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Original article

Dicationic dibenzofuran derivatives as anti-*Pneumocystis carinii* pneumonia agents: synthesis, DNA binding affinity, and anti-*P. carinii* activity in an immunosuppressed rat model

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Abstract – Previous work from our laboratory shows that compounds with two cations linked by a carbazole spacer were highly potent anti-*P. carinii* agents. A prodrug approach designed to increase oral activity of the dicationic carbazoles by converting amidine groups to amidoxime groups was unsuccessful. The ring nitrogen was implicated as playing a role in the lack of activity of carbazole amidoximes. The current study was designed to determine if replacement of the carbazole ring nitrogen by isosteric oxygen to form dibenzofurans would improve effectiveness of amidoxime prodrugs. Eight dibenzofuran dicationic derivatives were synthesized and evaluated for anti-*P. carinii* activity in an immunosuppressed rat model. Since DNA binding has been hypothesized to play a key role in antimicrobial activity of dicationic compounds, the compounds were examined for their binding affinity to calf thymus DNA and a poly-dA•poly-dT oligomer. While several of the compounds were more potent anti-*P. carinii* agents than pentamidine, the corresponding amidoximes were significantly less effective than the amidoxime of pentamidine. No direct quantitative correlation was determined between DNA binding affinity and anti-*P. carinii* activity, but all active compounds were strong DNA binding agents. © Elsevier, Paris

***Pneumocystis carinii* pneumonia / dibenzofurans / amidines / amidoximes / DNA**

1. Introduction

Dicationic molecules (pentamidine related) have been shown to be active against a number of pathogenic organisms including: *Pneumocystis carinii* [1–5], *Plasmodium falciparum* [6], *Giardia lamblia* [7], *Leishmania mexicana amazonensis* [6], *Cryptosporidium parvum* [8], *Toxoplasma gondii* [9], *Candida albicans* [10], and *Cryptococcus neoformans* [10]. Previous studies in our laboratory directed toward the development of dicationic molecules as potential antimicrobial agents have: (1) determined the potential effect of metabolism on antimicrobial activity and toxicity of pentamidine and related compounds [11–15]; (2) provided evidence for potential mechanisms of antimicrobial activity [7, 16–21] and toxicity [22] of these molecules; (3) shown the compounds to be active against a number of pathogenic organisms [3, 5–9, 17, 20, 23–28]; (4) determined the feasibility of utilizing amidoximes as prodrugs for diamidine compounds in order to increase bioavailability [29, 30]. A major effort in our laboratory has been directed toward the clinical development of a safe, effective and orally available dicationic molecule for the treatment of an important AIDS-related opportunistic infection, *Pneumocystis carinii* pneumonia (PCP).

While a large number of the dicationic molecules were found to be active against *P. carinii* and have low toxicity

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Abbreviations: HPLC: high-performance liquid chromatography; DMF: N,N-dimethylformamide; DMSO: dimethylsulfoxide; TFA: trifluoroacetic acid

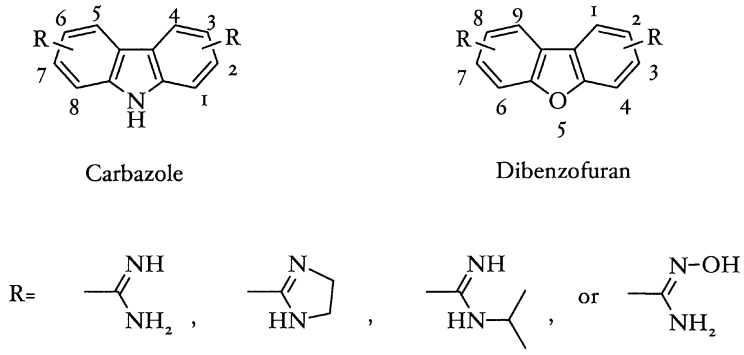


Figure 1. Bases corresponding to carbazole and dibenzofuran dications.

in an animal model of the disease, a major challenge has been to develop an orally active drug. A promising approach to obtaining orally active dicationic molecules was the synthesis of prodrugs containing amidoximes in place of amidine moieties. The amidoxime is less basic than the parent amidine (pK_a = 8–9 for amidoximes compared to pK_a = 12–13 for amidines) and has improved oral uptake [29]. While the amidoxime is devoid of in vitro antimicrobial activity, it is rapidly metabolized to the corresponding active amidine [29–36]. Previous studies showed that this approach was highly effective for pentamidine [29]. However, it was disappointing to find that several of the more potent and less toxic dicationic molecules were ineffective anti-PCP agents when given orally as the amidoxime prodrug. An unexplained correlation was observed in a series of prodrug experiments [29, 30] when it was found that the amidoxime prodrugs that were ineffective in treating PCP were those having a nitrogen in the moiety linking the two dicationic groups [29, 30]. The lack of activity of prodrugs with the cations linked by a carbazole spacer *(figure 1)* was particularly disappointing since the dicationic carbazoles had proved to be highly potent antimicrobial agents with low toxicity in animal models [4]. The current study was designed to determine if replacement of the carbazole nitrogen by oxygen to form a dibenzofuran would lead to improved effectiveness of amidoxime prodrugs. The current study contains the synthesis and anti-PCP testing of a series of dicationic dibenzofurans *(figure 1)* and selected amidoxime derivatives. Because DNA minor groove binding is hypothesized to play a major role in the antimicrobial activity of these molecules [7, 16–20], tests were also performed to determine the binding affinity of the molecules with calf thymus DNA and a poly-dA•poly-dT oligomer.

2. Chemistry

Diamidine **1** was first made by Moffatt in 1950 [37], while the other dications synthesized in this paper are novel. The 2,8-disubstituted dibenzofurans *(figure 2)* were synthesized from the commercially available dibenzofuran **9**. 2,8-Dibromodibenzofuran **10** [38], the major product of bromination of dibenzofuran **9**, underwent nucleophilic reaction with copper(I) cyanide in refluxing *N*,*N*-dimethylformamide to provide dicyanodibenzofuran **11** [39]. The diimidate intermediate **12** was prepared from **11** through Pinner reaction [24, 40–43] by reacting with ethanol in hydrogen chloride saturated 1,4-dioxane at room temperature for 9–14 days. The diimidate **12** was isolated without being characterized after high conversion was determined by HPLC and IR. The dried diimidate **12** reacted with excess ammonia, ethylenediamine, isopropylamine, and hydroxylamine in anhydrous ethanol to give the final compounds diamidine **1**, diimidazoline **2**, di(isopropylamidine) **3**, and diamidoxime **4** respectively. All these dications were in hydrochloride salt forms after final purification.

3,7-Disubstituted dibenzofuran derivatives *(figure 3)* could not be synthesized effectively from the dibenzofuran ring. Therefore, de novo furan ring preparation was necessary to obtain the desired products. Ullman coupling reaction [44] of 2,5-dibromonitrobenzene **13** with copper powder in *N*,*N*-dimethylformamide at 120 °C gave compound **14**. Selective nucleophilic substitution of **14** by reacting with sodium methoxide between 0 °C and

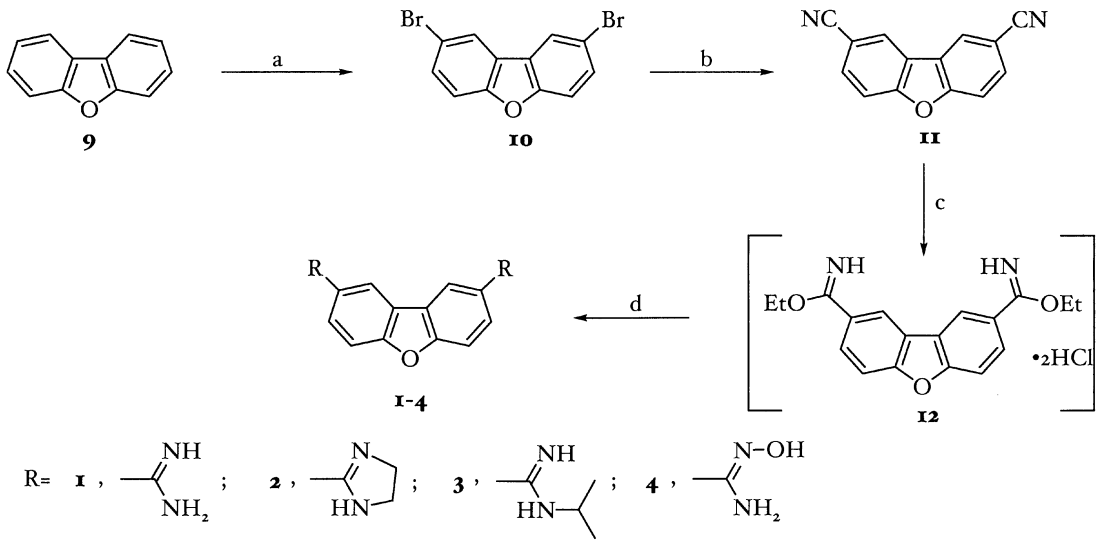


Figure 2. Synthesis of 2,8-disubstituted dibenzofuran dications. Keys: (a) Br_2/AcOH/reflux; (b) CuCN/DMF/reflux; (c) EtOH/HCl/1,4-dioxane/room temperature; (d) Appropriate amine/EtOH.

room temperature provided the methoxy compound **15**. Since the aromatic nitro group is a better leaving group than the aromatic bromo group, selective substitution could be achieved [45, 46]. However, significant impurities were found in the reaction mixture, resulting from different combinations of methoxy substitution. The key condition for obtaining the desired product **15** was temperature control (0 °C at the beginning of the reaction, then increased to room temperature as the reaction slowed). The nitro group of compound **15** was reduced to the corresponding amine **16** using 5% ruthenium on carbon as a catalyst in ethanol at 65–70 °C [47]. The dibenzofuran ring was formed with minor modifications of a reported procedure [48]. The amino group in compound **16** was first diazotized in sodium nitrite and aqueous sulfuric acid solution. The diazonium salt **17** obtained in situ was heated to reflux to give 3,7-dibromodibenzofuran **18**. The 3,7-dicyanodibenzofuran **19** was prepared from **18**, by reacting with copper(I) cyanide in refluxing *N*,*N*-dimethylformamide. Pinner reaction [24, 40–43] of compound **19** with ethanol in HCl saturated 1,4-dioxane at room temperature for 2 weeks gave the intermediate 3,7-diimidatedibenzofuran **20**, which was isolated without characterization. **20** was reacted with either excess ammonia, ethylenediamine, isopropylamine, or hydroxylamine in ethanol solution to give the corresponding diamidine **5**, diimidazoline **6**, di(isopropylamidine) **7**, or diamidoxime **8**.

3. Results and discussion

3.1. Activity against P. carinii pneumonia

The activity of compounds **1**–**8** against PCP in the rat model is shown in *table I*. Compounds **1**–**3** and **5**–**7** in the initial screen were given once daily by tail vein injection at a dose of 10 mmol/kg/d for 14 days, with activities compared to pentamidine at approximately one-half the effective i.v. dose of pentamidine, which is 22 mmol/kg/d. Two of the six dicationic compounds (**3**,**7**) were found to be highly potent at the screening dose, reducing the parasite load by over 99%. Both highly active compounds contained isopropyl substituted diamidines, with the cationic moieties of **3** located para and those of **7** meta to the furan oxygen atom. The 2,8-diamidine **1** was found to be comparable in activity to pentamidine at its screening dose and more active than pentamidine when given at the same dose as pentamidine. In contrast, the 3,7-diamidine **5** was found to be inactive. The 2,8-diimidazoline **2** and 3,7-diimidazoline **6** were both inactive at the screening dose. The observed toxicities of compounds **1**–**3** and **5**–**7** were found to be less than or equivalent to that of pentamidine at the doses used.

Compounds **4** and **8** were synthesized to test if diamidoximes would be effective prodrugs of the dibenzofuran diamidines. Both the diamidoximes were screened for

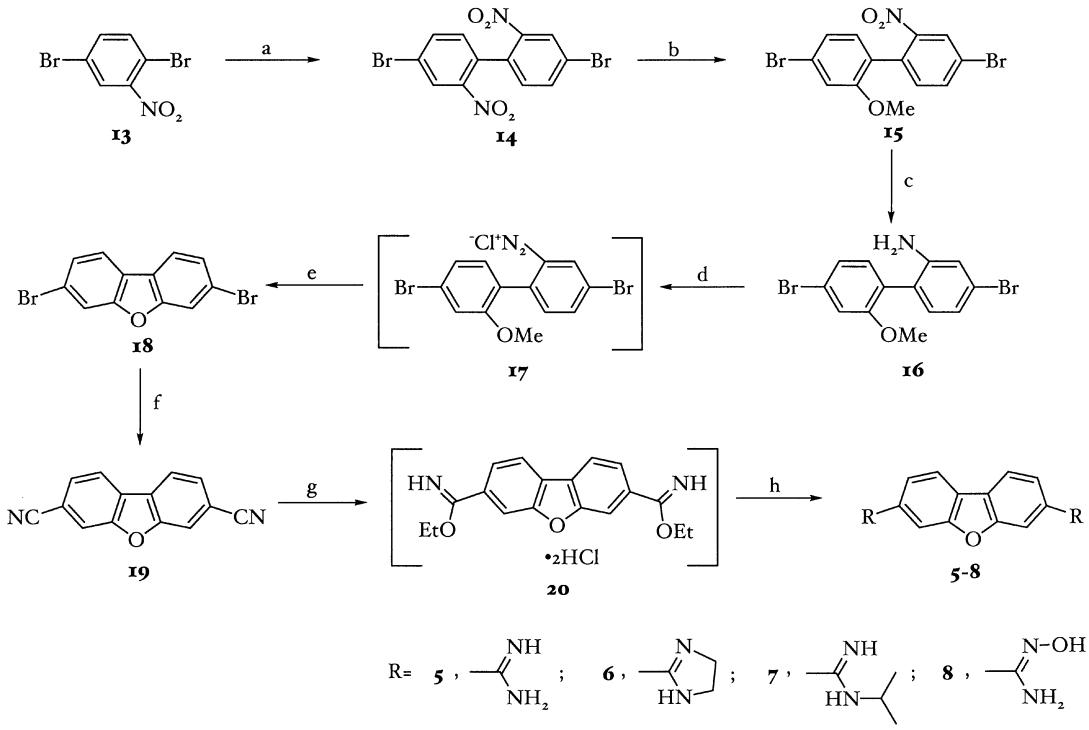


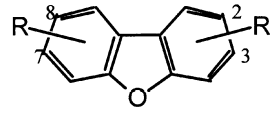
Figure 3. Synthesis of 3,7-disubstituted dibenzofuran dications. Key: (a) Cu/DMF/120 °C; (b) NaOMe/MeOH/DMF; (c) NH_2NH_2•H_2O/5% Ru/C/EtOH; (d) $NaNO_2/H_2SO_4$/< 5 °C; (e) Reflux; (f) CuCN/DMF/reflux; (g) EtOH/HCl/1,4-dioxane; (h) Appropriate amine/EtOH.

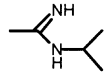
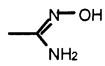
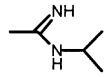
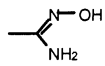
anti-*P. carinii* activity at 10 mmol/kg/d by tail vein injection and 33 mmol/kg/d by oral gavage for 14 days. The diamidoximes **4** and **8** were compared for efficacy with pentamidoxime, an effective prodrug. Not surprisingly, 3,7-diamidoxime **8** was not active since the corresponding diamidine **5** was not active. Compound **4** had only moderate activity against PCP, while the corresponding diamidine **1** proved to be potent. Further studies of diamidoxime metabolism are being performed using cultured cell lines and cell-free enzyme preparations to understand more about the metabolic mechanisms of prodrug activation. Preliminary results showed that the conversion of 2,8-dibenzofuran diamidoxime to diamidine is less efficient than conversion of pentamidoxime to pentamidine by whole cells. We hypothesize that either transport or enzyme activity plays a key role in the conversion of diamidoximes to diamidines.

3.2. DNA binding

Previous studies with dicationic carbazoles indicated that carbazoles with the cations substituted meta to the ring heterocycle (2,7-substituted carbazole) bound to the DNA minor groove in a different orientation from carbazoles with the cations para to the ring nitrogen (3,6-disubstituted carbazoles) [49]. As an example, 3,6-di(2-imidazolinyl)carbazole bound in the minor groove via hydrogen bonds from the two imidazoline groups resulting in an orientation with the ring nitrogen pointing away from the DNA surface. The corresponding 2,7-disubstituted compound was only able to establish two hydrogen bonds with the surface of the minor groove by flipping over and forming hydrogen bonds with the DNA through one of the imidazoline groups and the ring nitrogen. It was anticipated that the 3,7-dicationic benzofurans (cations meta to ring oxygen) would correspond with the 2,7-disubstituted carbazoles in orientation relative to the DNA surface, and therefore, without the hydrogen bond contributed by the ring nitrogen of the carbazole, would be less potent DNA binders than the corresponding 2,7-disubstituted carbazoles. Comparing the DNA binding results in *table I* with previous results from the carbazole study indicate only a modest reduction in the ΔT_ms of dibenzofurans compared to the carbazoles. Furthermore, judging from the carbazole studies it was anticipated that the 2,8-disubstituted dibenzofurans (two hydrogen bond donors) would have highly increased

Table I. Anti-*P. carinii* pneumonia activity and DNA binding affinity of dibenzofuran dications.



Compound	R		*Pneumocystis carinii*			DNA binding ΔT_m (°C) [d]	
			Dose [a]	Toxicity [b]	% Saline control ± standard error [c]	Calf thymus DNA	poly-dA•poly-dT
Pentamidine	–	–	22.0	++	0.80 ± 0.17	10.7	12.8 [51]
Pentamidoxime	–	–	22.0	+	0.11 ± 0.02	0.5	ND
			33.0 *	0	13.76 ± 9.12		
1	2, 8		10.0	+	7.15 ± 3.81	14.5	10.0
			22.0	++	0.10 ± 0.02		
2	2, 8		10.0	+	97.36 ± 31.92	15.0	14.9
3	2, 8		10.0	+	0.50 ± 0.27	8.1	3.2
4	2, 8		10.0	+	15.76 ± 14.60	0.5	ND
			33.0 *		45.06 ± 21.61		
5	3, 7		10.0	+	182.65 ± 38.32	12.7	10.0
6	3, 7		10.0	+	219.93 ± 39.28	14.0	8.6
7	3, 7		10.0	+	0.28 ± 0.27	12.7	1.8
8	3, 7		10.0	+	165.58 ± 108.73	0.9	0.5
			33.0 *	0	150.39 ± 54.05		

[a] Dose units were μmol/kg/day. Each compound was administered to 8 rats once daily for 14 days. Doses marked with (*) were by oral gavage, all others were i.v. via tail vein.
[b] Toxicity scores were subjective evalutions of overt toxicity in dexamethasone immunosuppressed rats. A score of 0 indicates no observable deleterious effects from dosing whereas ++ indicates hypotension, ataxia, and dyspnea.
[c] Cysts/g lung scores for the saline and pentamidine control groups were pooled across experiments. Scores for each compound were reported as % of the pooled saline score. Saline: 47.53×10^6 cysts/g lung (n = 45). Pentamidine: 0.38×10^6 cysts/g lung (n = 42).
[d] Change in melting point determined on calf thymus DNA and a poly-dA•poly-dT oligomer.

DNA binding potency than the corresponding 3,7-disubstituted dibenzofurans (one hydrogen bond donor). As can be seen from *table I*, the 3,7-disubstituted dibenzofurans were found to have ΔT_ms in the same range as the 2,8-disubstituted analogues. We are currently studying the DNA interactions of 3,7-di(2-imidazolinyl)dibenzofuran utilizing UV-VIS absorbency, fluorescence, kinetic measurement and circular dichroism in conjunction with NMR spectroscopy in an attempt to rationalize this unexpected finding.

Another interesting and unexpected result from the DNA binding studies was the relatively low affinity for poly-dA•poly-dT exhibited by two of the molecules (Compounds **3** and **7**). While these two molecules exhibited good affinity for calf thymus DNA, they were found to have low affinity for poly-dA•poly-dT. Both compounds had isopropyl-substituted amidines as the cationic group and were potent anti-*P. carinii* agents. The large differential observed between binding of calf thymus DNA and poly-dA•poly-dT for these molecules may represent a different mode of binding as a result of GC base pair interactions. Since the combination of strong DNA affinity and low poly-dA•poly-dT appears to correlate with potent anti-*P. carinii* activity this would be an exciting area for future study.

4. Biological studies

The activity of these compounds against *P. carinii* pneumonia in the rat model was determined using a standard procedure [5]. The binding affinities of the compounds with calf thymus DNA and poly-dA•poly-dT were measured by thermal melting experiments as reported [49].

5. Experimental protocol

Uncorrected melting points were measured on a Thomas Hoover capillary melting point apparatus. IR spectra were recorded in Nujol mulls or KBr pellets on a Perkin-Elmer 1320 spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on Bruker AC 300, Varian XL 400, and Bruker AMX 500 spectrometers. Chemical shifts were expressed in parts per million downfield relative to tetramethylsilane (TMS) or sodium 3-(trimethylsilyl) propionate. Anhydrous ethanol was distilled over Mg (with trace I_2) immediately before use. Reaction products were dried over P_2O_5 at room temperature, 40 °C or 77 °C at 0.2 mm Hg. Unless stated otherwise, reactions were monitored by TLC on silica gel or by reverse phase HPLC. HPLC chromatograms were recorded on a Hewlett-Packard 1090 chromatograph using UV detection (230 nm). Chromatographic data were recorded and analyzed with a Hewlett-Packard 3396 integrator. Mobile phase consisted of mixtures of acetonitrile in water containing 10 mM tetramethylammonium chloride, 10 mM sodium heptanesulfonate, and 2.2 mM phosphoric acid (Methods 1, and 2) or 10 mM potassium phosphate (Methods 3 and 4). HPLC method 1 employed a Dupont Zorbax Rx-C8 column (5.0 μ, 4.6 mm × 25 cm) with a flow rate of 1.5 mL /min. The concentration of acetonitrile was maintained at 3.75% from 0 to 0.5 min, increased to 45% following a linear gradient in 19.5 min, increased to 67.5% following a linear gradient over 5 min, then maintained at 67.5% for 7 min. HPLC methods 2, 3, and 4 employed a Dupont Zorbax SB-C8 column (3.5 μ, 3.0 mm × 15 cm) with a flow rate of 0.6 mL /min. In methods 2 and 3, the concentration of acetonitrile was maintained at 3.75% from 0 to 0.5 min, increased to 45% following a linear gradient in 13 min, immediately increased to 67.5% following a linear gradient over 3 min, then maintained at 67.5% for 4.5 min. In method 4, the concentration of acetonitrile was maintained at 5% from 0 to 0.5 min, increased to 47% following a linear gradient in 13 min, immediately increased to 72.5% following a linear gradient over 3 min, then maintained at 72.5% for 4.5 min. Electron impact mass spectra were recorded on a VG 70-SE or a VG 70-SEQ Hybrid spectrometer. FAB mass spectra were recorded on a VG 70-SEQ Hybrid spectrometer (Cesium ion gun, 30 KV). Microanalyses were performed by Atlantic Microlab, Norcross, GA., and all were within ± 0.4% of the theoretical values. Compounds **9** and **13** were purchased from Aldrich Chemical Co., Milwaukee, WI.

5.1. 2,8-Diamidinodibenzofuran dihydrochloride **1**

A stirred suspension of 2,8-dicyanodibenzofuran (**11**, 2.07 g, 9.50 mmol) in anhydrous EtOH (3.5 mL, 60 mmol) and 1,4-dioxane (100 mL) was cooled in an ice-salt bath and saturated with dry HCl gas at such a rate that the reaction temperature was maintained below 5 °C. The flask was then tightly sealed and the mixture was maintained at room temperature for 2 weeks, until no or only a small nitrile band (2230 cm^{-1}) was detected by IR analysis, and no starting material was detected by HPLC. The reaction mixture was purged with dry N_2 gas and diluted with ether (100–200 mL). The crude diimidate was filtered off and dried under N_2, and then added immediately to a solution of ethanolic ammonia (50 mL). The resultant suspension was stirred at 50–60 °C overnight in a tightly stoppered flask. The crude product was filtered off and recrystallized several times from water–acetone to give a white powder (1.96 g, 63.3%): m.p. > 300 °C [Lit. [37] > 320 °C]; HPLC method 4 t_R = 9.48 min (98.1 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.64 (s, 4 H), 9.38 (s, 4 H), 8.79 (s, 2 H), 8.09 (s, 4 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.5, 158.7, 128.8, 123.9, 123.0, 122.3, 112.7; FAB-MS *m/z* 253 (MH^+ of free base). Anal. ($C_{14}H_{12}N_4O$•2HCl•1.5H_2O) C, H, N.

5.2. 2,8-Di(2-imidazolinyl)dibenzofuran dihydrochloride **2**

A stirred suspension of 2,8-dicyanodibenzofuran (**11**, 2.06 g, 9.46 mmol) in anhydrous EtOH (10.0 mL, 170 mmol) and 1,4-dioxane (300 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 3 weeks. A mixture of half of the diimidate and ethylene diamine (22.74 g, 278.5 mmol) in anhydrous ethanol (80 mL) was refluxed under N_2 for 5.5 h. The reaction mixture was passed through decolorizing carbon (1 cm thick), and the filtrate was evaporated. The residue was further purified several times by recrystallization from 3 N HCl–acetone to give a white powder (0.33 g, 17%): m.p. 299–302 °C (dec.); HPLC method 1 t_R = 14.65 min (100 area%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.87 (s, 8 H), 7.96 (d, J = 8.6 Hz, 2 H), 8.14 (d, J = 8.6 Hz, 2 H), 8.77 (s, 2 H); FAB-MS m/z 305 (MH^+ of free base). Anal. ($C_{18}H_{16}N_4O$•2HCl•1.4H_2O) C, H, N.

5.3. 2,8-Di(N-isopropylamidino)dibenzofuran dihydrochloride **3**

A stirred suspension of 2,8-dicyanodibenzofuran (**11**, 2.00 g, 9.17 mmol) in anhydrous EtOH (4.3 mL, 73 mmol) and 1,4-dioxane (100 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. A mixture of the diimidate and freshly distilled (KOH) isopropylamine (10 mL, 120 mmol) in ethanol (20 mL) was stirred at room temperature for 4 days. The crude product was filtered off and was recrystallized several times from water–acetone to give a pale yellow powder (1.58 g, 42.0%): m.p. 279–282 °C; HPLC method 3 t_R = 11.34 min (97.4 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (d, J = 7.6 Hz, 2 H), 9.69 (s, 2 H), 9.33 (s, 2 H), 8.70 (d, J = 1.7 Hz, 2 H), 8.06 (d, J = 8.7, 2 H), d 7.98 (dd, J_1 = 8.7 Hz, J_2 = 1.7 Hz, 2 H), 4.18 (m, 2 H), 1.33 (m, 12 H); FAB-MS m/z 337 (MH^+ of free base). Anal. ($C_{20}H_{24}N_4O$•2HCl•0.90H_2O) C, H, N, Cl.

5.4. 2,8-Di(N-hydroxylamidino)dibenzofuran dihydrochloride **4**

A stirred suspension of 2,8-dicyanodibenzofuran (**11**, 3.38 g, 15.5 mmol) in anhydrous EtOH (7.5 mL, 170 mmol) and 1,4-dioxane (110 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. The crude diimidate was stirred overnight at 40 °C in a solution of hydroxylamine, prepared from hydroxylamine hydrochloride (8.62 g, 124 mmol) and sodium ethoxide (21 wt.%, 46.3 mL, 124 mmol) in anhydrous ethanol (100 mL). The reaction mixture was filtered through Celite 545 and the filtrate was diluted with ether. The resultant precipitate was filtered off and was recrystallized several times from 3–6 N HCl–acetone to give a white solid (0.55 g, 10%): m.p. 300–305 °C; HPLC method 2 t_R = 9.86 (100 area%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.22 (br s, 1 H), 11.39 (br s, 2 H), 9.13 (br s, 4 H), 8.69 (d, J = 1.7 Hz, 2 H), d 8.06 (d, J = 8.7 Hz, 2 H), d 7.99 (dd, J_1 = 8.7 Hz, J_2 = 1.7 Hz, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.91, 157.82, 128.09, 123.04, 122.53, 121.52, 112.53; FAB-MS m/z 285 (MH^+ of free base). Anal. ($C_{14}H_{12}N_4O_3$•2HCl•0.85H_2O) C, H, N.

5.5. 3,7-Diamidinodibenzofuran dihydrochloride **5**

A stirred suspension of 3,7-dicyanodibenzofuran (**19**, 2.20 g, 10.1 mmol) in anhydrous EtOH (4.6 mL, 81 mmol) and 1,4-dioxane (100 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. A mixture of half of the diimidate and ethanolic ammonia solution (80 mL) was stirred at 50–60 °C overnight. The crude product was filtered off, and dilution of the filtrate with ether resulted in further precipitation of product. The combined solids were recrystallized several times from 1 N HCl–acetone to give an off-white powder (0.33 g, 18%): m.p. > 300 °C; HPLC method 3 t_R = 9.06 min (99.9 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 4 H), 9.48 (s, 4 H), 9.56 (d, J = 8.2 Hz, 2 H), 8.44 (s, 2 H), 8.00 (d, J = 8.2 Hz, 2 H); FAB-MS m/z 253 (MH^+). Anal. ($C_{14}H_{12}N_4O$•2HCl) C, H, N, Cl.

5.6. 3,7-Di(2-imidazolinyl)dibenzofuran dihydrochloride **6**

A stirred suspension of 3,7-dicyanodibenzofuran (**19**, 2.20 g, 10.1 mmol) in anhydrous EtOH (4.6 mL, 81 mmol) and 1,4-dioxane (100 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. A mixture of half of the diimidate and ethylene diamine (2.02 mL, 30.2 mmol) in ethanol (80 mL) was refluxed overnight under nitrogen. The crude product was filtered off, and recrystallized from 1 N HCl–acetone to give a white solid (0.55 g, 29%): m.p. > 300 °C; HPLC method 3 t_R = 9.61 min (95.3 area%); ^{1}H NMR (300 MHz, TFA-*d*) δ 4.26 (s, 8 H), 7.86 (d, J = 8.2 Hz, 2 H), 8.17 (s, 2 H), 8.28 (d, J = 8.2 Hz, 2 H), 11.50 (s, 4 H); FAB-MS m/z 305 (MH^+). Anal. ($C_{18}H_{16}N_4O$•2HCl•0.30H_2O) C, H, N, Cl.

5.7. 3,7-Di(isopropylamidino)dibenzofuran dihydrochloride **7**

A stirred suspension of 3,7-dicyanodibenzofuran (**19**, 1.00 g, 4.58 mmol) in anhydrous EtOH (2.2 mL, 37 mmol) and 1,4-dioxane (80 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. A mixture of the diimidate and freshly distilled (KOH) isopropylamine (3.1 mL, 37 mmol) in ethanol (20 mL) was stirred at room temperature for 2 days. The crude product was filtered off, and dilution of the filtrate with ether resulted in further precipitation of product. The combined solids were recrystallized from water–acetone to give a pale yellow solid (1.21 g, 64.8%): m.p. > 300 °C; HPLC method 3 t_R = 10.86 min (95.9 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.54 (br s, 6 H), 8.51 (d, J = 8.1 Hz, 2 H), 8.27 (s, 2 H), 7.86 (dd, J_1 = 8.1 Hz, J_2 = 1.0 Hz, 2 H), 4.14 (m, 2 H), 1.32 (d, J = 6.3 Hz, 12 H); FAB-MS *m*/*z* 337 (MH^+). Anal. ($C_{20}H_{24}N_4O$•2HCl•0.30H_2O) C, H, N, Cl.

5.8. 3,7-Di(N-hydroxylamidino)dibenzofuran dihydrochloride **8**

A stirred suspension of 3,7-dicyanodibenzofuran (**19**, 2.00 g, 9.17 mmol) in anhydrous EtOH (4.2 mL, 73 mmol) and 1,4-dioxane (100 mL) was saturated with dry HCl gas as described above. The crude diimidate was collected after 2 weeks. A mixture of the crude diimidate in a solution of hydroxylamine, prepared from hydroxylamine hydrochloride (5.095 g, 73.32 mmol) and sodium ethoxide (21 wt.%, 27.4 mL, 73.3 mmol) in anhydrous ethanol (100 mL) was stirred at 40–50 °C overnight. The crude product was filtered off and recrystallized several times from 1 N HCl–acetone to give a white solid (0.38 g, 12%): m.p. > 300 °C; HPLC method 3 t_R = 8.83 min (99.6 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.3–11.6 (br s, 2 H), 8.8–9.6 (br s, 4 H), 8.51 (d, J = 8.4 Hz, 2 H), 8.29 (s, 2 H), 7.89 (d, J = 8.4 Hz, 2 H); FAB-MS *m*/*z* 285 (MH^+). Anal. ($C_{14}H_{12}N_4O_3$•2HCl•0.30H_2O) C, H, N, Cl.

5.9. 2,8-Dicyanodibenzofuran **11**

A mixture of 2,8-dibromodibenzofuran (**10**, 10.93 g, 33.53 mmol) and copper (I) cyanide (8.91 g, 102 mmol) in DMF (80 mL) was refluxed under N_2 for 9 h. The reaction mixture was poured into ice-water (300 mL). The precipitated solid was filtered off and stirred overnight in a solution of ethylenediamine (50 mL) in water (300 mL). The solid was filtered off, washed with water, then stirred in 10% sodium cyanide solution (100 mL) overnight. The solid was further purified by suspension in hot ethanol (100 mL) to give a white powder (6.97 g, 95.2%): m.p. 298–300 °C (dec.) (Lit. [37] 299 °C); HPLC method 1 t_R = 24.07 min (97.3 area%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.7 Hz, 2 H), 8.12 (dd, J_1 = 8.7 Hz, J_2 = 1.4 Hz, 2 H), 8.85 (d, J = 1.4 Hz, 2 H). Anal. ($C_{14}H_6N_2O$•0.4H_2O) C, H, N.

5.10. 4,4'-Dibromo-2,2'-dinitrobiphenyl **14**

A suspension of 2,5-dibromonitrobenzene (**13**, 50.0 g, 178 mmol) and copper powder (100 mesh, 25.0 g, 391 mmol) in DMF (300 mL) was stirred at 137 °C (oil bath) under N_2 for 2 h. The mixture was poured into toluene (1000 mL) and stirred for 4 h. Then the mixture was passed through Celite 545. The filtrate was collected and washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was recrystallized from ethanol (650 mL) to give a pale yellow solid (31.33 g, 87.57%): m.p. 143–146 °C (Lit. 150 °C [50] and 146–148 °C [44]); HPLC method 3 t_R = 18.70 min (97.2%); NMR (300 MHz, $CDCl_3$) δ 8.40 (d, J = 2.0 Hz, 2 H), 7.85 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 2 H), 7.18 (d, J = 8.2 Hz, 2 H).

5.11. 2-Methoxy-2'-nitro-4,4'-dibromobiphenyl **15**

A solution of sodium methoxide in methanol (22.7 mL, 99.4 mmol) was added dropwise to a solution of 4,4'-dibromo-2,2'-dinitrobiphenyl (**14**, 33.28 g, 82.79 mmol) in DMF (250 mL) cooled in an ice-bath. The reaction mixture was stirred overnight at room temperature under N_2. The reaction mixture was then poured into ice-water (700 mL). The precipitated solid was collected and recrystallized from acetonitrile (200 mL) and methanol (100 mL) to give olive green crystals (18.88 g, 59.0%): m.p. 127–129 °C; HPLC method 3 t_R = 19.57 min (96.5 area%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3 H), 7.04 (d, J = 1.6 Hz, 1 H), 7.14 (d, J = 8.1 Hz, 1 H), 7.23 (dd, J_1 = 1.6 Hz, J_2 = 7.3 Hz, 1 H), 7.26 (d, J = 7.3 Hz, 1 H), 7.76 (dd, J_1 = 1.9 Hz, J_2 = 8.1 Hz, 1 H), 8.09 (d, J = 1.9 Hz, 1 H); EI-MS *m*/*z* 385 (M^+). Anal. ($C_{13}H_9O_3NBr_2$) C, H, N.

5.12. 2-Methoxy-2'-amino-4,4'-dibromobiphenyl **16**

A solution of hydrazine hydrate (19.4 mL, 400 mmol) in ethanol (20 mL) was added dropwise into a suspension of 2-methoxy-2'-nitro-4,4'-dibromobiphenyl (**15**, 25.77 g, 66.59 mmol) and 5% Ru/C (2.69 g, 1.33 mmol) in ethanol (250 mL) maintained at 65–70 °C. The reaction mixture was then refluxed for 7 h. The hot reaction mixture was passed through Celite 545 (1 cm thick), and the filtrate was evaporated to give an off-white powder

(22.69 g, 95.46%): m.p. 94.5–97 °C; HPLC method 3 t_R = 18.57 min (97.9 area%); ^{1}H NMR δ 3.40 (br s, 2 H), 3.80 (s, 3 H), 6.9–7.2 (m, 6 H); EI-MS *m/z* 355 (M^+). Anal. ($C_{13}H_{11}ONBr_2$) C, H, N.

5.13. 3,7-Dibromo-dibenzofuran **18**

A solution of sodium nitrite (4.17 g, 60.5 mmol) in water (55.7 mL) was added slowly to a suspension of 2-methoxy-2'-amino-4,4'-dibromobiphenyl (**16**, 21.60 g, 60.50 mmol) in H_2SO_4 (14.82 g, 151.3 mmol) and water (52.7 mL), maintained below 2 °C. The mixture was stirred for 2 h at 0 °C, and then an excess of urea was added to destroy the unreacted nitrous acid. The reaction mixture was stirred overnight at room temperature, then stirred for 24 h at 70 °C. The solid formed was collected and recrystallized from benzene (120 mL) and methanol (100 mL) to give a beige solid (10.27g, 52.09%): m.p. 199–200.5 °C; HPLC method 3 t_R = 20.99 min (100 area%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, J = 8.2 Hz, 2 H), 7.74 (d, J = 1.2 Hz, 2 H), 7.49 (dd, J_1 = 8.2 Hz, J_2 = 1.2 Hz, 2 H); EI-Ms *m/z* 324 (M^+). Anal. ($C_{12}H_6OBr_2$) C, H, N.

5.14. 3,7-Dicyano-dibenzofuran **19**

A suspension of 3,7-dibromo-dibenzofuran (**18**, 10.27 g, 31.51 mmol) and copper(I) cyanide (8.23 g, 94.5 mmol) in DMF (80 mL) was refluxed under N_2 for 6 h. The reaction mixture was poured into ice-water (300 mL). The precipitated solid was collected and stirred for 5 h in a solution of ethylenediamine (50 mL) in water (300 mL). The solid was filtered off, washed with water, then stirred in 10% sodium cyanide solution (100 mL) for 4 h. The solid was further purified by suspension in hot ethanol (100 mL) to give a pale yellow powder (6.69 g, 97.43%): m.p. 322–325 °C (dec.); HPLC method 3 t_R = 16.56 min (96.6 area%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, J = 8.3 Hz, 2 H), 8.48 (s, 2 H), 7.95 (dd, J_1 = 8.3 Hz, J_2 = 1.0 Hz, 2 H); EI-MS *m/z* 218 (M^+). Anal. ($C_{14}H_6N_2$•0.23H_2O) C, H, N.

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