Lefkovits, I., Pernis, B., Eds.; Academic Press: San Diego, CA, 1990; pp 221−234.
(74) Werbovetz, K.; Brendle, J.; Sackett, D. Purification, characterization, and drug susceptibility of tubulin from *Leishmania*. *Mol. Biochem. Parasitol.* **1999**, *98*, 53–65.
(75) Werbovetz, K.; Sackett, D.; 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*-propylsulfonilamide (GB-II-5) against kinetoplastid parasites. *Mol. Pharmacol.* **2003**, *64*, 1325–1333.
(76) Sperandeo, N. R.; Brun, R. Synthesis and biological evaluation of pyrazolylnaphthoquinones as new potential antiprotozoal and cytotoxic agents. *ChemBioChem* **2003**, *4*, 69–72.

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