Extended Aromatic Furan Amidino Derivatives as Anti-*Pneumocystis carinii* Agents

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The syntheses of nine new derivatives of 2,5-bis[4-(*N*-alkylamidino)phenyl]furans with extended aromatic systems are reported. The interaction of these dicationic furans with poly(dA)*poly-(dT) and with the duplex oligomers d(CGCGAATTCGCG)$_2$ and d(GCGAATTCGC)$_2$ was determined by T_m measurement, and the effectiveness of these compounds against the immunosuppressed rat model of *Pneumocystis carinii* was evaluated. At a screening dose of 10 μmol/kg, 4 of the 12 amidino furans described here are more active than the parent compound **1**. In general, extension of the aromatic system in the absence of a substitution of the amidino nitrogens resulted in higher affinity for DNA than the parent compound as judged by the larger ΔT_m values and suggests enhanced van der Waals interactions in the amidino furan−DNA complex. Three of the compounds, **3**, **5**, and **11**, yield cysts counts of less than 0.1% of control when administered at a dosage of 10 μmol/kg. Compound **3**, which does not have an extended aromatic system, is the most active derivative. Although a direct correlation between anti-*P. carinii* activity and DNA binding affinity was not observed, all compounds which have significant activity have large ΔT_m values.

Introduction

Dicationic unfused aromatic amidine molecules similar to pentamidine are known to bind to the minor groove of DNA at AT-rich sites and to be effective agents against many opportunistic organisms such as *Pneumocystis carinii*, *Giardia lamblia*, and *Cryptosporidium parvum*.[1−6] Several hypotheses have been proposed to explain the mode of action of these compounds; however, there is strong evidence to support the binding of these compounds to DNA and subsequent inhibition of DNA-dependent enzymes as the mode of action.[7] Various minor groove binding compounds are known to interfere with the function of topoisomerases I and II and to intervene in transcription control.[8,9] Studies with *G. lamblia* and diamidino bis-benzimidazole molecules showed a strong correlation between DNA minor groove binding affinity, microbial topoisomerase II inhibition, and biological activity.[4,10] Other studies with *P. carinii* and diamidino bis-benzimidazole compounds showed selective inhibition of microbial topoisomerase II over mammaliam topoisomerase II. However, a strong correlation between anti-*P. carinii* activity and microbial enzyme inhibition was not observed.[11] Recently, furan diamidines have been found to inhibit an endo/exonuclease isolated from *P. carinii*.[12]

The compound 2,5-bis(4-amidinophenyl)furan (**1**) and substituted amidino derivatives have been studied extensively by our laboratory. Previously, **1** has been shown to be effective in vivo at a submicromole per kilogram body weight dosage against *P. carinii* in the immunosuppressed rat model[1,6] and has been shown to be effective in vivo against *Trypanosoma rhodesiense* in both the mouse and simian models.[13,14] Furthermore, **1** has been shown to bind in the minor groove of DNA at AT rich sites and to bind weakly at GC sites.[1,15,16] Other diamidino compounds, such as berenil and DAPI, also exhibit sequence-dependent dual binding modes.[17,18] However, the binding mode of **1** at GC sites is currently a matter of debate.[19,20]

Several factors have been shown to be important in minor groove binding affinity, such as electrostatic, hydrogen-bonding, and van der Waals interactions, as well as the radius of curvature of the molecule.[21,22] The crystal structure of **1** with d(CGCGAATTCGCG)$_2$ indicates the molecule sits in the minor groove of the AT region with the amidines within hydrogen-bonding distance of thymine carbonyls and the extended aromatic system in van der Waals contact with the sides of the groove. Also, the curvature of the molecule mimics the curvature of the minor groove.[1] The crystal structure of 2,5-bis[4-(*N*-isopropylamidino)phenyl]furan (**2**) and 2,5-bis[4-(*N*-cyclopropylamidino)phenyl]furan (**3**) with the same sequence also exhibits a good fit with the AATT site.[23] Furthermore, **2** and **3** have been shown to be more effective against *P. carinii* than pentamidine and **1**.[6] In addition to binding to DNA, some diamidino furans also bind to RNA, although the mode of binding is thought to be intercalation.[24] It is postulated that the intercalation binding of these compounds with nucleic acids may be involved in toxicity and drug loss.

Biophysical studies of other minor groove binding compounds as well as the X-ray crystallographic data for **1**−**3** demonstrate the importance of van der Waals contact with the minor groove for binding affinity. On

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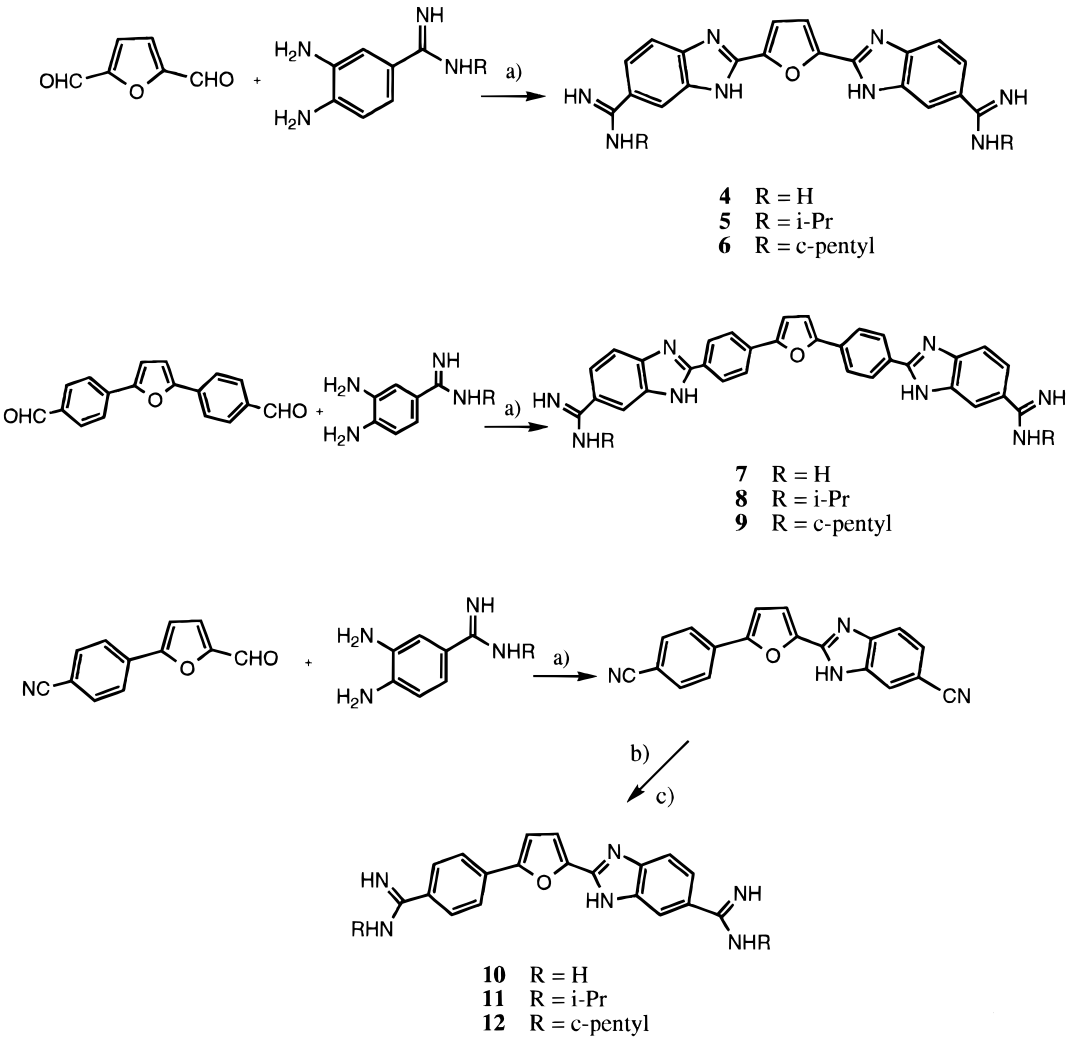
Scheme 1[a]



[a] (a) Benzoquinone, EtOH; (b) HCl, EtOH; (c) RNH_2, EtOH.

the basis of these studies and in order to increase the overall efficacy, decrease the toxicity, and improve the minor groove binding affinity, derivatives of **1**−**3** with extended aromatic systems were synthesized. Three modifications were made to increase the potential for van der Waals interactions: (1) one phenyl ring was replaced with a benzimidazole ring; (2) both phenyl rings were replaced with benzimidazole rings; and (3) benzimidazole rings were added in addition to the phenyl rings. This report describes the synthesis of these extended analogues of **1**−**3**, the determination of their DNA binding affinity, and the evaluation of their effectiveness against *P. carinii* pneumonia in the immunosuppressed rat model.

Chemistry

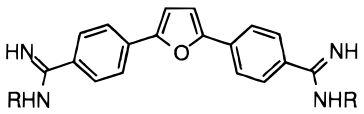
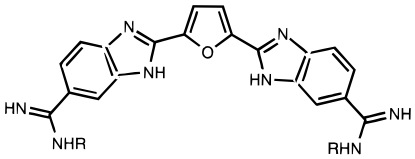
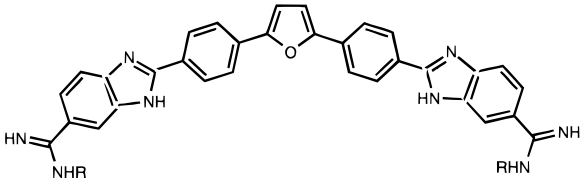
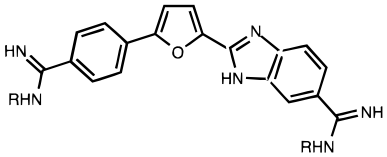
The syntheses of the new compounds **4**−**12** were achieved employing a similar approach for the three different systems. The approaches used are outlined in Scheme 1. The key step in each was formation of benzimidazole rings. In each case an aldehyde was allowed to react with a substituted phenylenediamine in the presence of 1,4-benzoquinone as the aromatizing agent. In the cases of preparation of **4**−**9**, the amidino unit was preformed and was the substituent on the phenylenediamine ring, whereas in the cases of **10**−**12**, the amidino units were formed after construction of the benzimidazole ring.

Biological Results

Table 1 contains the results of thermal melting experiments with poly(dA)*poly(dT) in MES 10 buffer at a ratio of 0.3 drug per phosphate, poly(dA)*poly(dT) in CAC 10 buffer at a ratio of 0.1 drug per phosphate, $d(CGCGAATTCGCG)_2$ in MES 10 buffer at a ratio of 1.0 drug per duplex, and $d(GCGAATTCGC)_2$ in CAC 10 buffer at a ratio of 1.0 drug per duplex. It was necessary to employ the smaller drug-to-phosphate ratio with polymeric DNA as affinity ranking was difficult at the 0.3 drug-to-phosphate ratio as several measurements exceeded the temperature limits for the spectrometer. Oligomeric studies were done with both $d(CGCGAATTCGCG)_2$ and $d(GCGAATTCGC)_2$ in order to compare the ΔT_m for a 10-base pair and a 12-base pair duplex with essentially the same binding site. As the melting transition for the 12-base pair duplex was gradual, there was some concern about hairpin formation and biphasic melting.

In general, extending the aromatic region increased the binding affinity, as measured by thermal melting

Table 1. Nucleic Acid Binding Results and in Vivo Activity of Dicationic Furans against *P. carinii*

compd	R	ΔT_m[a] (DNA)	ΔT_m[b] (DNA)	ΔT_m[c] (oligomer)	ΔT_m[d] (oligomer)	dosage[e] (μmol/kg/day)	cyst/g of lung[e] (% of control)	toxicity[e]
saline							100.0 ± 7.66[f]	
pentamidine						22.0	1.82 ± 0.38	
								
1	H	18.8	25.0	8.3	11.7	13.3	0.73 ± 0.32	0
						2.7	6.74 ± 3.3	0
						0.3	6.42 ± 3.11	0
						0.03	26.23 ± 4.64	0
2	$CH(CH_3)_2$	22.9	>28.0	11.7	14.4	10.8	0.24 ± 0.22	0
						2.2	10.04 ± 8.62	0
						1.1	14.00 ± 5.59	0
						0.2	201.74 ± 72.96	0
3		>28.5	>28.0	12.8	15.8	9.4	0.04 ± 0.03	+
						1.9	0.09 ± 0.06	0
						0.9	0.13 ± 0.03	0
						0.2	111.65 ± 45.60	0
								
4	H	>28.5	>28.0	11.7	14.4	10.0	1.80 ± 0.57	+
5	$CH(CH_3)_2$	21.9	>28.0	10.0	12.9	10.0	0.11 ± 0.03	0
						2.0	31.77 ± 11.65	0
6		**	**	11.7	11.7	3.8	53.81 ± 53.16	++
								
7	H	**		**		7.1	180.10 ± 18.14	+
8	$CH(CH_3)_2$	**		**		5.0	NA	++++
9		7.1		**		6.1	NA	++++
								
10	H	22.4	24.3	10.0	11.4	5.0	50.98 ± 11.95	0
11	$CH(CH_3)_2$	20.1	23.2	9.0	10.0	10.0	0.13 ± 0.04	+
12		21.8	**	9.7	9.7	2.5	0.82 ± 0.42	++

[a] Increase in thermal melting of poly(dA)·poly(dT) in 0.01 M cacodylate and 0.1 drug per base. [b] Increase in thermal melting of poly(dA)·poly(dT), 0.3 drug per base, see ref 24. [c] Increase in thermal melting of the oligomer $d(GCGAATTCGC)_2$ in 0.01 M cacodylate and 1.0 drug per duplex. [d] Increase in thermal melting of the oligomer $d(CGCGAATTCGCG)_2$, see ref 31. [e] Evaluation of iv dosage of the furan dications against *P. carinii* in rats as described in ref 2. [f] Mean cyst count for pooled controls: saline (n = 125) 53.41 ± 4.09 cysts/g of lung tissue; pentamidine (n = 135) 0.93 ± 0.14 cysts/g of lung tissue. ** DNA−drug complex precipitated.

experimentation with these compounds and AT-rich DNA. Specifically, for the unsubstituted amidino derivatives with $d(GCGAATTCGC)_2$, the phenyl benzimidazole amidine **10** has a greater DNA binding affinity that **1**, and the bis-benzimidazole amidine **4** has a greater DNA binding affinity than **10**. As expected, extending the aromatic system resulted in increased DNA binding affinity most likely due to the increased potential for van der Waals interactions. The trend did not hold true for the highly extended diphenyl bis-benzimidazole analogue **7** as thermal melting result suggests that **7** slightly destabilized $d(GCGAATTCGC)_2$. However, UV−visible titration of **7** with $d(GCGAATTCGC)_2$ in ethanol, water, and buffer (CAC 10) indicates that the compound tends to self-stack as the solvent becomes more polar and that the compound does interact with the DNA (Figure 1). Based on this result, it was concluded that **7** was self-stacking under the

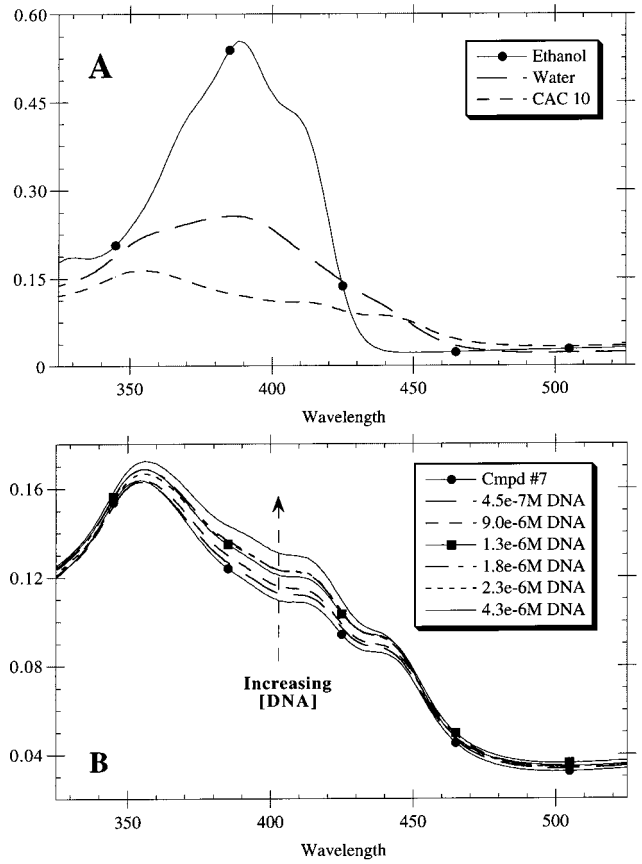


Figure 1. Spectral properties of **7** and titration of **7** with d(GCGAATTCGC)$_2$: (A) UV−vis spectra of 1×10^{-5} M **7** in water, ethanol, and CAC 10 buffer; (B) titration of 1×10^{-5} M **7** in CAC 10 buffer with d(GCGAATTCGC)$_2$.

conditions of the experiment which explained the negligible T_m and that **7** did have some affinity for this sequence.

For the extended *N*-alkyl-substituted derivatives, an increase in DNA binding affinity was observed. However, the increase was not as great for the extended analogues relative to the increase seen when the isopropyl and cyclopentyl substituents were added to **1**. Specifically, **5** and **11** have a smaller ΔT_m with both d(CGCGAATTCGCG)$_2$ and d(GCGAATTCGC)$_2$ compared to **2**; **6** and **12** have a smaller ΔT_m with both d(CGCGAATTCGCG)$_2$ and d(GCGAATTCGC)$_2$ compared to **3**. An explanation for this result could be that with the extended aromatic system, the bulky alkyl groups at the termini of the molecule are no longer easily accommodated by a 4-base pair binding site and/or an additional entropy cost exists for the orientation of the *N*-alkyl side chains. Again, for the corresponding highly extended diphenyl bis-benzimidazole derivatives, either a negligible or a negative ΔT_m was observed with d(GCGAATTCGC)$_2$.

Because the extended diphenyl bis-benzimidazoles **7**−**9** produced negligible ΔT_m with both poly(dA)*poly(dT) and oligomeric DNA with 4-base pair AT binding sites, oligomers with longer AT tracts were employed to discern the length of the AT tract needed to enhance binding affinity of these molecules. Table 2 shows the results of **1**, **4**, **7**, and **10** with three oligomers of 4-, 6-, and 8-base pair AT tract length: The highly extended **7** does not exhibit any appreciable binding affinity until the AT tract is extended to 8 base pairs. However, for an 8-base pair AT tract, **7** exhibits stronger binding affinity than **1**, **4**, or **10**. At a ratio of 1.0 drug to binding site, the ΔT_m of **7** is 11.5 °C greater than that of compound **10**, 14.7 °C greater than that of **4,** and 22.2 °C greater than that of **1**. Given the large hydrophobic area of the central portion of **7** relative to the other three systems, one possible explanation for the behavior of **7** is that under the conditions of the experiment stacking interactions are greater than the drug−DNA interactions until the binding site is enlarged to 8 AT base pairs. The 8-base pair binding site most likely provides the best fit for the highly extended **7**.

Table 2. ΔT_m for Selected Compounds with Oligomers Containing 4-, 6-, and 8-AT Base Pair Binding Sites

	compound no.			
	1[a]	**4**[a]	**7**[a]	**10**[a]
d(GCGAATTCGC)$_2$	8.3	11.7	**	10.0
d(CGAAATTTCG)$_2$	12.6	17.1	−1.5	14.1
d(GAAAATTTTCGAAAATTTTC)$_2$	7.3	18.0	29.5	14.8

[a] Experiments were performed using CAC 10 buffer and were done at a ratio of 1.0 drug per binding site. ** Drug−DNA complex precipitated.

Results for in vivo testing for *P. carinii* pneumonia are also shown in Table 1. At a dosage of approximately 10 μM/kg/day compounds **1**−**5**, **11**, and **12** demonstrate excellent activity ranging from 0.04% to 1.80% cysts relative to the saline control. Recently, Boykin et al. reported the anti-*P. carinii* activity of analogues of **1**, which had been substituted at the amidino nitrogens with alkyl groups.[6] They found that all compounds which had a large ΔT_m value with d(CGCGAATTCGCG) showed strong activity against *P. carinii* at a dosage of approximately 10 μM/kg/day. For the present studies three compounds (**2**−**4**) produced a large ΔT_m with d(CGCGAATTCGCG), and all three of these compounds showed excellent activity and no or little toxicity at a dosage of approximately 10 μM/kg/day.

Compounds **7**−**9** were not included in the above analysis as they produced negligible ΔT_m values with d(GCGAATTCGC)$_2$, although spectroscopic results indicate these compounds do have affinity for this sequence. With oligomers containing an 8-base pair AT tract, all three compounds produced ΔT_m values over 29 °C, an indication of strong affinity for AT tract DNA. However, all three of these compounds were either inactive (**7**) or too toxic to be evaluated for anti-*P. carinii* activity (**8** and **9**). Given the large affinity for DNA, a possible explanation for the toxicity and inactivity of these highly extended compounds is nonspecific binding to other biomolecules.

For the present set of compounds, it was concluded that while DNA binding affinity was clearly important for drug activity, a direct correlation between AT tract DNA binding affinity and activity was not evident. Other factors which could be important in determining drug activity are cell membrane permeability and nonspecific binding. Furthermore, it was concluded that extending the length of the compound did not increase the efficacy of this class of compounds against *P. carinii*.

Experimental Section

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus or a Fisher-Johns

apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded employing a Varian GX400 spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are reported in hertz. Mass spectra were recorded on a VG Instruments 70-SE spectrometer (Georgia Institute of Technology, Atlanta, GA). IR spectra were recorded using a Michelson 100 (Bomem, Inc.) instrument. Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ±0.4 of the theoretical values. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific. The syntheses of **1**−**3** have been previously reported.[6,13] Thermal melting experiments and DNA preparations were done as previously described.[25]

4: 2,5-Bis[2-(5-amidinobenzimidazoyl)]furan Hydrochloride. A solution of furan-2,5-dicaboxaldehyde[26] (0.248 g, 0.002 mol), 4-amidino-1,2-phenylenediamine hydrochloride hydrate[27] (0.8 g, 0.004 mol), and 1,4-benzoquinone (0.432 g, 0.004 mol) in ethanol (40 mL) was heated at reflux for 4 h (under nitrogen).[28] The reaction mixture was cooled to room temperature, and the dark solid was collected by filtration, washed with cold ethanol and anhydrous ether, and dried to yield 0.61 g (80%) of the free base. This solid was dissolved in hot ethanol (300 mL) and filtered. The filtrate volume was reduced to 70 mL and acidified with HCl−saturated ethanol. After standing overnight in the refrigerator, the green solid was collected by filtration, washed with anhydrous ether, and dried under vacuum to yield 0.4 g (52%) of a yellow-green powder solid, mp > 300 °C. ^{1}H NMR (DMSO-d_6): 9.30 (s, 4H), 8.95 (s, 4H), 8.19 (s, 2H), 7.81 (d, 2H, J = 8.8), 7.72 (d, 2H, J = 8.4), 7.60 (s, 2H). ^{13}C NMR (DMSO-d_6/D_2O): 166.8, 146.3, 146.1, 142.2, 139.7, 123.4, 122.7, 117.1, 116.1, 115.4. MS (FAB): m/z 385 (M^+ + 1). HRMS: calcd mass (free base), 385.1525 (M^+ + 1); observed mass, 385.1535. Anal. ($C_{20}H_{16}N_8O\cdot 2HCl\cdot 1.5H_2O$) C, H, N.

5: 2,5-Bis[2-(5-(*N*-isopropylamidino)benzimidazoyl)]furan Hydrochloride. A protocol similar to that described above was employed for the condensation of 2,5-furandicarboxaldehyde[26] and 4-(*N*-isopropylamidino)-1,2-phenylenediamine[27] to give a 54% yield of a yellow-green powder, mp > 300 °C. ^{1}H NMR (DMSO-d_6): 9.60 (s, 1H), 9.58 (s, 1H,), 9.45 (s, 2H), 9.04 (s, 2H), 8.06 (s, 2H), 7.82 (d, 2H, J = 8.4), 7.69 (s, 2H), 7.62 (d, 2H, J = 8.2), 4.09 (m, 2H, J = 7.0), 1.32 (d, 12H, J = 6.3). ^{13}C NMR (DMSO-d_6/D_2O): 162.8, 145.9, 145.1, 140.9, 138.5, 124.5, 124.0, 116.9, 115.9, 115.8, 45.9, 21.7. MS (FAB): m/z 469 (M^+ + 1). HRMS: calcd mass (free base), 469.2464 (M^+ + 1); observed mass, 469.2475. Anal. ($C_{26}H_{28}N_8O\cdot 3HCl\cdot 2.5H_2O$) C, H, N.

6: 2,5-Bis[2-(5-(*N*-cyclopentylamidino)benzimidazoyl)]furan Hydrochloride. Distilled cyclopentylamine (1.83 g, 0.021 mol) was added to a stirred suspension of the imidate ester hydrochloride (4.91 g, 0.02 mol) (formed from 4-cyano-2-nitroaniline under Pinner type conditions) in 30 mL of dry ethanol; the mixture was stirred for 12 h at room temperature and for 1 h at 50 °C. The solvent was removed under reduced pressure, and the residual thick oily mass was triturated with dry ether and dried under vacuum to yield 4.7 g (95%), mp 168−179 °C dec. ^{1}H NMR (DMSO-d_6): 9.29 (br, 3H), 8.45 (d, 2H, J = 2.4), 8.03 (s, 2H), 7.77 (dd, 1H, J = 2.4, 8.8), 7.20 (d, 1H, J = 8.8), 4.20 (quintet, 1H, J = 6.0), 2.04−2.0 (m, 2H), 1.73−1.65 (m, 4H), 1.57−1.49 (m, 2H). ^{13}C NMR (DMSO-d_6): 160 4, 148.5, 134.1, 129.4, 127.3, 118.9, 114.6, 54.1, 31.2, 23.5.

The 4-(*N*-cyclopentylamidino)-2-nitroaniline (mp 238−240 °C dec) was used directly without further characterization (5.0 g, 0.02 mol), and 1.0 g of 10% Pd/C in 130 mL of dry methanol was subjected to hydrogenation at 50 psi for approximately 1 h. The catalyst was filtered over Celite and washed with hot methanol, the solvent of the filtrate was removed under reduced pressure, the residue was triturated with dry ether, and the solid was filtered and dried under vacuum at 45 °C for 24 h. The yield of light brown hygroscopic solid was 3.91 g (72%), mp 170−178 °C. ^{1}H NMR (DMSO-d_6): 8.97 (br s, 1H), 8.82 (br s, 1H), 8.64 (brs, 1H), 6.89 (s, 1H), 6.88 (d, 1H, J = 8.4), 6.59 (d, 1H, J = 8.4), 5.40−4.92 (br, 2H), 4.17 (m, 1 H), 3.63 (br, 2H), 2.10−1.98 (m, 2H), 1.82−1.76 (m, 4H). ^{13}C NMR (DMSO-d_6): 162.4, 140.8, 134.0, 118.4, 115.6, 113.0, 112.5, 53.7, 31.3, 23.5. MS (FAB): 219 (M^+ + 1). Anal. ($C_{12}H_{18}N_4\cdot HCl\cdot H_2O$) C, H, N.

A protocol similar to that described above was employed for the condensation of 2,5-furandicarboxaldehyde[26] and 4-(*N*-cyclopentylamidino)-1,2-phenylenediamine to give a 77% yield of a yellow-green powder, mp 287−289 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.07 (s, 2H), 7.82 (d, 2H, J = 8.4), 7.66 (s, 2H), 7.63 (d, 2H, J = 8.4), 4.22−4.14 (m, 2H), 2.14−2.04 (m, 4H), 1.82−1.67 (m, 8H), 1.64−1.56 (m, 4H).^{13}C NMR (DMSO-d_6/D_2O): 163.0, 145.4, 144.5, 140.4, 137.7, 123.9, 123.2, 116.4, 115.5, 115.2, 54.6, 31.5, 23.7. MS (FAB): 521 (M^+ + 1). Anal. ($C_{30}H_{32}N_8O\cdot 4HCl$) C, H, N.

7: 4,4′-Bis[2-(5-amidinobenzimidazoyl)]-2,5-diphenylfuran Hydrochloride. 2,5-Bis(4-formylphenyl)furan was prepared by reduction of 2,5-bis(4-cyanophenyl)furan[29,30] (1.12 g, 0.004 mol) using 1 M DIBAL in ($CHCl_3$:ether). ^{1}H NMR (DMSO-d_6): 10.0 (s, 2H), 8.05 (d, 4H, J = 7.5), 7.97 (d, 4H, J = 7.5), 7.37 (s, 2H).^{13}C NMR (DMSO-d_6): 192.0, 125.7, 135.0, 134.6, 130.1, 123.9, 111.6. MS: m/e 276. Anal. ($C_{18}H_{12}O_3$) C, H.

A protocol similar to that described above was employed for the condensation of 2,5-bis(4-formylphenyl)furan and 4-amidino-1,2-phenylenediamine[27] to give a 68% yield of a yellow-brown powder, mp 355−7 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.35 (d, 4H, J = 8), 8.21 (s, 2H), 8.06 (d, 4H, J = 8), 7.84 (d, 2H, J = 8.4), 7.34 (d, 2H, J = 8.4), 7.29 (s, 2H). ^{13}C NMR (DMSO-d_6/D_2O/90 °C): 165.9, 143.2, 152.7, 140.8, 137.9, 132.2, 127.9, 126.5, 124.1, 122.7, 121.8, 115.9, 114.7, 110.4. MS (FAB): 537 (M^+ + 1). Anal. ($C_{32}H_{24}N_8O\cdot 4HCl\cdot H_2O$) C, H, N.

8: 4,4′-Bis[2-(5-(*N*-isopropylamidino)benzimidazoyl)]-2,5-diphenylfuran Hydrochloride. A protocol similar to that described above was employed for the condensation of 2,5-bis(4-formylphenyl)furan and 4-(*N*-isopropylamidino)-1,2-phenylenediamine[27] to give a 75% yield of yellow solid, mp 290−292 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.28 (d, 4H, J = 6.8), 8.1 (s, 2H), 7.95 (d, 4H, J = 6.8), 7.80 (d, 2H, J = 8.4), 7.62 (d, 2H, J = 8.4), 7.2 (s, 2H), 4.09 (septet, 2H, J = 6.4), 1.32 (d, 12H, J = 6.4). ^{13}C NMR (DMSO-d_6/D_2O): 162.3, 162.2, 152.8, 152.4, 138.9, 133.0, 132.9, 128.3, 125.1, 124.4, 123.7, 115.6, 114.8, 111.1, 45.5, 21.1. MS (FAB): 621 (M^+ + 1). Anal. ($C_{38}H_{36}N_8O\cdot 4HCl\cdot 2H_2O$) C, H, N.

9: 4,4′-Bis[2-(5-(*N*-cyclopentylamidino)benzimidazoyl)]-2,5-diphenylfuran Hydrochloride. A protocol similar to that described above was employed for the condensation of 4-(*N*-cyclopentylamidino)-1,2-phenylenediamine with 2,5-bis-(4-formylphenyl)furan to yield (77%) a yellow solid, mp 295−297 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.30 (d, 4H, J = 8.4), 8.05 (d, 4H, J = 8.4), 8.01 (s, 1H), 7.77 (d, 2H, J = 8.4), 7.56 (d, 2H, J = 8.4), 7.27 (s, 2H), 4.15 (br, 2H), 2.13−2.03 (m, 4H), 1.81−1.55 (m, 12H). ^{13}C NMR (DMSO-d_6/D_2O): 163.7, 154.0, 153.1, 141.2, 138.6, 132.8, 127.9, 126.1, 124.6, 123.3, 123.1, 115.9, 115.7, 111.0, 55.7, 32.3, 24.4. MS (FAB): 673 (M^+ + 1). Anal. Calcd for $C_{42}H_{40}N_8O\cdot 4HCl$: C, 61.61; H, 5.42; N, 13.69. Found: C, 62.28; H, 5.74; N, 13.62.

10: 2-(4-Amidinophenyl)-5-[2-(5-amidinobenzimidazoyl)]furan Hydrochloride. A solution of 5-(4-cyanophenyl)furan-2-carboxaldehyde[29,30] (1.88 g, 0.0152 mol), 3,4-diaminobenzonitrile[27] (2 g, 0.0152 mol), and benzoquinone (1.64 g, 0.0152 mol) in ethanol (150 mL) was allowed to reflux under nitrogen for 4 h. After cooling solid was collected by filtration and recrystallized from chloroform, yield 3.75 g (79%) of yellow solid, mp 157−160 °C dec. ^{1}H NMR (DMSO-d_6): 13.65 (brs, 1H), 8.14 (brs, 1H), 8.11 (d, 2H, J = 8.4), 7.98 (d, 2H, J = 8.1), 7.76 (d, 1H, J = 8.4), 7.62 (dd, 1H, J = 8.4, 1.5), 7.49 (d, 1H, J = 3.9), 7.46 (d, 1H, J = 3.9). ^{13}C NMR (DMSO-d_6): 153.0, 145.8, 145.2, 133.1, 132.9, 125.9, 125.9, 124.5, 119.8, 118.7, 114.4, 111.6, 110.2, 104.3. MS: m/e 310 (M^+). HRMS: calcd mass, 310.0855; observed mass, 310.0803. Anal. ($C_{19}H_{10}N_4O\cdot H_2O$) C, H, N.

A suspension of 2-(5-cyano-2-benzimidazolyl)-5-(4-cyanophenyl)furan (0.5 g, 0.0015 mol) in 50 mL of absolute ethanol was chilled to 0−5 °C and was saturated with HCl (g). The flask was stoppered, and contents were stirred at room

temperature until an IR spectra indicated the disappearance of the nitrile peak. Anhydrous ether was added to the suspension, and solid was collected by filtration and washed with dry ether. Diimidate ester suspended in absolute ethanol (50 mL) was added, and suspension was chilled to 0−5 °C and saturated with NH_3 (g). The flask was stoppered, and contents were stirred at room temperature for 3 days. Anhydrous ether was added to the suspension, and solid was collected by filtration and washed with anhydrous ether. Free base was suspended in absolute ethanol saturated with HCL (25 mL), and the mixture was heated at reflux for 1 h. After cooling solid was collected by filtration, washed with ether and acetone, and dried in a vacuum at 90 °C for 3 days to yield 0.46 g (68%) of yellow powder, mp 284−290 °C. ^{1}H NMR (DMSO-d_6/D_2O): 8.18 (d, 2H, J = 8.7), 8.16 (d, 1H, J = 1.7), 7.98 (d, 2H, J = 8.7), 7.80 (d, 1H, J = 8.6), 7.68 (dd, 1H, J = 8.4, 1.8), 7.51 (d, 1H, J = 2.9), 7.48 (d, 1H, J = 3.8). ^{13}C NMR (D_2O/DMSO-d_6): 166.4, 165.5, 154.3, 146.4, 144.3, 142.2, 139.2, 134.7, 128.9, 126.0, 125.2, 123.0, 121.5, 116.2, 116.1, 112.1. MS (FAB): m/z 345 ($M^+ + 1$). HRMS: calcd mass (free base), 345.1464 ($M^+ + 1$); observed mass, 345.1490. Anal. ($C_{19}H_{16}N_6O$·2HCl·1.5H_2O) C, H, N.

11: 2-[4-(*N*-Isopropylamidino)phenyl]-5-[2-(5-(*N*-isopropylamidino)benzimidazoyl)]furan Hydrochloride. A suspension of 2-(5-cyano-2-benzimidazolyl)-5-(4-cyanophenyl)-furan (0.5 g, 0.0016 mol) in 50 mL of absolute ethanol was chilled to 0−5 °C and was saturated with HCl (g). The flask was stoppered, and the contents were stirred at room temperature until an IR spectra indicated the disappearance of the nitrile peak. Anhydrous ether was added to the suspension, and solid was collected by filtration and washed with dry ether. Diimidate ester was suspended in absolute ethanol (50 mL), and isopropylamine (2.1 mL, 25 mmol) was added. Mixture was stirred for 72 h at room temperature, and ether was added. The form solid was collected by filtration and dried in a vacuum at 90 °C for 48 h. Free base was suspended in absolute ethanol saturated with HCl, and the mixture was heated at 2 h. After cooling dry ether was added and solid collected by filtration, washed with acetone and dry ether, and dried in a vacuum at 90 °C for 48 h, yield 0.52 g (65%) of yellow crystals, mp 268−285 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.14 (d 2H, J = 8.2), 8.06 (s, 1H), 7.86 (d, 2H, J = 7.9), 7.83 (d, 1H, J = 8.5), 7.63 (d, 1H, J = 8.4), 7.51 (d, 1H, J = 3.1), 7.42 (d, 1H, J = 3.0), 4.06 (m, 2H), 1.36 (m, 12H). ^{13}C NMR (D_2O/DMSO-d_6): 163.4, 163.0, 156.4, 144.6, 141.8, 139.1, 136.2, 134.1, 129.4, 129.1, 125.9, 125.5, 124.9, 119.2, 116.2, 115.8, 112.5, 47.6, 47.5, 22.1, 22.0. MS (FAB): m/z 428 ($M^+ + 1$). HRMS: calcd mass (free base), 428.2325; observed mass, 428.2353. Anal. ($C_{25}H_{28}N_6O$·2HCl·1.5H_2O) C, H, N.

12: 2-[4-(*N*-Cyclopentylamidino)phenyl]-5-[2-(5-(*N*-cyclopentylamidino)benzimidazoyl)]furan Hydrochloride. A protocol similar to that above for **11** was employed to yield 0.6 g (71%) of a yellow solid, mp 239−241 °C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.11 (d, 2H, J = 8.4), 8.06 (d, 1H, J = 1.2), 7.82 (d, 2H, J = 8.4), 7.80 (d, 1H, J = 7.9), 7.65 (d, 1H, J = 7.9), 7.61 (d, 1H, J = 7.9), 7.41 (d, 1H, J = 3.0), 7.4 (d, 1H, J = 3.0), 4.14 (m, 2H), 2.5−2.15 (m, 4H), 1.81−1.63 (m, 8H), 1.61−1.55 (m, 4H). ^{13}C NMR (D_2O/DMSO-d_6): 163.1, 162.4, 155.0, 144.6, 143.1, 139.5, 136.7, 133.3, 129.5, 128.7, 124.8, 124.3, 122.1, 117.1, 116.1, 115.1, 111.4, 54.8, 51.7, 31.7, 30.8, 23.9, 23.7. MS (FAB): m/z 481 ($M^+ + 1$). Anal. ($C_{29}H_{32}N_6O$·3HCl·1.0H_2O) C, H, N.

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