Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 683–691
Bioorganic & Medicinal Chemistry

Synthesis and antiprotozoal activity of novel bis-benzamidino imidazo[1,2-*a*]pyridines and 5,6,7,8-tetrahydro-imidazo[1,2-*a*]pyridines

Mohamed A. Ismail,[a] Reem K. Arafa,[a] Tanja Wenzler,[b] Reto Brun,[b] Farial A. Tanious,[a] W. David Wilson[a] and David W. Boykin[a,*]

[a]*Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA*
[b]*Parasite Chemotherapy, Swiss Tropical Institute, Basel CH4002, Switzerland*

Received 11 September 2007; revised 8 October 2007; accepted 12 October 2007
Available online 18 October 2007

Abstract—The key dinitrile intermediates **4a–d** were synthesized by reaction of phenacyl bromide **1** and the appropriate 2-amino-5-bromopyridines to yield **3a–d**. Suzuki coupling of **3a–d** with 4-cyanophenylboronic acid yielded the 2,6-bis(4-cyanophenyl)-imidazo[1,2-*a*]pyridine derivatives **4a–d**. The bis-amidoximes **5a–d**, obtained from **4a–d** by the action of hydroxylamine, were converted to the bis-*O*-acetoxyamidoximes which on catalytic hydrogenation in a mixture of ethanol/ethyl acetate gave the acetate salts of 2,6-bis[4-(amidinophenyl)]-imidazo[1,2-*a*]pyridines **7a–d**. In contrast, catalytic hydrogenation of the bis-*O*-acetoxyamidoxime of **5a** in glacial acetic acid gave the saturated analogue 2,6-bis[4-(amidinophenyl)]-5,6,7,8-tetrahydro-imidazo[1,2-*a*]pyridine **8**. *O*-Methylation of the amidoximes **5a–d** gave the *N*-methoxyamidines **6a–d**. The diamidines showed strong DNA binding affinity, were very active in vitro against *T. b. r.* exhibiting IC_{50} values between 7 and 38 nM, but were less effective against *P. f.* with IC_{50} values between 23 and 92 nM. Two of the diamidines **7c** and **7d** were slightly more active than furamidine but less active than azafuramidine in the *T. b. r.* STIB900 mouse model. Only one prodrug **6b** showed moderate activity in the same mouse model.
© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

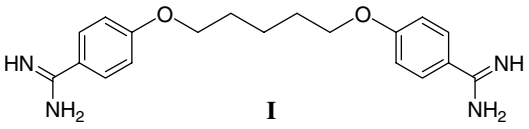
Dicationic diamidine derivatives have long been known to exhibit broad-spectrum antiprotozoal activity.[1] A significant number of these compounds possesses effectiveness against the protozoan diseases caused by *Trypanosoma brucei* and *Plasmodium* sp.[1] Despite this fact, pentamidine (**I**) is the only compound of this class to see clinical use.[1] A prodrug of furamidine [2,5-bis(4-amidinophenyl)furan] (**IIa**), pafuramidine [2,5-bis[4-(methoxyamidino)phenyl]furan] (**IIb**), is currently in two Phase III clinical trials as an oral drug versus human African trypanosomiasis (HAT) and against AIDS-related *Pneumocystsis jiroveci* pneumonia.[1,2] A closely related prodrug **IId** of the azafuramidine **IIc** is effective in a model of late stage HAT and is under evaluation for clinical advancement.[3] The antiparasitic action of such diamidines is hypothesized to arise from their binding to the minor groove of DNA at AT rich sites.[2,4–9] It has been further suggested that the DNA binding leads to inhibition of DNA dependent enzymes and possibly direct inhibition of transcription.[9–13] At least in part, the selectivity of the diamidines seems likely due to involvement of amidine transporters for uptake[14] and strong binding to kinetoplast DNA once in the parasite.[1b]

Previous bis-benzamidine drug discovery efforts have focused on variations in the linker between the benzamidine units ranging from oxyalkyl groups for pentamidine analogues to aryl/heteroaryl rings for furamidine types. Extensive numbers of 5- and 6-membered ring systems have been investigated as a linker for the bis-benzamidines of furamidine (replacement of furan).[1] However, larger linker units such as two fused 5-ring units or a fused 5–6-membered ring system does not appear to have been investigated. Larger appropriately designed linkers can be envisioned to be potent minor groove binders and potentially show useful antiprotozoan activity.[15] As an initial effort to explore the

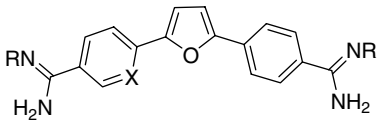
Keywords: Diamidines; Antiprotozoal agents; Minor-groove binders; Imidazo[1,2-*a*]pyridines; Prodrugs.
* Corresponding author. Tel.: +1 404 413 5498; fax: +1 404 413 5505; e-mail: dboykin@gsu.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.042

efficacy of triaryl bis-benzamidine molecules with larger linkers we have elected to study a series of bis-benzamidine imidazo[1,2-*a*]pyridines. Imidazo[1,2-*a*]pyridine units are important building blocks in both natural and synthetic bioactive compounds[16,17] and we have found that an amidino imidazo[1,2-*a*]pyridine as a replacement of one of the benzamidine units yields highly effective antiprotozoan agents.[18] All of these factors lead us to synthesize bis-benzamidino imidazo[1,2-*a*]pyridine and 5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridines for evaluation of their DNA affinity and their antiprotozoan activity. In addition, *N*-methoxy amidine analogues were prepared as potential prodrugs for their corresponding diamidines.



I



IIa R = H; X = CH
IIb R = OMe; X = CH
IIc R = H; X = N
IId R = OMe; X = N

2. Results and discussion

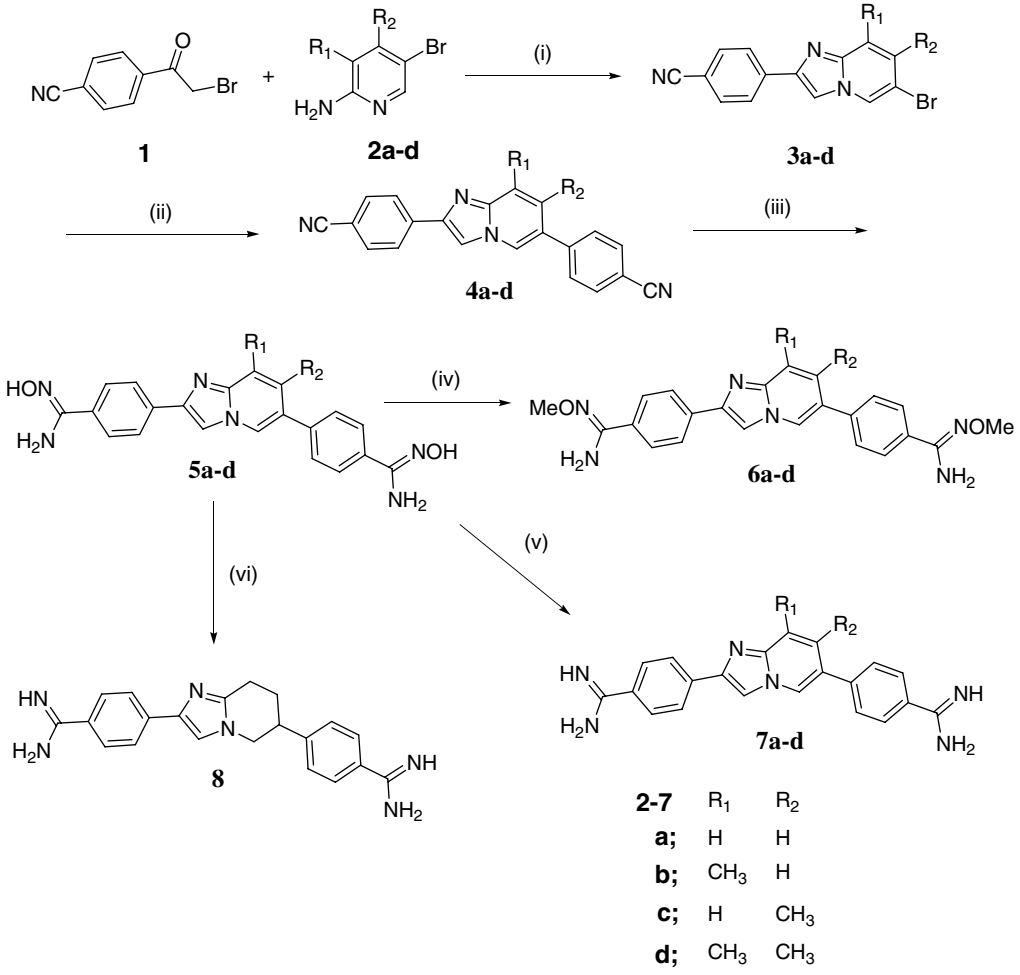
2.1. Chemistry

As illustrated in Scheme 1, the key 2,6-bis(4-cyanophenyl)-imidazo[1,2-*a*]pyridine intermediates **4a**–**d** were prepared in two steps. The first step involves condensation between 4-cyanophenacyl bromide **1** and the appropriate 2-aminopyridine to yield, after cyclization, the 4-(6-bromo-imidazo[1,2-*a*]pyridin-2-yl)-benzonitriles **3a**–**d**. In the second step, a Suzuki coupling reaction between **3a**–**d** and 4-cyanophenylboronic acid furnished 2,6-bis(4-cyanophenyl)imidazo[1,2-*a*]pyridine derivatives **4a**–**d** in high yields. The dinitriles **4a**–**d** were converted to the corresponding bis-amidoximes **5a**–**d** via reaction with hydroxylamine in DMSO solution. Thereafter, the acetate salts of the 2,6-bis[4-(amidinophenyl)]imidazo[1,2-*a*]pyridine derivatives **7a**–**d** were obtained by transformation of the corresponding bis-amidoximes **5a**–**d** to their bis-*O*-acetoxyamidoxime counterparts, without isolation, followed by catalytic hydrogenation in a mixture of ethanol/ethyl acetate. Interestingly, catalytic hydrogenation of the bis-*O*-acetoxyamidoxime of **5a** in glacial acetic acid furnished the saturated diamidine 2,6-bis[4-(amidinophenyl)]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine **8** (Scheme 1). These results indicate that proper choice of the hydrogenation solvent(s) determines whether the product will be saturated or unsaturated. A previous publication from our laboratory as well as recent study of the effect of solvent on the course of catalytic hydrogenation of other nitrogen heterocycles further illustrates the importance of solvent selection for this reaction.[18,19] Nevertheless, these hydrogenations are also quite sensitive to substrate substituents since attempts to reduce bis-*O*-acetoxyamidoximes of the analogues **5b**–**d** by acetic acid aided catalytic hydrogenation failed demonstrating that these solvent effects are also quite sensitive to substrate substituents.

Potential prodrugs, the *N*-methoxyamidines **6a**–**d**, were prepared by *O*-methylation of their corresponding bis-amidoxime precursors **5a**–**d** using dimethyl sulfate in aqueous lithium hydroxide solution in reasonable yields (Scheme 1). The hydrochloride salts of the 2,6-bis[4-(*N*-methoxyamidinophenyl)]-imidazo[1,2-*a*]pyridine derivatives **6a**–**d** were obtained by passing hydrogen chloride gas into ethanolic solutions of their free bases.

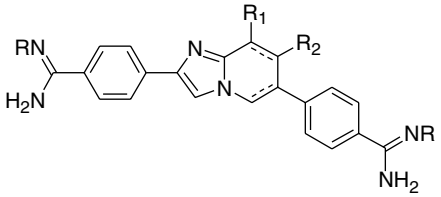
2.2. Biology

The results for the evaluation of the new diamidines against *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*) and their DNA binding affinities are shown in Table 1. For comparative purposes the analogous data for furamidine (**IIa**) and an azafuramidine (**IIc**) are also included in Table 1. The DNA affinities for the diamidines **7a**–**d** and **8** are represented by the ΔT_m values for their complexes with poly(dA-dT). The ΔT_m values for **7a**–**d** are shown as >28 °C which indicates that under the conditions of this experiment the complexes are quite stable and did not melt. Clearly, the interaction of **7a**–**d** with DNA is quite favorable as the affinity is greater than that of furamidine (ΔT_m = 25 °C). The interaction of **8** with DNA is strong (ΔT_m = 22.9 °C) but not as favorable as that for furamidine or **7a**–**d**. The lower affinity for **8** which contains a partially unsaturated linker is not unexpected as the non-planar linker would not be expected to have as favorable van der Waals contacts as its planar counterparts **7a**–**d**. These results demonstrate that larger linkers when appropriately arrayed can lead to potent DNA binders. CD spectra of **7a** and **8** on titration into a solution of a polyd(A-T)$_2$ DNA sequence were monitored from 220 to 450 nm. At the maximum absorption wavelength of the compounds, where the DNA CD signals do not interfere, positive induced CD signals were observed around 330 nm (Fig. 1). Neither **7a** nor **8** has intrinsic CD signals and the signals shown in Figure 1 are for the bound compound. Such positive induced CD signals are generally a characteristic of binding in the minor groove of DNA as observed for other minor groove binding compounds.[20,21] Presumably, the conjugated compound **7a** shows a stronger induced CD signal than **8** because it can slide more deeply into the minor groove and interact better with walls of the minor groove and the base pairs at the floor of the groove. The CD results were obtained at relatively high concentration, with respect to K_d, in order to obtain satisfactory signals but they can still be used to approximate K_d values. For **7a** K_d ~20 nM with DNA concentration in base pairs and for **8** K_d ~250 nM, significantly less than for **7** in agreement with ΔT_m values. The CD results thus show that



Scheme 1. Reagents and conditions: (i) EtOH, reflux; (ii) 4-cyanophenyl boronic acid, $Pd(PPh_3)_4$; (iii) $NH_2OH{\cdot}HCl/K$–*O*–*t*-Bu, DMSO; (iv) LiOH/$(CH_3)_2SO_4$; (v) (a) Ac_2O/AcOH, (b) H_2/Pd-C, EtOH (vi) (a) Ac_2O/AcOH, (b) H_2/Pd-C, AcOH.

Table 1. DNA affinities and in vitro anti-protozoan data



Code	Pyridine ring	R	R_1	R_2	ΔT_m[a] (°C)	*T. b. r.* IC_{50} (nM)[b]	*P. f.* IC_{50} (nM)[b]	Cytotoxicity IC_{50} (μM)
IIa	NA	NA	NA	NA	25	4.3	15.5	6400
IIc	NA	NA	NA	NA	19.3	6.5	6.5	77,900
8	Saturated	H	H	H	22.9	14	85	8900
7a	Unsaturated	H	H	H	>28	7.5	92	2500
6a	Unsaturated	OMe	H	H		825	181	6900
7b	Unsaturated	H	CH_3	H	>28	38	23	7400
6b	Unsaturated	OMe	CH_3	H		2367	614	9500
7c	Unsaturated	H	H	CH_3	>28	13	27	6800
6c	Unsaturated	OMe	H	CH_3		1739	880	17,000
7d	Unsaturated	H	CH_3	CH_3	>28	15	63	4700
6d	Unsaturated	OMe	CH_3	CH_3		7207	3249	>146,800

[a] See Ref. 22.
[b] Average of duplicate determinations, see Ref. 22.

these compounds bind very strongly in the DNA minor groove.

The diamidines **7a–d** and **8** are very effective against *T. b. r.*, their IC_{50} values range from 7 to 38 nM,

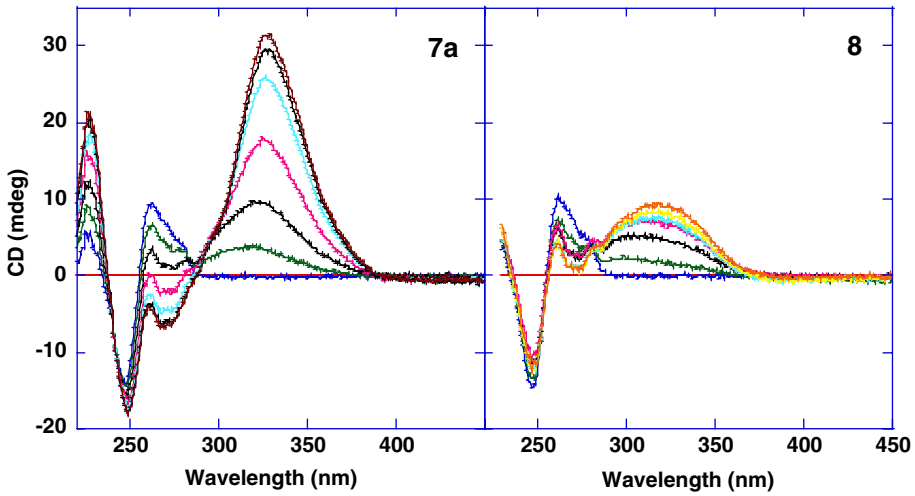
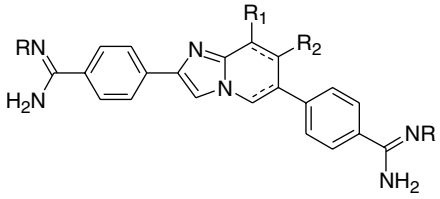


Figure 1. Induced CD signals for **7a** and **8** with the polyd(A-T)$_2$ DNA duplex in MES 10 buffer at 25 °C. Molar ratios of compounds to DNA base pairs are 0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 for **7a**, and 0.10, 0.20, 0.30, 0.40, 0.50, and 0.60 for **8**.

however they are not quite as potent as furamidine (IC_{50} = 4.3 nM). The activity of **7a–d** and **8** against *P. f.* is reduced from that against *T. b. r.* as the IC_{50} values range from 23 to 92 nM. These compounds are also less active against *P. f.* than furamidine (IC_{50} = 15.5 nM). As anticipated, the activities of the prodrugs in the in vitro screens were low. The selectivity indices for **7a–d** and **8** are quite reasonable ranging from 194 to 635 based on their cytotoxicity to rat L-6 myoblast cells. Based upon these results the compounds were advanced to an animal model for *T. b. r.*

Table 2 contains the results for evaluation of these compounds in the STIB900 model for acute African trypanosomasis. To select and discriminate the compounds with superior activity over the first generation compound furamidine, it was decided to reduce the standard screening dose for the diamidines to 5 mg/kg ip and 25 mg/kg po for prodrugs. This new condition makes the model more stringent and enables better discrimination among active compounds. On intraperitoneal dosing at 5 mg/kg all of the diamidines, **7a–d** and **8**, extend the survival time of the treated animals 5 days

Table 2. In vivo anti-trypanosomal activity of imidazo[1,2-*a*]pyridines and 5,6,7,8-tetrahydro-imidazo[1,2-*a*]pyridine analogues in the STIB900 mouse model[a]



Code	Pyridine ring	R	R_1	R_2	Dosage route	Dosage[b] (mg/kg)	Cures[c]	Survival (days)[d]
IIa	NA	NA	NA	NA	ip	5	0/4	35.5
IIb	NA	NA	NA	NA	po	25	1/4	>60
IIc	NA	NA	NA	NA	ip	5	3/4	>54.5
IId	NA	NA	NA	NA	po	25	4/4	>60
						5	4/4	>60
8	Saturated	H	H	H	ip	20	4/4	>60
						5	0/4	31.25
7a	Unsaturated	H	H	H	ip	20	0/4	22
6a	Unsaturated	OMe	H	H	po	25	0/4	10.75
7b	Unsaturated	H	CH_3	H	ip	5	0/4	14.75
6b	Unsaturated	OMe	CH_3	H	po	25	1/4	>20.25
7c	Unsaturated	H	H	CH_3	ip	5	1/4	>37.25
6c	Unsaturated	OMe	H	CH_3	po	25	0/4	14.5
7d	Unsaturated	H	CH_3	CH_3	ip	5	1/4	>39.5
6d	Unsaturated	OMe	CH_3	CH_3	po	25	0/4	15.5

[a] See Ref. 22 for details of STIB900 model.
[b] Dosage was for 4 days; ip, intraperitoneal; po, oral.
[c] Number of mice that survive and are parasite free for 60 days.
[d] Average days of survival; untreated control animals expire between day 7 and 9 post-infection.

or more beyond that of untreated controls. Compounds **7c** and **7d** provide 1/4 cures at a dose of 5 mg/kg. Thus, these two compounds are more effective in vivo than furamidine which at this dosage provided no cures, but not as effective as the azafuramidine **IIc** which yields 3/4 cures at the same dose. Compound **8** at a dosage of 20 mg/kg gave 4/4 cures, whereas **7a** at the same dose failed to provide cures. Only one of the prodrugs **6b** provided 1/4 cures at the standard screening dose of 25 mg/kg po. This result is comparable to that of pafuramidine (**IIb**) which also gives 1/4 cures at this dosage but was inferior to the prodrug of an azafuramidine **IId** which provides 4/4 cures at both the screening dose and remarkably at the low dose of 5 mg/kg po.

The strategy to replace the furan linker with the larger imidazo[1,2-*a*]pyridine unit worked quite well as judged by the high DNA affinities observed and by the fact that these molecules were found to be effective antiprotozoal agents on both in vitro and in vivo evaluation. The observed DNA affinities for **7a**–**d** are superior to that of furamidine and their antiprotozoal activity is comparable to that of furamidine. These results clearly suggest that other linkers larger than single 5- or 6-ring systems merit careful exploration.

3. Experimental

3.1. Biology

3.2. Efficacy evaluations

In vitro assays with *T. b. r*. STIB 900 and *P. f*. K1 strain as well as the efficacy study in an acute mouse model for *T. b. r*. STIB 900 were carried out as previously reported.[22]

3.3. T_m measurements

Thermal melting experiments were conducted with a Cary 300 spectrophotometer. Cuvettes for the experiment are mounted in a thermal block and the solution temperatures are monitored by a thermistor in the reference cuvette. Temperatures were maintained under computer control and are increased at 0.5 °C/min. The experiments were conducted in 1 cm path length quartz curvettes in CAC 10 buffer (cacodylic acid 10 mM, EDTA 1 mM, NaCl 100 mM with NaOH added to give pH 7.0). The concentrations of DNA were determined by measuring the absorbance at 260 nm. A ratio of 0.3 mole compound per mole of DNA was used for the complex and DNA with no compound was used as a control.[23]

3.4. Circular dichroism (CD)

CD spectra were collected with a Jasco J-810 spectrometer at different ratios of compound to DNA at 25 °C in MES 10 buffer. A DNA solution in a 1-cm quartz cuvette was first scanned over a desired wavelength range. Compounds **7a** and **8** at increasing ratios were then titrated into the same cuvette and the complexes rescanned under same conditions. Changes in CD spectra as a function of added compound concentration were used to approximate K_d values for binding by using published methods.[24]

3.5. Chemistry

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^{1}H and ^{13}C NMR spectra were recorded employing a Varian Unity Plus 300 spectrometer (Varian, Inc., Palo Alto, California), and chemical shifts (δ) are in ppm relative to TMS as the internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (VG Analytical, Ltd., Manchester, United Kingdom). Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ±0.4 of the theoretical values. The compounds reported as salts were frequently analyzed correctly for fractional moles of water and/or ethanol of solvation. In each case, proton NMR showed the presence of indicated solvent(s). All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, Frontier Scientific or Lancaster Synthesis, Inc.

3.5.1. 4-(6-Bromo-imidazo[1,2-*a*]pyridin-2-yl)-benzonitrile (3a). A mixture of 2-amino-5-bromopyridine (1.73 g, 10 mmol) and 4-cyanophenacyl bromide (2.24 g, 10 mmol) in ethanol (50 mL) was heated at reflux for 24 h. The precipitated salt was filtered, suspended in water, and neutralized with aqueous $NaHCO_3$ solution. The free base precipitate was filtered and dried to furnish **3a** in 62% yield, mp 218–219 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.41 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 8.15 (d, J = 8.4 Hz, 2H), 8.53 (s, 1H), 8.92 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 143.5, 143.0, 137.9, 132.7, 128.5, 127.1, 126.1, 118.8, 117.9, 111.3, 110.0, 106.4. EIMS (*m*/*z*, rel. int.); 298 (M^+, 100). Anal. Calcd for $C_{14}H_8BrN_3$: C, 56.40; H, 2.70. Found C, 56.33; H, 2.74.

3.5.2. 2,6-Bis(4-cyanophenyl)-imidazo[1,2-*a*]pyridine (4a). To a stirred solution of **3a** (2.98 g, 10 mmol) and tetrakis(triphenylphosphine) palladium (350 mg) in toluene (20 mL) under a nitrogen atmosphere was added 10 mL of a 2 M aqueous solution of Na_2CO_3 followed by 4-cyanophenylboronic acid (1.75 g, 12 mmol) in 10 mL of methanol. The vigorously stirred mixture was warmed to 80 °C for 24 h, then cooled, and the precipitate was filtered. The precipitate was partitioned between methylene chloride (500 mL) and 2 M aqueous Na_2CO_3 (50 mL) containing 6 mL of concentrated ammonia. The organic layer was dried (Na_2SO_4) and then concentrated to dryness under reduced pressure to afford **4a** in 69% yield, mp 292–294 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.70 (s, 2H), 7.88 (d, J = 7.8 Hz, 2H), 7.94 (s, 4H), 8.17 (d, J = 7.8 Hz, 2H), 8.55 (s, 1H), 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 144.4,

143.2, 140.9, 138.0, 132.8, 132.6, 127.2, 126.1, 125.4, 125.0, 123.7, 118.8, 118.5, 117.0, 111.6, 110.2, 109.9. EIMS (*m*/*z*, rel. int.); 320 (M^+, 100), 293 (5), 191 (3), 179 (10), 160 (15). HRMS calcd for $C_{21}H_{12}N_4$: 320.10620. Observed: 320.10640. Anal. Calcd for $C_{21}H_{12}N_4$: C, 78.73; H, 3.78. Found C, 78.46; H, 3.93.

3.5.3. 2,6-Bis[4-(*N*-hydroxyamidinophenyl)]-imidazo[1,2-*a*]pyridine (5a). A mixture of hydroxylamine hydrochloride (5.2 g, 75 mmol) in anhydrous DMSO (40 mL) was cooled to 5 °C under nitrogen and potassium *t*-butoxide (8.4 g, 75 mmol) was added in portions. The mixture was stirred for 30 min. This mixture was added to dinitrile **4a** (2.40 g, 7.5 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then poured slowly onto ice-water. The precipitate was filtered and washed with water to afford **5a** (free base) in 95% yield, mp 251–253 °C. ^{1}H NMR (DMSO-d_6); δ 5.88 (s, 2H), 5.91 (s, 2H), 7.67 (s, 2H), 7.75–7.83 (m, 6H), 8.00 (d, J = 8.4 Hz, 2H), 8.44 (s, 1H), 8.94 (s, 1H), 9.70 (s, 1H), 9.73 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 150.6, 150.3, 144.4, 144.2, 136.8, 134.1, 132.55, 132.50, 126.0, 125.9, 125.7, 125.2, 124.8, 124.6, 124.0, 116.6, 109.8.

3.5.4. 2,6-Bis[4-(*N*-methoxyamidinophenyl)]-imidazo[1,2-*a*]pyridine (6a). To a suspension of the amidoxime **5a** (386 mg, 1 mmol) in DMF (10 mL) was added LiOH·H_2O (252 mg, 6 mmol, in 3 mL H_2O) which was followed by dimethylsulfate (630 mg, 5 mmol). The reaction mixture was stirred overnight after which it was poured onto ice/water and the precipitate was filtered, washed with water, and chromatographed on silica gel using hexanes/EtOAc (50:50) to give compound **6a** in 73% yield, mp 192–194 °C. ^{1}H NMR (DMSO-d_6); δ 3.75 (s, 3H), 3.76 (s, 3H), 6.08 (br s, 2H), 6.13 (br s, 2H), 7.64–7.66 (m, 2H), 7.73–7.81 (m, 6H), 7.98 (d, J = 7.8 Hz, 2H), 8.43 (s, 1H), 8.92 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 150.7, 150.5, 144.3, 144.1, 137.2, 134.5, 131.6, 131.5, 126.1, 125.9, 125.8, 125.0, 124.6, 124.4, 123.8, 116.4, 109.7, 60.4, 60.3. *Hydrochloride salt of* **6a**. Mp 236–238 °C, ^{1}H NMR (D_2O/DMSO-d_6); δ 3.83 (s, 3H), 3.84 (s, 3H), 7.91–7.94 (m, 6H), 8.03 (d, J = 8.7 Hz, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.28 (d, J = 8.7 Hz, 1H), 8.84 (s, 1H), 9.34 (s, 1H). Anal. Calcd for $C_{23}H_{22}N_6O_2$–3.0HCl–0.25H_2O–0. 25C_2H_5OH: C, 52.28; H, 5.04; N, 15.56. Found C, 52.51; H, 4.98; N, 15.25.

3.5.5. 2,6-Bis[4-(amidinophenyl)]-imidazo[1,2-*a*]pyridine acetate salt (7a). To a solution of **5a** (386 mg, 1 mmol) in glacial acetic acid (10 mL) was slowly added acetic anhydride (0.35 mL). After stirring for overnight TLC indicated complete consumption of the starting material, then the solvent was evaporated under reduced pressure. To the formed product (the product of acylation step) in a mixture of ethanol/EtOAc (50 mL, 1:1) was added 10% palladium on carbon (80 mg), then the mixture was placed on Parr hydrogenation apparatus at 50 psi for 4 h at room temperature. The reaction mixture was filtered through hyflo and the filter pad washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **7a** in 76% yield mp 259–261 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.97 (s, 3× CH_3), 7.67 (s, 2H), 7.77–7.88 (m, 6H), 8.07 (d, J = 8.4 Hz, 2H), 8.48 (s, 1H), 8.94 (s, 1H). Anal. Calcd for $C_{21}H_{18}N_6$–3.0AcOH–1.9H_2O: C, 57.01; H, 5.98; N, 14.78. Found: C, 56.68; H, 5.98; N, 15.02.

3.5.6. 2,6-Bis[4-(amidinophenyl)]-5,6,7,8-tetrahydro-imidazo[1,2-*a*]pyridine acetate salt (8). To a solution of **5a** (386 mg, 1 mmol) in glacial acetic acid (10 mL) was slowly added acetic anhydride (0.35 mL). After stirring for overnight TLC indicated complete consumption of the starting material, then the solvent was evaporated under reduced pressure. To the formed product (the product of acylation step) in glacial acetic acid (20 mL) was added 10% palladium on carbon (80 mg), then the mixture was placed on Parr hydrogenation apparatus at 50 psi for 6 h at room temperature. The reaction mixture was filtered through hyflo and the filter pad washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **8** in 84% yield, mp 234–236 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.75 (s, 3× CH_3), 2.15 (br s, 3H), 2.95 (br s, 2H), 4.09 (br s, 1H), 4.27 (br s, 1H), 7.62 (m, 3H), 7.77–7.82 (m, 4H), 7.89–7.92 (m, 2H). Anal. Calcd for $C_{21}H_{22}N_6$–3.0AcOH–1.9H_2O: C, 56.61; H, 6.65; N, 14.67. Found: C, 56.49; H, 6.30; N, 14.58.

Free base of **8**. Mp 254–255 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 2.13–2.20 (m, 3H), 2.91 (m, 1H), 3.34 (br s, 1H), 4.03 (m, 1H), 4.27 (m, 1H), 7.62 (m, 3H), 7.77–7.82 (m, 4H), 7.89–7.92 (m, 2H). ^{13}C NMR (DMSO-d_6); δ 162.3, 144.2, 143.6, 138.7, 136.2, 126.8, 126.7, 123.4, 115.4, 99.4, 49.7, 27.6, 23.7. FABMS (*m*/*z*, rel. int., thioglycerol); 359 (M^++1, 75), 324 (100), 291(5), 273 (15), 237 (20). HRMS calcd for $C_{21}H_{23}N_6$: 359.1984. Observed: 359.1980.

3.5.7. 2-(4-Cyanophenyl)-6-bromo-8-methyl-imidazo[1,2-*a*]pyridine (3b). The same procedure described for **3a** was used employing 4-cyanophenacyl bromide and 2-amino-3-methyl-5-bromopyridine. Yield 71%, mp 209–210 °C. ^{1}H NMR (DMSO-d_6); δ 2.56 (s, 3H), 7.53 (s, 1H), 7.95 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 8.1 Hz, 2H), 8.60 (s, 1H), 8.88 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 143.3, 140.4, 133.3, 133.0, 128.1, 127.4, 127.0, 125.9, 119.2, 112.8, 111.2, 108.2, 16.7. MS (ESI) *m*/*e* (rel. int.); 312 (M^+, 10), 230 (100), 217 (60), 205 (50). Anal. Calcd for $C_{15}H_{10}BrN_3$: C, 57.71; H, 3.23. Found C, 57.93; H, 3.35.

3.5.8. 2,6-Bis(4-cyanophenyl)-8-methyl-imidazo[1,2-*a*]pyridine (4b). The same procedure described for **4a** was used starting with **3b**. Yield 73%, mp 283–285 °C. ^{1}H NMR (DMSO-d_6); δ 2.60 (s, 3H), 7.53 (s, 1H), 7.85–7.93 (m, 6H), 8.17 (d, J = 8.4 Hz, 2H), 8.53 (s, 1H), 8.87 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 144.9, 142.5, 141.1, 138.1, 132.6, 132.4, 127.0, 126.6, 126.0, 123.6, 123.3, 122.9, 118.6, 118.4, 111.9, 110.0, 109.7, 16.3. MS (ESI) *m*/*e* (rel. int.); 335 (M^++1, 100), 310 (30), 279 (20), 234 (40). Anal. Calcd for $C_{22}H_{14}N_4$: C, 79.02; H, 4.22. Found C, 78.68; H, 4.45.

3.5.9. 2,6-Bis[4-(*N*-hydroxyamidino-phenyl)]-8-methyl-imidazo[1,2-*a*]pyridine (5b). The same procedure described for **5a** was used starting with **4b**. Yield 92%, mp >300 °C. ^{1}H NMR (DMSO-d_6); δ 2.60 (s, 3H), 6.22 (s, 2H), 6.40 (s, 2H), 7.51 (s, 1H), 7.76–7.90 (m, 6H), 8.03 (d, J = 8.1 Hz, 2H), 8.44 (s, 1H), 8.80 (s, 1H), 9.93 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 152.0, 151.2, 144.7, 143.5, 137.5, 134.8, 131.6, 131.1, 126.2, 126.1, 125.3, 124.6, 123.5, 121.9, 110.5, 16.8. MS (ESI) *m/e* (rel. int.); 401 (M^++1, 75), 339 (20), 266 (10), 201 (100).

3.5.10. 2,6-Bis[4-(*N*-methoxyamidinophenyl)]-8-methyl-imidazo[1,2-*a*]pyridine (6b). The same procedure described for **6a** was used starting with **5b**, the crude product was chromatographed on silica gel eluting with hexanes/EtOAc (30:70). *Hydrochloride salt of* **6b**. Mp 286–288 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 2.80 (s, 3H), 3.85, 3.86 (2s, 6H), 7.93–7.98 (m, 6H), 8.18 (s, 1H), 8.32 (d, J = 8.4 Hz, 2H), 8.86 (s, 1H), 9.24 (s, 1H). ^{13}C NMR (D_2O/DMSO-d_6); δ 158.1, 157.0, 141.0, 139.6, 135.8, 132.3, 131.0, 129.2, 128.9, 128.6, 128.0, 127.6, 126.7, 124.8, 124.1, 113.8, 64.0, 63.7, 17.1. MS (ESI) *m/e* (rel. int.); 429 (M^++1, 60), 240 (55), 215 (100). HRMS calcd for $C_{24}H_{25}N_6O_2$: 429.2039. Observed: 429.2043. Anal. Calcd for $C_{24}H_{24}N_6O_2$–3.0HCl–1.5H_2O: C, 51.02; H, 5.35; N, 14.87. Found: C, 50.92; H, 5.25; N, 14.50.

3.5.11. 2,6-Bis[4-(amidinophenyl)]-8-methyl-imidazo[1,2-*a*]pyridine acetate salt (7b). The same procedure described for **7a** was used starting with **5b**. Yield 81%, mp 241–243 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.88 (s, 3× CH_3), 2.63 (s, 3H), 7.53 (s, 1H), 7.89–8.00 (m, 5H), 8.07–8.18 (m, 3H), 8.53 (s, 1H), 8.86 (s, 1H). Anal. Calcd for $C_{22}H_{20}N_6$–3.0AcOH–1.7H_2O: C, 58.06; H, 6.16; N, 14.51. Found: C, 57.90; H, 5.93; N, 14.76.

3.5.12. 2-(4-Cyanophenyl)-6-bromo-7-methyl-imidazo[1,2-*a*]pyridine (3c). The same procedure described for **3a** was used employing 4-cyanophenacyl bromide and 2-amino-4-methyl-5-bromopyridine. Yield 56%, mp 253–255 °C. ^{1}H NMR (DMSO-d_6); δ 2.49 (s, 3H), 7.61 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 8.16 (d, J = 8.4 Hz, 2H), 8.42 (s, 1H), 8.88 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 144.1, 142.7, 137.8, 134.7, 132.1, 126.4, 125.8, 118.3, 115.6, 110.0, 109.9, 109.6, 21.3. MS (ESI) *m/e* (rel. int.); 312 (M^+, 100), 293 (10). Anal. Calcd for $C_{15}H_{10}BrN_3$: C, 57.71; H, 3.23. Found: C, 57.94; H, 3.11.

3.5.13. 2,6-Bis(4-cyanophenyl)-7-methyl-imidazo[1,2-*a*]pyridine (4c). The same procedure described for **4a** was used starting with **3c**. Yield 94%, mp 220–222 °C. ^{1}H NMR (DMSO-d_6); δ 2.21 (s, 3H), 7.53 (s, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 8.1 Hz, 2H), 8.12 (d, J = 8.4 Hz, 2H), 8.45–8.47 (m, 2H). ^{13}C NMR (DMSO-d_6); δ 144.9, 142.8, 141.8, 138.4, 134.5, 132.7, 132.3, 130.7, 126.9, 126.1, 125.9, 119.0, 118.7, 116.1, 110.8, 110.6, 109.7, 20.2. MS (ESI) *m/e* (rel. int.); 335 (M^++1, 100), 241 (5). Anal. Calcd for $C_{22}H_{14}N_4$: C, 79.02; H, 4.22. Found: C, 79.18; H, 4.13.

3.5.14. 2,6-Bis[4-(*N*-hydroxyamidino-phenyl)]-7-methyl-imidazo[1,2-*a*]pyridine (5c). The same procedure described for **5a** was used starting with **4c**. Yield 93%, mp 187–189 °C. ^{1}H NMR (DMSO-d_6); δ 2.24 (s, 3H), 5.81 (s, 2H), 5.86 (s, 2H), 7.43–7.49 (m, 3H), 7.71–7.78 (m, 4H), 7.93 (d, J = 8.4 Hz, 2H), 8.32 (s, 1H), 8.40 (s, 1H), 9.66 (s, 1H), 9.71 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 150.8, 150.7, 144.8, 144.1, 137.6, 134.5, 134.4, 132.7, 132.5, 129.4, 127.6, 125.8, 125.5, 125.3, 115.7, 109.0, 20.5. MS (ESI) *m/e* (rel. int.); 401 (M^++1, 100), 386 (15).

3.5.15. 2,6-Bis[4-(*N*-methoxyamidinophenyl)]-7-methyl-imidazo[1,2-*a*]pyridine (6c). The same procedure described for **6a** was used starting with **5c**, the crude product was chromatographed on silica gel eluting with hexanes/EtOAc (30:70). Yield 85%, mp 112–114 °C. ^{1}H NMR (DMSO-d_6); δ 2.24 (s, 3H), 3.75, 3.76 (2s, 6H), 6.05 (s, 2H), 6.12 (s, 2H), 7.44–7.49 (m, 3H), 7.71–7.77 (m, 4H), 7.94 (d, J = 8.4 Hz, 2H), 8.34 (s, 1H), 8.40 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 150.7, 150.6, 144.5, 143.9, 137.7, 134.7, 133.9, 131.6, 131.3, 128.9, 127.2, 125.7, 125.5, 124.9, 115.4, 108.7, 60.3, 19.9. *Hydrochloride salt of* **6c**. Mp 133–135 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 2.42 (s, 3H), 3.83, 3.85 (2s, 6H), 7.65 (d, J = 8.1 Hz, 2H), 7.91–7.95 (m, 5H), 8.21 (d, J = 8.1 Hz, 2H), 8.85 (s, 1H), 8.87 (s, 1H). MS (ESI) *m/e* (rel. int.); 429 (M^++1, 100). HRMS calcd for $C_{24}H_{25}N_6O_2$: 429.2039. Observed: 429.2026. Anal. Calcd for $C_{24}H_{24}N_6O_2$–3.0HCl–2.8H_2O–0. 75C_2H_5OH: C, 49.17; H, 6.00; N, 13.49. Found: C, 49.53; H, 5.95; N, 13.11.

3.5.16. 2,6-Bis[4-(amidinophenyl)]-7-methyl-imidazo[1,2-*a*]pyridine acetate salt (7c). The same procedure described for **7a** was used starting with **5c**. Mp 241–243 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.72 (s, 3× CH_3), 2.26 (s, 3H), 7.57 (s, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.85–7.92 (m, 4H), 8.14 (d, J = 8.4 Hz, 2H), 8.48 (s, 1H), 8.49 (s, 1H). MS (ESI) *m/e* (rel. int.); 369 (M^+, 100). HRMS calcd for $C_{22}H_{21}N_6$: 369.1828. Observed: 369.1826. Anal. Calcd for $C_{22}H_{20}N_6$-3.0AcOH-0.5H_2O: C, 60.31; H, 5.96; N, 15.07. Found: C, 60.22; H, 5.86; N, 15.08.

3.5.17. 2-(4-Cyanophenyl)-6-bromo-7,8-dimethyl-imidazo[1,2-*a*]pyridine (3d). The same procedure described for **3a** was used employing 4-cyanophenacyl bromide and 2-amino-3,4-dimethyl-5-bromopyridine. Yield 64%, mp 210–212 °C. ^{1}H NMR (DMSO-d_6); δ 2.35 (s, 3H), 2.56 (s, 3H), 7.87 (d, J = 8.1 Hz, 2H), 8.11 (d, J = 8.1 Hz, 2H), 8.41 (s, 1H), 8.80 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 144.6, 141.6, 137.6, 132.7, 131.7, 126.1, 124.5, 124.1, 118.9, 111.7, 111.0, 109.9, 18.4, 14.1. MS (ESI) *m/e* (rel. int.); 326 (M^+, 100), 241 (10). Anal. Calcd for $C_{16}H_{12}BrN_3$: C, 58.91; H, 3.71. Found: C, 58.73; H, 4.02.

3.5.18. 2,6-Bis(4-cyanophenyl)-7,8-dimethyl-imidazo[1,2-*a*]pyridine (4d). The same procedure described for **4a** was used starting with **3d**. Yield 84%, mp 229–231 °C. ^{1}H NMR (DMSO-d_6); δ 2.12 (s, 3H), 2.57 (s, 3H), 7.61 (d, J = 8.1 Hz, 2H), 7.84 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 8.1 Hz, 2H), 8.31 (s, 1H),

8.42 (s, 1H). MS (ESI) *m/e* (rel. int.); 349 (M^++1, 100). Anal. Calcd for $C_{23}H_{16}N_4$: C, 79.29; H, 4.63. Found: C, 78.96; H, 4.81.

3.5.19. 2,6-Bis[4-(*N*-hydroxyamidinophenyl)]-7,8-dimethylimidazo[1,2-*a*]pyridine (5d). The same procedure described for **5a** was used starting with **4d**. Yield 94%, mp 180–181.5 °C. ^{1}H NMR (DMSO-d_6); δ 2.14 (s, 3H), 2.56 (s, 3H), 5.85 (s, 2H), 5.90 (s, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.72–7.79 (m, 4H), 7.97 (d, J = 8.1 Hz, 2H), 8.26 (s, 1H), 8.31 (s, 1H), 9.65 (s, 1H), 9.71 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 150.7, 150.5, 145.2, 143.4, 138.3, 134.5, 132.4, 132.2, 130.1, 129.4, 127.9, 125.6, 125.3, 125.1, 123.1, 122.6, 109.3, 16.4, 13.5. MS (ESI) *m/e* (rel. int.); 415 (M^++1, 100), 281 (25), 208 (52). HRMS calcd for $C_{23}H_{23}N_6O_2$: 415.1882. Observed: 415.1873.

3.5.20. 2,6-Bis[4-(*N*-methoxyamidinophenyl)]-7,8-dimethylimidazo[1,2-*a*]pyridine (6d). The same procedure described for **6a** was used starting with **5d**. Yield 59%, mp 140–141 °C. ^{1}H NMR (DMSO-d_6); δ 2.14 (s, 3H), 2.57 (s, 3H), 3.75 (s, 6H), 6.06 (s, 2H), 6.13 (s, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.71–7.77 (m, 4H), 7.98 (d, J = 8.4 Hz, 2H), 8.26 (s, 1H), 8.33 (s, 1H). *Hydrochloride salt of* **6d**. Mp 230–232 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 2.26 (s, 3H), 2.64 (s, 3H), 3.82, 3.84 (2s, 6H), 7.62 (d, J = 8.1 Hz, 2H), 7.83–7.88 (m, 4H), 8.12 (d, J = 8.1 Hz, 2H), 8.66 (s, 1H), 8.70 (s, 1H) ^{13}C NMR (D_2O/DMSO-d_6); δ 157.4, 155.7, 142.0, 140.6, 140.3, 135.1, 132.2, 130.8, 130.0, 129.8, 128.5, 128.4, 127.4, 125.5, 121.0, 112.9, 63.7, 63.2, 17.6, 14.1. MS (ESI) *m/e* (rel. int.); 443 (M^++1, 100), 295 (32). HRMS calcd for $C_{25}H_{27}N_6O_2$: 443.2195. Observed: 443.2195. Anal. Calcd for $C_{25}H_{26}N_6O_2$–3.0HCl–2.75H_2O–0.25C_2H_5OH: C, 49.97; H, 5.91; N, 13.71. Found: C, 49.99; H, 5.84; N, 13.42.

3.5.21. 2,6-Bis[4-(amidinophenyl)]-7,8-dimethyl-imidazo[1,2-*a*]pyridine acetate salt (7d). The same procedure described for **7a** was used starting with **5d**. Mp 260–262 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.71 (s, 2.8× CH_3), 2.14 (s, 3H), 2.55 (s, 3H), 7.58 (d, J = 8.4 Hz, 2H), 7.84 (m, 4H), 8.12 (d, J = 8.4 Hz, 2H), 8.26 (s, 1H), 8.38 (s, 1H). MS (ESI) *m/e* (rel. int.); 383 (M^++1, 100). HRMS calcd for $C_{23}H_{23}N_6$: 383.1984. Observed: 383.2001. Anal. Calcd for $C_{23}H_{22}N_6$–2.8AcOH: C, 62.38; H, 6.07; N, 15.26. Found: C, 62.29; H, 5.97; N, 15.33.

Acknowledgments

We thank Guy Riccio of STI for the technical assistance with the *T. b. r.* animal model. This work was supported by the Bill and Melinda Gates Foundation and by NIH Grants GM61587 and AI46365.

References and notes

1. (a) Tidwell, R. R.; Boykin, D. W. Dicationic DNA Minor Groove Binders as Antimicrobial Agents. In *Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes*; Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Wiley-VCH: New York, 2003; Vol. 2, pp 414–460; (b) Wilson, W. D.; Nguyen, B.; Tanious, F. A.; Mathis, A.; Hall, J. E.; Stephens, C. E.; Boykin, D. W. *Curr. Med. Chem.-Anti-Cancer Agents* **2005**, *5*, 389; (c) Soeiro, M. N. C.; de Souza, E. M.; Stephens, C. E.; Boykin, D. W. *Expert Opin. Invest. Drugs* **2005**, *14*, 957; (d) Dardonville, C. *Expert Ther. Pat.* **2005**, *15*, 1241; (e) Werbovetz, K. A. *Curr. Opin. Invest. Drugs* **2006**, *7*, 147.
2. (a) Fairlamb, A. H. *Trends Parasitol.* **2003**, *19*, 488; (b) Bouteille, B.; Oukem, O.; Bisser, S.; Dumas, M. *Fundam. Clin. Pharmacol.* **2003**, *17*, 171; (c) Yeramian, P. D.; Castagnini, L. A.; Allen, J. A.; Umesh, L.; Gotuzzo, E. Efficacy and Safety of DB289, A New Oral Drug for Treatment of *Pneumocystis carinii* Pneumonia (PCP) in AIDS Patients. Presented at the 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Meeting, September 14–17, 2003, Chicago, IL.
3. Ansede, J. H.; Voyksner, R. D.; Ismail, M. A.; Boykin, D. W.; Tidwell, R. R.; Hall, J. E. *Xenobiotica* **2005**, *35*, 211.
4. Boykin, D. W.; Kumar, A.; Spychala, J.; Zhou, M.; Lombardy, R.; Wilson, W. D.; Dykstra, C. C.; Jones, S. K.; Hall, J. E.; Tidwell, R. R.; Laughton, C.; Nunn, C. M.; Neidle, S. *J. Med. Chem.* **1995**, *38*, 912.
5. Boykin, D. W.; Kumar, A.; Xiao, G.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R. *J. Med. Chem.* **1998**, *41*, 124.
6. Francesconi, I.; Wilson, W. D.; Tanious, F. A., et al. *J. Med. Chem.* **1999**, *42*, 2260.
7. Laughton, C. A.; Tanious, F. A.; Nunn, C. M.; Boykin, D. W.; Wilson, W. D.; Neidle, S. *Biochemistry* **1996**, *35*, 5655.
8. Trent, J. O.; Clark, G. R.; Kumar, A.; Wilson, W. D.; Boykin, D. W.; Hall, J. E.; Tidwell, R. R.; Blagburn, B. L.; Neidle, S. *J. Med. Chem.* **1996**, *39*, 4554.
9. Wilson, W. D.; Tanious, F. A.; Ding, D.; Kumar, A.; Boykin, D. W.; Colson, P.; Houssier, C.; Bailly, C. *J. Am. Chem. Soc.* **1998**, *120*, 10310.
10. Bailly, C.; Dassonneville, L.; Carrascol, C.; Lucasl, D.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *Anti-Cancer Drug Des.* **1999**, *14*, 47.
11. Fitzgerald, D. J.; Anderson, J. N. *J. Biol. Chem.* **1999**, *274*, 27128.
12. Dykstra, C. C.; McClernon, D. R.; Elwell, L. P.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1994**, *38*, 1890.
13. Henderson, D.; Hurley, L. H. *Nature Med.* **1995**, *1*, 525.
14. (a) Lanteri, C. A.; Stewart, M. L.; Brock, J. M.; Alibu, V. P.; Meshnick, S. R.; Tidwell, R. R. *Mol. Pharmacol.* **2006**, *70*, 1585; (b) Mathis, A. M.; Holman, J. L.; Sturk, L. M.; Ismail, M. A.; Boykin, D. W.; Tidwell, R. R.; Hall, J. E. *Antimicrob. Agents Chemother* **2006**, *50*, 2185.
15. Nguyen, N.; Boykin, D. W.; Wilson, W. D. DNA Minor Groove Interactions of Antiparasitic Diamidines: Re-evaluation of the Crescent-Shape Concept in Groove-Binding. In *Synthetic and Biophysical Studies of DNA Binding Compounds*. Lee, M., Strekows, L., Eds.; Transworld Research Network: Trivandrum, India, 2007; Chapter 2.
16. Hamdouchi, C.; Blas, J.; Prado, M.; Gruber, J.; Heinz, B. A.; Vance, L. *J. Med. Chem.* **1999**, *42*, 50.
17. (a) Hamama, W. S.; Zoorob, H. H. *Tetrahedron* **2002**, *58*, 6143; (b) Sundberg, R.; Biswas, S.; Murthi, K. K.; Rowe, D. *J. Med. Chem.* **1998**, *41*, 4317.
18. Ismail, M. A.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2004**, *47*, 3658.
19. Skupinska, K. A.; McEachern, E. J.; Skerij, R. T.; Bridger, G. J. *J. Org. Chem.* **2002**, *67*, 7890.

20. Rodger, A.; Nordén, B. *Circular Dichroism and Linear Dichroism*; Oxford University Press: New York, 1997.
21. Lyng, R.; Rodger, A.; Norden, B. *Biopolymers* **1992**, *32*, 1201–1214.
22. Ismail, M. A.; Brun, R.; Easterbrook, J. D.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2003**, *46*, 4761.
23. Wilson, W. D.; Tanious, F. A.; Fernandez-Saiz, M.; Rigl, C. T. Evaluation of Drug–Nucleic Acid Interactions by Thermal Melting Curves. In *Methods in Mol. Biol.: Drug–DNA Interaction Protocols*, K. Fox, Ed., 1997; pp 90, 219.
24. McCoubrey, A.; Latham, H. C.; Cook, P. R.; Rodger, A.; Lowe, G. *FEBS Lett.* **1996**, *380*, 73–78.