2,5-Bis[4-(*N*-alkylamidino)phenyl]furans as Anti-*Pneumocystis carinii* Agents

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The syntheses of 12 new 2,5-bis[4-(*N*-alkylamidino)phenyl]furans are reported. The interaction of these dicationic furans with poly(dA-dT) and with the duplex oligomer d(CGCGAATTCGCG)$_2$ was determined by T_m measurements, and the effectiveness of these compounds against the immunosuppressed rat model of *Pneumocystis carinii* was evaluated. At the screening dose of 10 μmol/kg, 9 of the 14 *N*-alkylamidino furans described here are more active than the parent compound **1**. Substitution of an alkyl group on the amidino nitrogen, except for in **9**, **13**, and **15**, 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 bis-amidine−DNA complex. Five of the compounds, **3**, **5**, **7**, **10**, and **12**, yield cyst counts of less than 0.1% of control when administered at a dosage of 10 μmol/kg. Five compounds, **1**, **6**, **8**, **10**, and **12**, show significant activity at a dosage of approximately 1 μmol/kg; **12** is the most active derivative, and it is approximately 100 times more effective than pentamidine in this animal model.

Introduction

A number of aromatic diamidines have been shown to bind to the minor groove of DNA and to exhibit useful antimicrobial activities.[1−4] A number of hypotheses for the mode of antimicrobial action of aryldiamidines have been proposed;[5] however, evidence is growing that these compounds function by complex formation with DNA and subsequent selective inhibition of DNA-dependent microbial enzymes. Intervention in transcription control has been demonstrated and seems to be a plausible mode of action for several structurally diverse minor-groove binders.[6] Several minor-groove binding compounds have been shown to interfere with the function of topoisomerase I and/or II.[7] Studies with diamidino bis-benzimidazoles show a direct correlation between topoisomerase II inhibition and anti-giardial activity.[8] Selective inhibition of topoisomerase II isolated from *Pneumocystis carinii* relative to the mammalian enzyme was noted for diamidino bis-benzimidazoles, although a strong correlation between anti-*Pneumocystis* activity and enzyme inhibition was not observed.[9]

Work from our laboratories has demonstrated the antimicrobial and nucleic acid binding properties of amidino and cyclic amidino 2,5-diarylfurans.[2,10] 2,5-Bis-(4-amidinophenyl)furan (**1**) was recently reported to be effective in vivo at submicromolar dosage against *P. carinii* in the immunosuppressed rat model.[2] Earlier, **1** was shown to be effective in vivo in both mouse and simian models for *Trypanosoma rhodesiense*.[1,11] An X-ray structure of the complex between **1** and d(CGC-GAATTCGCG)$_2$ clearly shows that **1** fits well into the DNA minor groove.[2] Other biophysical studies have demonstrated that these compounds strongly bind to the minor groove of DNA at AT-rich sites and weakly bind by intercalation at GC sites of DNA.[10] Similar dual binding modes have been reported for the related diamidines DAPI and berenil.[12−14] The existence of an intercalation component to the DNA binding of **1** continues to be debated.[15,16] We recently published the X-ray structures of the complexes between 2,5-bis[4-(*N*-isopropylamindino)phenyl]furan (**6**) and 2,5-bis[4-(*N*-cyclopropylamidino)phenyl]furan (**7**) and d(CGCGAAT-TCGCG)$_2$ and a preliminary report of their anti-*P. carinii* activity.[17] The X-ray structures for **1**, **6**, and **7** demonstrated the excellent fit of this class of compounds in the minor groove at the AATT site.[2,17,18] Moreover, it has been shown that these types of furan amidines also bind to RNA; however, the binding to RNA is thought to be by intercalation.[19] The intercalation binding of **1** and its analogues to nucleic acids may be a source of toxicity and/or drug loss for these compounds. Based upon literature reports for related minor-groove binders and our preliminary studies, it was postulated that the mode of action of these furan dications involves interference of the normal function of the pathogen's DNA-dependent enzymes, perhaps topoisomerase II.[2]

The strong DNA affinity and effective antimicrobial activity of **1** led us to consider other modifications of this structure with the goal of improved efficacy and reduced toxicity. From biophysical studies of other minor-groove binding compounds[20] and analysis of X-ray crystallographic data for DNA complexes of **1**, **6**, and **7**,[17,18] we have concluded that groups which provide increased van der Waals interactions with the walls of the minor groove significantly enhance binding affinity. A theoretical study of minor-groove binding molecules reached the same conclusion.[21] This report describes the expansion of the series of bis-*N*-alkyl analogues of

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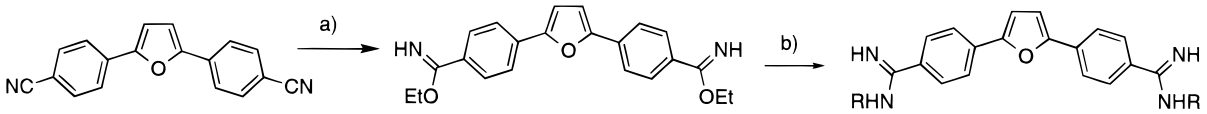
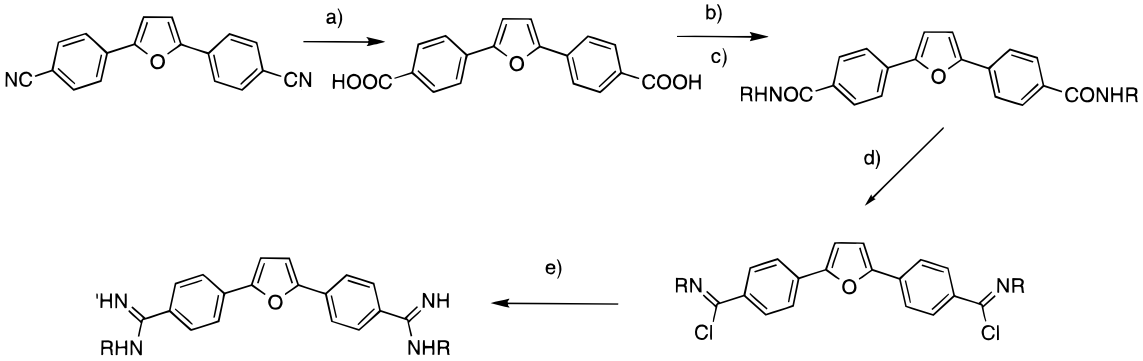
Scheme 1[a]



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

Scheme 2[a]



[a] (a) NaOH, CH_3OH, H_2O; (b) $SOCl_2$, C_6H_6; (c) RNH_2, CH_2Cl_2; (d) $SOCl_2$, CH_2Cl_2; (e) NH_3, CH_2Cl_2.

1 with varying size of the alkyl groups, the determination of their DNA binding affinity, and the evaluation of their effectiveness in the immunosuppressed rat model for *P. carinii* pneumonia.

Chemistry

The synthesis of all the 2,5-bis[4-(*N*-alkylamidino)-phenyl]furans, except **2** and **3**, was achieved in a straightforward manner starting from 2,5-bis(4-cyanophenyl)furan and employing a classical Pinner-type approach for conversion of the nitrile function into the amidino one.[1] The synthetic steps are outlined in Scheme 1. Due to problems associated with obtaining and using anhydrous methylamine and ethylamine, preparation of **2** and **3** was achieved by the indirect route outlined in Scheme 2.

Biological Results

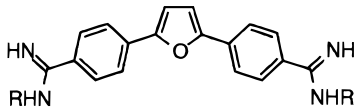
Table 1 contains the results from assessing the affinity of the dications for poly(dA-dT) and d(CGC-GAATTCGCG)$_2$. It was necessary to employ the oligomer in order to rank the relative DNA binding affinity since several of the compounds (**6**, **7**, **10**−**12**) bind so strongly to poly(dA-dT) that melting was not observed within the temperature limits of the experiment. Substitution of an alkyl group on the amidino nitrogen, except for **9**, **13**, and **15**, resulted in higher affinity for DNA than the parent compound (compare the ΔT_m values for the oligomer in Table 1). Not only does the minor groove accommodate rather bulky alkyl groups at the termini of the dicatonic molecule (cf. **12**, **14**), but these groups clearly enhance the binding affinity. These results are consistent with enhanced van der Waals interactions by the alkyl groups of greater surface area with the walls of the DNA minor groove. It is noteworthy that the three compounds (**9**, **13**, **15**) which exhibit significantly lower affinities for the oligomer than **1** are ones in which it seems likely that entropic factors contribute to the lower binding affinity. The fact that the ΔT_m value for the 3-pentyl compound **13** is approximately one-half the value of that for the cyclopentyl analogue **12** clearly illustrates this point.

Most of the compounds, including the previously reported parent compound **1**, in Table 1 are more active than pentamidine, with no overt toxicity at the screening dose, in the immunosuppressed rat model for *P. carinii* pneumonia. Nine of the 14 *N*-alkylamidino furans are more active at the screening dose than the parent compound **1**. Five of the compounds, **3**, **5**, **7**, **10**, and **12**, yield cyst counts of less than 0.1% of control when administered at a dosage of approximately 10 μmol/kg. These five compounds are more active by factors of 10−20 times that of **1**. Five compounds, **1**, **6**, **8**, **10**, and **12**, show significant activity at a dosage of approximately 1 μmol/kg. The most active compound of these five is **12** which is approximately 100 times more effective than pentamidine in this animal model.

It appears for this series of furans that enhanced DNA affinity indeed leads to improved anti-PCP activity. We recently reported a similar relationship between cytotoxicity data for several of these compounds and DNA binding affinity.[22] All the compounds with ΔT_m values of 14 °C or higher, **3**, **6**, **10**, **12**, and **14**, show excellent anti-*P. carinii* activity at the 10 μM dosage level. However, at lower dosage all these compounds do not show comparable activity; for example, note the results for **12** and **14** which exhibit similar ΔT_m values, yet at low dosages the anti-PCP activity of **14** is significantly less than that of **12**. Recently, Hilldebrandt et al. demonstrated that several of the alkylamidino furans listed in Table 1 inhibited an endo/exonuclease isolated from *P. carinii* and thereby identified a new potential mode of action for these types of compounds.[23] Correlations were noted between nuclease inhibition, DNA binding affinity, and anti-*P. carinii* activity; when the data for new compounds reported here are added to the previously reported result, the correlation is retained (not shown).[23] Thus, it appears that the mode of anti-*P. carinii* action of these dicationic furans may involve inhibition of an endo/exonuclease.

In summary, the design strategy to increase minor-groove DNA binding by enhancing the van der Waals interactions with the wall of the groove by addition of moderately bulky alkyl groups to the termini of the 2,5-

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



compd	R	ΔT_m DNA[a]	ΔT_m oligomer[b]	dosage[c] (μmol/kg/day)	cysts/g of lung[c] (% of control)	toxicity[c]
saline					100.0 ± 8.13[d]	
pentamidine				22.0	1.46 ± 0.21	++
				13.3	0.61 ± 0.27	0
1	H	25	11.7	2.7	5.65 ± 2.76	0
				0.3	5.38 ± 2.61	0
				0.03	21.97 ± 3.89	0
2	CH_3			10.6	0.16 ± 0.13	+
				0.2	85.37 ± 24.4	0
3	CH_2CH_3			10.4	0.05 ± 0.02	0
4	CH_2CH_2OH	21.1	11.0	10.4	0.60 ± 0.28	0
5	$CH_2CH_2CH_3$	25.9	13.0	10.6	0.08 ± 0.04	0
				0.2	107.83 ± 26.47	0
6	$CH(CH_3)_2$	>28	14.4	10.8	0.20 ± 0.18	0
				2.2	8.41 ± 7.22	0
				1.1	11.73 ± 4.68	0
				0.2	169.0 ± 61.1	0
7	*c*-($CH_2CH_2CH_2$)	>28	12.4	10.8	0.03 ± 0.01	+
				0.2	129.0 ± 17.9	0
8	CH_2-*c*-($CH_2CH_2CH_2$)	26.8	13.3	10.8	0.24 ± 0.14	0
				0.5	3.25 ± 0.79	0
9	$CH_2CH(CH_3)_2$	21.4	10.0	10.0	2.20 ± 1.35	0
10	*c*-($CH_2CH_2CH_2CH_2$)	>28	14.8	9.9	0.05 ± 0.01	+
				1.0	0.67 ± 0.25	0
11	$CH_2CH_2CH(CH_3)_2$	>28	11.2	9.7	2.38 ± 0.89	0
12	*c*-($CH_2CH_2CH_2CH_2CH_2$)	>28	15.8	9.4	0.03 ± 0.02	+
				1.9	0.08 ± 0.05	0
				0.9	0.11 ± 0.02	0
				0.2	93.5 ± 38.2	0
13	$CH(CH_2CH_3)_2$	20.4	10.0	4.8	24.43 ± 14.7	++
14	*c*-(C_6H_{12})	ppt	15.4	9.1	0.02 ± 0.01	0
				0.9	26.28 ± 5.51	0
15	CH_2-*c*-(C_6H_{12})	21.4	10.6	8.7	43.9 ± 14.23	0

[a] Increase in thermal melting of polyA·polyT; see ref 19. [b] Increase in thermal melting of the oligomer d(GCGCAATTGCGC)$_2$; see ref 24. [c] Evaluation of iv dosage of the furan dications against *P. carinii* in rats as described in ref 4b. [d] Mean cyst count for pooled controls; saline (n = 142) = 63.77 ± 5.18 cysts/g of lung tissue; pentamidine (n = 135) = 0.93 ± 0.14 cysts/g of lung tissue.

diarylfuran system appears to be successful. Furthermore, the improved DNA affinity has led to enhancement of in vivo activity in the immunosuppressed rat model for *P. carinii* pneumonia, and compound **12** has emerged as a highly effective candidate for further evaluation against *P. carinii* infections.

Experimental Section

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded employing a Varian GX400 spectrometer and chemical shifts (δ) are in ppm relative to TMS unless otherwise noted. 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 synthesis of **6** and **7** was reported previously.[17] T_m values for compound−DNA complexes were determined as previously described.[2,3,19,24]

2,5-Bis[4-(*N*-methylamidino)phenyl]furan (2). To a slurry of 2,5-bis(4-cyanophenyl)furan[25] (5.4 g, 0.02 mol) in 30 mL of methanol was added 100 mL of 30% NaOH, and the mixture was heated at reflux for 40 h. The mixture was cooled, 100 mL of cold water was added, and the pH was adjusted to a value of 2 with concentrated HCl. The solid was filtered, washed with water and ether, and dried under vacuum at 85 °C to yield 4.65 g (75%) of 2,5-bis(4-carboxyphenyl)furan: mp 394−396 °C; ^{1}H NMR (DMSO-d_6/D$_2$O/70 °C) δ 8.42 (d, 4H, J = 8 Hz) 8.34 (d, 4H, J = 8 Hz), 7.67 (s, 2H); ^{13}C NMR (DMSO-d_6/70 °C) δ 166.6, 152.5, 133.1, 130.1, 129.7, 123.2, 110.2; MS m/e 308 (M$^+$). Anal. ($C_{18}H_{12}O_5$) C,H.

A suspension of 2,5-bis(4-carboxyphenyl)furan (3.08 g, 0.01 mol), 75 mL of dry benzene, thionyl chloride (7.08 g, 0.06 mol), and 4 drops of DMF was allowed to reflux for 2.5 h. The solvent was removed under vacuum, and the residue which resulted was triturated with benzene; the benzene was removed under vacuum and the residue triturated with dry ether. The yellow solid was filtered and dried under vacuum at 60 °C to yield 2.9 g (84%) of the diacid chloride: mp 358−360 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 4H, J = 7.6 Hz), 7.86 (d, 4H, J = 7.6 Hz), 7.02 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 167.4, 153.1, 135.8, 132.0, 128.9, 123.8, 111.3; MS m/e 345 (M$^+$). The diacid chloride was used directly in the next step without further characterization.

To a suspension of the diacid chloride (0.69 g, 0.002 mol) in 50 mL of dry CH_2Cl_2 was added methylamine (0.186 g, 0.004 mol), and the mixture was allowed to stir at room temperature for 7 h. The solvent was removed under reduced pressure, and water was added. The solid was filtered and washed with 10% HCl, 10% $NaHCO_3$, and water. After drying the solid was recrystallized from $CHCl_3$−ether to give a white solid (0.53 g, 80%): mp 282−284 °C; ^{1}H NMR (DMSO-d_6) δ 8.29 (br s, 2H), 7.92 (d, 4H, J = 8 Hz), 7.88 (d, 4H, J = 8 Hz), 7.17 (s, 2H), 2.82 (br s, 6H); ^{13}C NMR (DMSO-d_6) δ 165.8, 152.4, 133.2, 131.8, 127.4, 123.0, 109.4; MS m/e 334 (M$^+$). Anal. ($C_{20}H_{18}N_2O_3$) C,H,N.

To a suspension of 2,5-bis[4-(*N*-methylcarbamoyl)phenyl]-furan (0.835 g, 0.0025 mol) in 40 mL of dry $CHCl_3$ and thionyl

chloride (1.2 g, 0.01 mol) was added 4−5 drops of DMF, and the mixture was allowed to reflux until the solution was clear (ca. 4 h). The solution was filtered hot, and the filtrate was distilled under reduced pressure to remove solvent. The residue was dissolved in dry CH_2Cl_2, the solvent removed under reduced pressure, and the solid triturated with dry hexane. The solid was filtered (under nitrogen), and the yellow solid was dried under vacuum at 40 °C to yield 0.88 g (81%) of the bis-imidoyl chloride. The bis-imidoyl chloride was used directly in the next step without further characterization.

A suspension of 0.88 g (0.002 mol) of the bis-imidoyl chloride in 45 mL of dry CH_2Cl_2 was saturated with dry ammonia gas at 0−5 °C. The mixture was stirred at room temperature for 24 h, and the solvent was removed under reduced pressure. The residue was treated with ice−water, and the pH of the slurry was adjusted to a value of 10 by adding 2 M NaOH. The resulting solid was filtered, washed with water, and dried under vacuum to yield 0.5 g (75%) of the free base of **2**: mp 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 7.81 (br s, 8H), 7.13 (s, 2H), 6.52−6.32 (br, 4H), 2.83 (s, 6H); MS *m/e* 332 (M^+).

The free base was converted into the salt by taking up 0.4 g (0.0012 mol) in 6 mL of methanol saturated with HCl and stirring for 1 h. After reducing the solvent volume under reduced pressure, addition of dry ether caused precipitation of the salt, which was filtered, washed with ether, and dried under vacuum to yield 0.44 g (90%) of yellow solid: mp 330−335 °C dec; ^{1}H NMR (DMSO-d_6/D_2O/40 °C) δ 8.01 (d, 4H, J = 8.4 Hz), 7.83 (d, 4H, J = 8.4 Hz), 7.31 (s, 2H), 3.02 (s, 6H); ^{13}C NMR (DMSO-d_6/D_2O/40 °C) δ 163.2, 152.6, 134.1, 130.1, 128.9, 127.4, 124.1, 111.5, 29.6. Anal. ($C_{20}H_{20}N_4O$·2HCl) C,H,N.

2,5-Bis[4-(*N*-ethylamidino)phenyl]furan (3). Following the procedure described above for **2**, 2,5-bis[4-(*N*-ethylcarbamoyl)phenyl]furan was prepared in an 85% yield: mp 309−311 °C dec; ^{1}H NMR (DMSO-d_6/75 °C) δ 8.22 (br s, 2H), 7.92 (d, 4H, J = 8.4 Hz), 7.86 (d, 4H, J = 8.4 Hz), 7.15 (s, 2H), 3.33 (q, 2H), 1.17 (t, 6H); ^{13}C NMR (DMSO-d_6/75 °C) δ 165.1, 152.3, 133.4, 131.7, 127.4, 122.8, 109.2, 33.7, 14.2; MS *m/e* 362 (M^+). Anal. ($C_{22}H_{22}N_2O_3$) C,H,N.

2,5-Bis[4-(*N*-ethylcarbamoyl)phenyl]furan was converted into the corresponding bis-imidoyl chloride which was then converted into the free base of **3** according to the procedure described above for **2** to yield a white solid (80%): mp 213−215 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.81 (br s, 8H), 7.12 (s, 2H), 6.43−6.21 (br, 4H), 3.19 (q, 4H, J = 7.6 Hz), 1.18 (t, 6H, J = 7.6 Hz); MS *m/e* 360 (M^+).

The free base of **3** was converted into the yellow dihydrochloride salt of **3** as described above for **2** in an 88% yield: mp 229−231 °C dec; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.96 (d, 4H, J = 8.4 Hz), 7.79 (d, 4H, J = 8.4 Hz), 7.26 (s, 2H), 3.42 (q, 4H, J = 7.2 Hz), 1.24 (t, 6H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6/D_2O) δ 162.6, 152.8, 134.3, 129.3, 127.8, 124.3, 111.8, 38.2, 13.2. Anal. ($C_{22}H_{24}N_4O$·2HCl·0.75H_2O) C,H,N.

2,5-Bis[4-[*N*-(2-hydroxyethyl)amidino]phenyl]furan (4). 2,5-Bis(4-cyanophenyl)furan[25] (2.79 g, 0.01 mol) was suspended in 80 mL of ethanol (distilled from magnesium metal) and stirred for ca. 1 week at room temperature to yield 3.93 g (90%) of the corresponding imidate ester, which was used directly without characterization. Dried (CaH_2) and freshly distilled aminoethanol (0.27 g, 0.0045 mol) was added to a suspension of imidate ester hydrochloride (0.65 g, 0.0015 mol) in 10 mL of ethanol, and the mixture was stirred under nitrogen at room temperature for 12 h. The solvent was distilled under vacuum, the residue was treated with ice−water, and the pale yellow solution was made basic with 1 M NaOH to pH 9. The solid was filtered, washed with water, dried, and crystallized from $CHCl_3$−ether (1:4) to yield a pale crystalline solid (0.43 g, 74%): mp >330 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.92 (q, 8H, J = 8.4 Hz), 7.20 (s, 2H), 4.13 (t, 4H, J = 9.2 Hz), 3.97 (t, 4H, J = 9.2); MS *m/e* 394 (M^+).

The free base (0.39 g, 0.001 mol) was dissolved in 5 mL of dry ethanol, treated with 7 mL of saturated ethanolic HCl, and heated at 40 °C for 2 h. The ethanol was removed under vacuum and the residue triturated with dry ether; the yellow crystalline solid was filtered, washed with ether, and dried in vacuo at 70 °C for 24 h to yield 0.43 g (88%): mp 190−192 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.99 (s, 2H), 9.57 (s, 2H), 9.19 (s, 2H), 8.06 (d, 4H, J = 7.93 Hz), 7.94 (d, 4H, J = 8.55 Hz), 7.4 (s, 2H), 3.69 (t, 4H, J = 4.9 Hz), 3.58 (t, 4H, J = 4.9 Hz); ^{13}C NMR (DMSO-d_6) δ 162.6, 152.4, 133.8, 129.1, 127.5, 123.6, 111.4, 58.4, 45.7. Anal. ($C_{22}H_{24}N_4O_3$·2HCl·1.25H_2O) C,H,N.

2,5-Bis[4-(*N*-*n*-propylamidino)phenyl]furan (5). The imidate ester was converted into the free base of **5** as described above to yield (86%), after recrystallization from $CHCl_3$−ether (1:8), a pale yellow solid: mp 187−188 °C; IR (KBr) 3261, 3062, 2949, 2929, 2874, 2602, 1542, 1497, 1364, 1196, 1149, 1022, 847, 790, 750, 685; ^{1}H NMR (DMSO-d_6) δ 7.84 (s, 8H), 7.17 (s, 2H), 6.6−6.38 (vbr, 4H), 3.09 (t, 4H, J = 6.9 Hz), 1.62 (tt, 4H, J = 6.9, 7.5 Hz), 0.96 (t, 6H, J = 7.5 Hz); MS *m/e* 388 (M^+).

The free base of **5** was converted into the yellow dihydrochloride salt of **5** as described above in an 89% yield: mp 227−230 °C dec; IR (KBr) 3399, 3228, 3042, 2878, 1670, 1614, 1501, 1375, 1292, 1127, 1027, 929, 849, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.11 (br s, 2H), 9.65 (s, 2H), 9.3 (d, 2H), 8.05 (d, 4H, J = 8.4 Hz), 7.95 (d, 4H, J = 8.4 Hz), 7.4 (s, 2H), 3.46 (q, H, J = 6.3 Hz), 1.7 (dqt, 4H, J = 7.2 Hz), 0.98 (t, 6H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 161.8, 152.2, 133.5, 128.8, 127.2, 123.4, 111.0, 43.9, 20.6, 10.8. Anal. ($C_{24}H_{28}N_4O$·2HCl·0.5H_2O) C,H,N.

2,5-Bis[4-[*N*-(cyclopropylmethyl)amidino]phenyl]furan (8). The imidate ester was converted into the free base of **8** as described above to yield (84%), after recrystallization from $CHCl_3$−ether (1:8), a pale yellow solid: mp 178−179 °C; ^{1}H NMR (DMSO-d_6) δ 7.83 (s, 8H), 7.14 (s, 2H), 6.34 (br, 4H), 3.09 (d, 4H, J = 6.0 Hz), 1.13−1.05 (m, 2H), 0.47−0.41 (m, 4H), 0.28−0.21 (m, 4H); MS *m/e* 412 (M^+).

The free base of **8** was converted into the yellow dihydrochloride salt of **8** as described above in a 92% yield: mp 243−245 °C; IR (KBr) 3390, 3245, 3197, 2865, 1673, 1614, 1503, 1380, 1111, 929, 765, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.24 (br s, 2H), 9.65 (s, 2H), 9.3 (s, 2H), 8.06 (d, 4H, J = 8.4 Hz), 7.97 (d, 4H, J = 8.4 Hz), 7.41 (s, 2H), 3.42 (t, 4H, J = 6.3 Hz), 1.26−1.06 (m, 2H), 0.58−0.51 (m, 4H), 0.44−0.38 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 163.4, 153.3, 134.9, 129.7, 128.2, 124.9, 112.3, 48.2, 9.8, 4.4. Anal. ($C_{26}H_{28}N_4O$·2HCl·0.5H_2O) C,H,N.

2,5-Bis[4-(*N*-isobutylamidino)phenyl]furan (9). The imidate ester was converted into the free base of **9** as described above to yield (86%), after recrystallization from ethanol−ether (1:5), a pale yellow solid: mp 178−179 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.84 (s, 8H), 6.65−6.31 (vbr, 4H), 2.94 (d, 4H, J = 7.7 Hz), 1.9 (sept, 2H, J = 7.7, 6.1 Hz), 0.96 (d, 12H, J = 6.1 Hz); ^{13}C NMR (DMSO-d_6/D_2O) δ 156.1, 152.6, 136.0, 130.6, 127.1, 122.9, 109.1, 53.4, 28.9, 20.9; MS *m/e* 416 (M^+).

The free base of **9** was converted into the yellow dihydrochloride salt of **9** as described above in a 94% yield: mp 293−295 °C dec; IR (KBr) 3412, 3234, 3092, 2990, 1669, 1612, 1569, 1500, 1379, 1291, 1132, 1023, 929, 851, 792, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.0 (br s, 2H), 9.58 (br s, 2H), 9.25 (br, 2H), 8.04 (d, 4H, J = 8.6 Hz), 7.94 (d, 4H, J = 8.6 Hz), 7.37 (s, 2H), 3.35 (d, 2H, J = 7.7 Hz), 2.08 (sept, 2H, J = 6.7 Hz), 1.0 (d, 12H, J = 6.7 Hz); ^{13}C NMR (DMSO-d_6) δ 162.2, 152.3, 133.6, 128.9, 127.4, 123.5, 111.2, 49.4, 26.8, 19.7. Anal. ($C_{26}H_{32}N_4O$·2HCl·0.5H_2O) C,H,N.

2,5-Bis[4-(*N*-cyclobutylamidino)phenyl]furan (10). The imidate ester was converted into the free base of **10** as described above to yield (76%), after recrystallization from ethanol−ether (1:5), a pale yellow solid: mp 208−210 °C dec; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.82 (q, 8H, J = 8.8 Hz), 7.13 (s, 2H), 4.08 (t, 2H, J = 7.6 Hz), 2.40−2.31 (m, 4H), 2.0−1.88 (m, 4H), 1.77−1.64 (m, 4H); MS *m/e* 412 (M^+).

The free base of **10** was converted into the yellow dihydrochloride salt of **10** as described above in a 90% yield: mp 293−295 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.2 (s, 2H), 10.18 (s, 2H), 9.6 (s, 2H), 9.1 (s, 2H), 8.04 (d, 4H, J = 8.8 Hz), 7.94 (d, 4H, J = 8.8 Hz), 7.38 (s, 2H), 4.44 (sext, 2H, J = 7.2 Hz), 2.51−2.42 (m, 4H), 2.37−2.26 (m, 4H), 1.88−1.78 (m, 2H), 1.77−1.60 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 160.7, 152.2, 133.6, 129.0, 127.0, 123.3, 110.0, 47.3, 28.4, 14.6. Anal. ($C_{26}H_{28}N_4O$·2HCl·H_2O) C,H,N.

2,5-Bis[4-(*N*-isopentylamidino)phenyl]furan (11). The imidate ester was converted into the free base of **11** as described above to yield (76%), after recrystallization from ethanol−ether, a white solid: mp 185−186 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.85 (d, 4H, J = 8.4 Hz), 7.81 (d, 4H, J = 8.4 Hz), 7.17 (s, 2H), 3.19 (t, 4H, J = 7.5 Hz), 1.74 (sept, 2H, J = 6.6 Hz), 1.52 (q, 4H, J = 6.6 Hz), 0.93 (d, 12H, J = 6.6 Hz); MS *m/e* 444 (M^+).

The free base of **11** was converted into the yellow dihydrochloride salt of **11** as described above in an 89% yield: mp 297−299 °C dec; IR (KBr) 3360, 3201, 2962, 2874, 1669, 1613, 1503, 1376, 1028, 847, 745 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.1−9.2 (vbr, 6H), 8.06 (d, 4H, J = 8.3 Hz), 7.89 (d, 4H, J = 8.3 Hz), 7.4 (s, 2H), 3.46 (t, 4H, J = 7.3 Hz), 1.72 (sept, 2H, J = 6.35 Hz), 1.6 (dt, 4H, J = 7.3, 6.35 Hz), 0.95 (d, 12H, J = 6.35 Hz); ^{13}C NMR (DMSO-d_6) δ 161.9, 152.3, 133.7, 128.9, 127.5, 123.5, 111.2, 41.1, 35.8, 25.2, 22.1. Anal. ($C_{28}H_{36}N_4O$·2HCl) C,H,N.

2,5-Bis[4-(*N*-cyclopentylamidino)phenyl]furan (12). The imidate ester was converted into the free base of **12** as described above to yield (76%), after recrystallization from ethanol−ether (1:6), a pale yellow solid: mp 200−201 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.84 (s, 8H), 7.18 (s, 2H), 3.9 (br, 2H), 1.94−1.91 (br m, 4H), 1.88−1.7 (br m, 4H), 1.69−1.45 (br m, 12H); ^{13}C NMR (DMSO-d_6) δ 156.5, 152.5, 135.9, 130.7, 127.2, 122.9, 109.1, 55.0, 33.0, 24.0; MS *m/e* 440 (M^+).

The free base of **12** was converted into the yellow dihydrochloride salt of **12** as described above in a 92% yield: mp 294−296 °C dec; IR (KBr) 3400, 3045, 2939, 2880, 1667, 1609, 1502, 1358, 1291, 1189, 1024, 929, 845, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.89 (s, 1H), 9.86 (s, 1H), 9.62 (s, 2H), 9.3 (s, 2H), 8.04 (d, 4H, J = 8.3 Hz), 7.9 (d, 4H, J = 8.3 Hz), 7.4 (s, 2H), 4.27 (br, 2H), 2.08−2.06 (m, 4H), 1.76−1.58 (m, 12H); ^{13}C NMR (DMSO-d_6) δ 161.6, 152.2, 133.4, 128.9, 127.4, 123.2, 110.7, 54.1, 31.0, 23.3. Anal. ($C_{28}H_{32}N_4O$·2HCl·H_2O) C,H,N.

2,5-Bis[4-(*N*-3-pentylamidino)phenyl]furan (13). The imidate ester was converted into the free base of **13** as described above to yield (76%), after recrystallization from $CHCl_3$−ether (1:3), a pale yellow solid: mp 155−156 °C; IR (KBr) 3245, 3120, 2962, 1593, 1544, 1380, 1194, 848, 784 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.81 (q, 8H, J = 8.4 Hz), 7.13 (s, 2H), 6.22 (br, 4H), 3.45 (br, 2H), 1.58−1.41 (m, 8H), 0.88 (t, 12H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 157.3, 152.5, 136.7, 130.5, 1127.0, 122.8, 108.8, 55.0, 27.3, 10.5; MS *m/e* 444 (M^+).

The free base of **13** was converted into the yellow dihydrochloride salt of **13** as described above in a 90% yield: mp >360 °C; IR (KBr) 3410, 3235, 3105, 1668, 1613, 1500, 1459, 1368, 1126, 1025 cm^{-1}; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.93 (d, 4H, J = 8.5 Hz), 7.76 (d, 4H, J = 8.5 Hz), 7.13 (s, 2H), 3.88−3.65 (m, 2H), 1.9−1.8 (m, 4H), 1.78−1.66 (m, 4H), 1.05 (t, 6H, J = 7.3 Hz); ^{13}C NMR (DMSO-d_6/D_2O) δ 165.1, 154.1, 136.2, 130.0, 129.0, 125.8, 112.9, 59.1, 27.8, 11.5. Anal. ($C_{28}H_{36}N_4O$·2HCl·1.5H_2O) C,H,N.

2,5-Bis[4-(*N*-cyclohexylamidino)phenyl]furan (14). The imidate ester was converted into the free base of **14** as described above to yield (78%), after recrystallization from ethanol−ether (1:6), a pale yellow solid: mp 270−275 °C dec; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.89 (d, 4H, J = 8.4 Hz), 7.8 (d, 4H, J = 8.4 Hz), 7.21 (s, 2H), 3.6 (br m, 2H), 1.9−1.57 (m, 5H), 1.41−1.14 (m, 5H); MS *m/e* 468.

The free base of **14** was converted into the yellow dihydrochloride salt of **14** as described above in a 91% yield: mp 298−300 °C dec; IR (KBr) 3410, 3192, 3091, 2931, 2853, 1669, 1611, 1502, 1369, 1056, 791, 747 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.65 (br s, 2H), 9.53 (br s, 2H), 9.28 (s, 2H), 8.07 (d, 4H, J = 8.3 Hz), 7.85 (d, 4H, J = 8.3 Hz), 7.43 (s, 2H), 3.75 (br m, 2H) 2.0−1.9 (m, 4H), 1.8−1.1 (m, 16H); ^{13}C NMR (DMSO-d_6) δ 161.1, 152.3, 133.6, 129.1, 127.7, 123.5, 111.2, 51.8, 30.9, 24.6, 24.1. Anal. ($C_{30}H_{36}N_4O$·2HCl·0.5H_2O) C,H,N.

2,5-Bis[4-[*N*-(cyclohexylmethyl)amidino]phenyl]furan (15). The imidate ester was converted into the free base of **15** as described above to yield (74%), after recrystallization from ethanol−ether (1:4), a pale yellow solid: mp 280−282 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.5−8.5 (br, 4H), 8.0 (d, 4H, J = 8.4 Hz), 7.88 (d, 4H, J = 8.4 Hz), 7.35 (s, 2H), 3.24 (d, 4H, J = 6.9 Hz), 1.80−1.64 (m, 12H), 1.30−1.15 (m, 8H), 1.08−0.93 (m, 4H); MS *m/e* 496 (M^+).

The free base of **15** was converted into the yellow dihydrochloride salt of **15** as described above in an 88% yield: mp 245−247 °C dec; IR (KBr) 3418, 3236, 3061, 2921, 2852, 1668, 1614, 1501, 1448, 1289, 1025, 929, 847, 791, 747 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.0 (br, 2H), 9.61 (br, 2H), 9.24 (br, 2H), 8.06 (d, 4H, J = 8.55 Hz), 7.93 (d, 4H, J = 8.55 Hz), 7.4 (s, 2H), 3.35 (t, 4H, J = 6.1 Hz), 1.81−1.65 (m, 12H), 1.26−1.14 (m, 6H), 1.09−1.0 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 162.1, 152.3, 133.6, 128.9, 127.4, 123.5, 111.2, 48.2, 35.9, 29.8, 25.7, 25.1. Anal. ($C_{32}H_{40}N_4O$·2HCl·0.25H_2O) C,H,N.

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