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Dicationic near-linear biphenyl benzimidazole derivatives as DNA-targeted antiprotozoal agents

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Abstract—A series of near-linear biphenyl benzimidazole diamidines **5a**–**h** were synthesized from their respective diamidoximes (**4a**–**h**), through the bis-*O*-acetoxyamidoxime, followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd–C. Compounds **4a**–**h** were obtained in three steps, starting with the Suzuki coupling reaction of the appropriate haloarylcarbonitriles **1a**–**g** or 4-bromo-2-fluorobenzaldehyde with 4-formylphenylboronic acid or 4-cyanophenylboronic acid to form the anticipated 4-formylbiphenyl carbonitrile analogues **2a**–**h**. Subsequent condensation of the formyl derivatives **2a**–**h** with 3,4-diaminobenzonitrile in the presence of sodium bisulfite or 1,4-benzoquinone gave the desired dinitriles **3a**–**h**, the precursors for **4a**–**h**. All the diamidines showed strong DNA affinities, as judged by high ΔT_m values with poly(dA.dT)$_2$. The compounds were quite active in vitro versus *Trypanosoma brucei rhodesiense*, giving IC_{50} values ranging from 3 to 37 nM. These compounds were even more active versus *Plasmodium falciparum*, exhibiting IC_{50} values ranging from 0.5 to 23 nM. The compounds showed moderate to good activity in vivo in the STIB900 model for acute African trypanosomiasis. The most active compounds **5b** and **e** gave 3/4 cures on an IP dosage of 20 mg/kg.

1. Introduction

The antimicrobial activity of aromatic diamidines was first reported in the 1930s.[1] Since that time, numerous dicationic systems have been investigated, with the major objective of discovering useful therapeutic agents. Despite these efforts, pentamidine (**I**), first reported in 1942,[2] is the only compound from this class that has seen significant clinical use. Currently, pentamidine is used against antimony-resistant leishmaniasis, primary stage human African trypanosomiasis (HAT), and for AIDS-related *Plasmodium jiroveci* pneumonia.[1] An orally effective prodrug of furamidine (**II**) is currently in Phase II clinical trials against malaria and pneumocystis pneumonia and scheduled for Phase III trails against HAT.[1,3–6] These dicationic molecules are thought to act by binding to the minor groove of DNA at AT-rich sites.[1] Minor groove binding has been suggested to cause inhibition of DNA-dependent enzymes or possibly even direct inhibition of transcription.[1,7–10] The selectivity of these molecules, at least for trypanosomes, likely includes a cell entry component involving amidine transporters[11] and may involve interaction with kinetoplast DNA.[1b] A key requirement in the designing of new minor groove binders has been that the molecular framework bearing the amidine units should present a crescent shape geometry complementary to the curve of the minor groove of DNA.[12] Van der Waals contacts with the walls of the groove have been shown to be an important contributor to binding affinity.[13–15] A recent theoretical analysis of the binding interactions of 25 minor groove binders has stressed the importance of small molecule curvature to provide energetically favorable Van der Waals contacts.[16] Pentamidine, furamidine, and many analogs answer this crescent shape requirement.[1,12,17,18] It has been suggested that high affinity minor groove binders either match the groove curve or easily assume low energy conformations that complement the groove on complex formation. Molecules that present too great or too small curvature are thought to lack significant contacts with the minor groove and thus exhibit reduced binding affinities.[12] Recently, we reported excellent DNA affinities and antiprotozoan activity of curved biphenyl benzimidazoles

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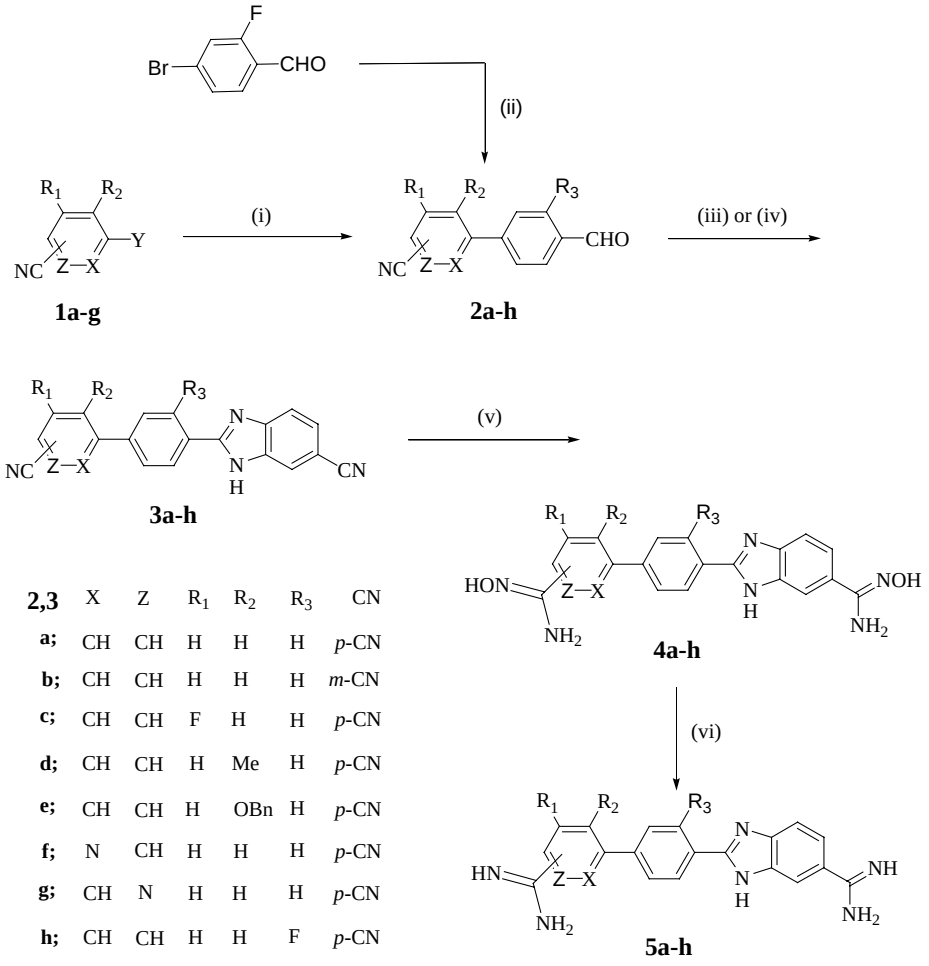
(**III**).[19] In addition to the study of curved molecules, recent reports have shown that the diamidine, CGP 40215A (**IV**), which has a near-linear linking framework, exhibits excellent anti-trypanosomal activity. Surprisingly, this linear compound also had strong minor groove binding affinity with AT base pair specificity.[20–22] Detailed analysis of binding data, crystal structure, and molecular dynamic simulations results has indicated that water-mediated interactions between CGP 40215A and the DNA minor groove in effect provide the needed curvature.[21,22] We reported about modest antimicrobial activity and strong DNA affinity for the linear dication 3,5-bis[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]pyridine.[23] In view of these cases of essentially linear dicationic molecules, which show promising antimicrobial activity and significant DNA minor groove affinity, as well as the promising properties of various benzimidazole aromatic diamidines,[19] we have undertaken a study of near-linear biphenyl benzimidazole dicationic systems. In this work, we have also replaced the terminal phenyl group with a pyridyl group. Such alterations in structure can potentially change the base pair recognition on DNA binding and yield different pharmacokinetic profiles. We report the synthesis of novel near-linear biphenyl benzimidazole dications and their initial evaluation as minor groove binders and anti-protozoan agents.

2. Results and discussion

2.1. Chemistry

Acetate salts of a series of benzimidazole aromatic diamidines **5a**–**h** were synthesized from their respective diamidoximes **4a**–**h**, through the corresponding bis-*O*-acetoxyamidoximes, followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd–C (Scheme 1). Compounds **4a**–**h** were obtained in three steps, starting with the Suzuki coupling reaction of the appropriate haloarylcarbonitriles **1a**–**g** or 4-bromo-2-fluorobenzaldehyde with 4-formylphenylboronic or 4-cyanophenylboronic acid to form the anticipated 4-formylbiphenyl carbonitrile analogues **2a**–**h**. Subsequent condensation of the formyl derivatives **2a**–**h** with 3,4-diaminobenzonitrile in the presence of an equimolar ratio of sodium bisulfite or 1,4-benzoquinone gave the desired dinitriles **3a**–**h**. The dinitriles were allowed to



2,3	X	Z	R_1	R_2	R_3	CN
a;	CH	CH	H	H	H	*p*-CN
b;	CH	CH	H	H	H	*m*-CN
c;	CH	CH	F	H	H	*p*-CN
d;	CH	CH	H	Me	H	*p*-CN
e;	CH	CH	H	OBn	H	*p*-CN
f;	N	CH	H	H	H	*p*-CN
g;	CH	N	H	H	H	*p*-CN
h;	CH	CH	H	H	F	*p*-CN

Scheme 1. Reagents and conditions: (i) 4-formylphenyl boronic acid, $Pd(PPh_3)_4$; (ii) 4-cyanolphenyl boronic acid, $Pd(PPh_3)_4$; (iii) 3,4-diaminobenzonitrile, sodium bisulfite, DMF, reflux; (iv) 3,4-diaminobenzonitrile, 1,4-benzoquinone, EtOH, reflux; (v) $NH_2OH{\cdot}HCl/KO$-*t*-Bu, DMSO; (vi) (a) $AcOH/Ac_2O$, (b) H_2/Pd–C, AcOH.

react at room temperature for 24 h with a mixture of hydroxylamine hydrochloride and potassium *tert*-butoxide in DMSO solution to furnish **4a**–**h** in excellent yield. Thus, we have described a straightforward methodology to obtain a variety of dicationic diphenylbenzimidazole derivatives for evaluation versus *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*).

2.2. Biology

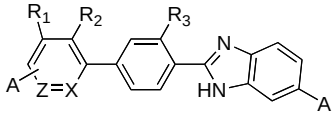
The DNA affinities and in vitro evaluation of the newly synthesized dicationic diphenylbenzimidazole derivatives against *T.b.r.* and *P.f.* are given in Table 1. The DNA affinities, as indicated by ΔT_m values for their complexes with polydA.polydT, are quite high with one exception. Based on the curvature arguments described above, it is quite surprising that these near-linear compounds have higher affinities for AT sequences in DNA than their curved isomers (compare values for **III** to **5a** and see Ref. 19). The drastic difference in the curvature of more 'classical' minor groove binding agents, such as DB75 (**II** in Fig. 1), and DB911 (**III** in Fig. 1), compared to DB921 (**5a**) can easily be seen in Fig. 2. The models in Fig. 2 are planar projections of ab initio energy-minimized, geometry-optimized structures for the three compounds and in the projections the curvature differences can easily be seen. The calculated torsion angles for **5a** are typical of the group of derivatives: approximately, 35–40° for the biphenyl junction, 10–15° for the benzimidazole-phenyl, and 35–40° for the amidine-aromatic angle. These angles are probably reduced when the compounds fit into the minor groove of DNA.

The one compound, **5d**, which shows a somewhat lower ΔT_m value than the other analogues in Table 1 has a methyl group located adjacent to the biphenyl ring junction. Energy minimization studies of the compound with and without the methyl group indicate a significantly larger torsion angle rotation for **5d**. Thus, it appears that such a torsion angle change is detrimental to minor groove binding in this system, as would be expected from the requirement that the aromatic system fit deeply into the minor groove to make favorable contacts with AT base pairs at the floor of the groove. Detailed DNA binding studies of these compounds have been initiated and preliminary results confirm the very strong binding and AT base pair specificity of the linear compounds. Based on the water-mediated interactions of **IV** (Fig. 1; CGP 40215A), we speculate that DB921 may also have an indirect, water-mediated interaction of the phenyl-amidine end of the molecule with AT base pairs. Such an interaction would provide a crescent shape to the compound–water system and could explain the strong DNA interactions of the linear compounds.

The IC_{50} values for the dicationic diphenylbenzimidazole derivatives against *T.b.r.* range from 3 to 37 nM. These near-linear analogs are generally more active in vitro against trypanosomes than their curved isomers (type III), which we recently reported.[19] These compounds were even more active in vitro versus *P.f.*, giving IC_{50} values ranging from 0.5 to 23 nM. Compound **5a**, with an IC_{50} value of 0.5 nM, is the most active dication against *P.f.*, which we have reported to date. There is quite good selectivity for the parasitic organisms by these dicationic compounds compared to their cytotoxicity for mammalian cells (see Table 1). The selectivity ratios range from near 522 to 34,000. Consequently, these compounds are quite promising candidates for animal model studies.

The results from studying these compounds in the STIB900 mouse model for acute African trypanosomasis are given in Table 2. All the near-linear molecules extend the life of the treated animals beyond that of the untreated controls. Four of the compounds **5b**–**e** provided one or more cures. Only compounds **5b** and **e** gave 3/4 cures, in contrast to the results that were obtained

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



Code	X	Z	R_1	R_2	R_3	A	ΔT_mpoly dA-dT[a]	IC_{50}		
								T.b.r.[b] (nM)	*P. f.*[b] (nM)	L6cells[c] (μM)
II	NA	NA	NA	NA	NA	*p*-(C=NH)NH_2	25	4.5	15.5	6.4
III	NA	NA	NA	NA	NA	*p*-(C=NH)NH_2	25.6	4.4	27.5	19.3
5a	CH	CH	H	H	H	*p*-(C=NH)NH_2	>28	10.6	0.5	17.0
5b	CH	CH	H	H	H	*m*-(C=NH)NH_2	>28	9.1	23	19.2
5c	CH	CH	F	H	H	*p*-(C=NH)NH_2	>27	37	1	21.9
5d	CH	CH	H	Me	H	*p*-(C=NH)NH_2	25.5	6	12.5	26.4
5e	CH	CH	H	OH	H	*p*-(C=NH)NH_2	~27–28	27	19.1	14.1
5f	N	CH	H	H	H	*p*-(C=NH)NH_2	~27–28	32	7.2	25.5
5g	CH	N	H	H	H	*p*-(C=NH)NH_2	>28	3	7	25.9
5h	CH	CH	H	H	F	*p*-(C=NH)NH_2	~27–28	8	1	30.8

[a] DNA: polydA.polydT; buffer MES10; compound/DNA ratio = 0.3. For compounds with ΔT_m values listed as >28 °C, no melting was observed at the highest temperature of the experiment, 96 °C. For compounds with ΔT_m values listed as ~27–28, the complex was beginning to melt at 96 °C but only part of the T_m curve could be obtained.

[b] The *T.b.r.* strain employed was STIB900 and the *P.f.* strain was K1; see Refs. 24 and 25. IC_{50} values are the average of duplicate determinations.

[c] Cytotoxicity was evaluated using cultured L-6 rat myoblast cells using the Alamar Blue assay, see Ref. 25.

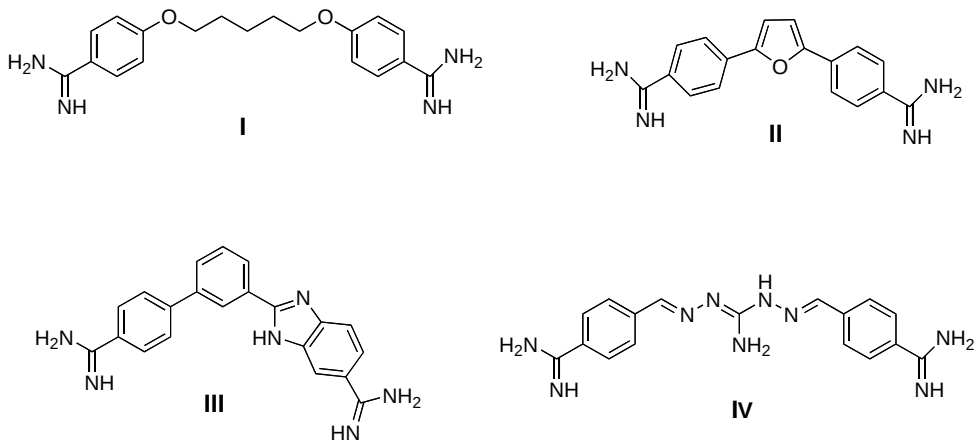


Figure 1. Structures of important dicationic antiprotozoan agents.

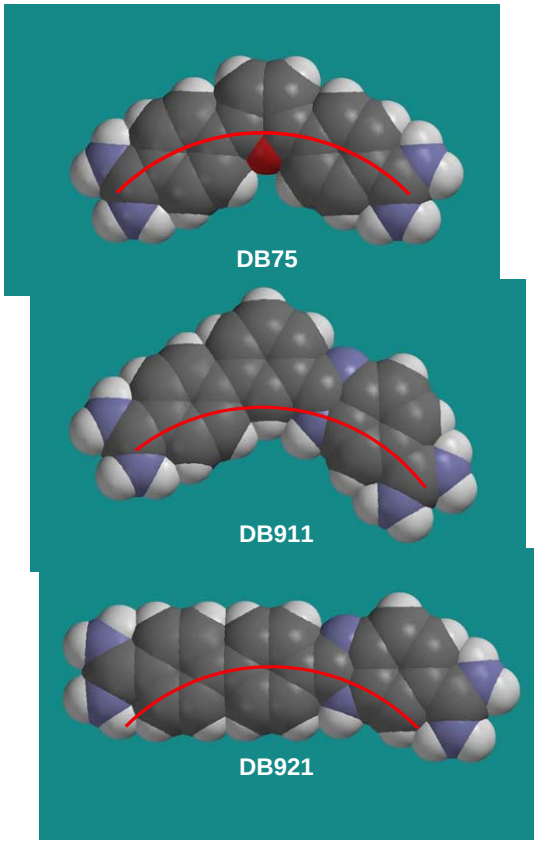


Figure 2. Energy minimized space filling models for DB75(II), DB911(III), and DB921(5a) are shown. The structures were projected onto a planar surface to assist in visualization of differences in overall compound molecular curvature. Equivalent arcs are shown overlaid on each model. The amidine–amidine curvature is essentially equivalent for DB75(II) and DB911(III), and is favorable for interactions with the DNA minor groove. Clearly DB921(5a) does not have the intrinsic molecular curvature to match the groove and its strong groove interactions must be the result of additional interactions which may be mediated by bound water molecules.

for the curved analogs III[19] where several compounds provided 4/4 cures. Thus, the in vivo efficacy of the near-linear molecules does not match that of their curved analogues, despite an overall superior intrinsic anti-trypanosamal activity. Factors, such as adsorption and distribution, are likely causes of these differences. To capitalize on the inherent activity of these near-linear compounds, various drug delivery strategies will have to be explored.

Table 2. In vivo anti-trypanosomal activity of dicationic compounds in the STIB900 mouse model[a]

Compound	Cures[b]	Survival (days)[c]
II	0/4	>39.5
III	4/4	>60
5a	0/4	36.5
5b	3/4	>51.5
5c	1/4	>33.5
5d	1/4	>42
5e	3/4	>51.5
5f	0/4	27
5g	0/4	11
5h	0/4	27.75

[a] See Ref. 24 for details of STIB900 model. Dosage was intraperitoneal at 20 mg/kg for 4 days.
[b] Number of mice that survive 60 days and are parasite free.
[c] Average days of survival; untreated controls died between day 7 and 8 post infection.

2.3. Experimental

2.3.1. Biology. In vitro assays with *T.b.r.* STIB 900 and *P.f.* K1 strain, as well as efficacy study in an acute mouse model for *T.b.r.* STIB 900, were carried out as previously reported.[24,25]

2.3.2. 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 a reference cuvette. Temperatures are under computer control and are increased at 0.5 °C/min. The experiments were conducted in 1 cm path length quartz curvettes in MES 10 buffer (MES 10 mM, EDTA 1 mM, and NaCl 100 mM). The concentrations of DNA were determined by measuring the absorbance at 260 nm. A ratio of 0.3 compound per base was used for the complex and DNA with no compound was used as a control.

2.3.3. Molecular modeling. The energy-minimized, geometry optimized structures were calculated by the Hartree–Fock 6-31G** method in the Spartan04 software package. Geometry-optimized, minimum-energy structures were also calculated with the Spartan04 MMFF molecular mechanics force field and similar structures were obtained as with the Hartree–Fock method. For curvature visualization, planar projections of energy-minimized structures were obtained by adjusting all dihedral angles to zero after energy minimization and comparing all compounds in the same plane.

2.4. Chemistry

Melting points were recorded on 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 measured on a Varian Unity Plus 300 spectrometer, and chemical shifts (δ) are, in ppm, relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (ion source ESI, unless otherwise stated). Elemental analyses were obtained from Atlantic Microlab (Norcross, GA). The compounds reported as salts 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, Fisher Scientific, Frontier, or Lancaster.

2.4.1. 4′-Formylbiphenyl-4-carbonitrile (2a). To a stirred solution of 4-bromobenzonitrile (1.82 g, 10 mmol) and tetrakis(triphenylphosphine) palladium (300 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-formylphenylboronic acid (1.80 g, 12 mmol) in 8 mL methanol. The vigorously stirred mixture was warmed to 80 °C for 12 h. The solvent was evaporated and the precipitate was partitioned between methylene chloride (200 mL) and 2 M aqueous Na_2CO_3 (25 mL) containing 5 mL of concentrated ammonia. The organic layer was dried (Na_2SO_4) and then concentrated to dryness under reduced pressure to afford **2a** in 87% yield, mp 150–150.5 °C (chromatography, SiO_2, hexanes/EtOAc, 80:20). ^{1}H NMR (DMSO-d_6); δ 7.98–8.05 (m, 8H), 10.09 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 143.7, 143.2, 135.9, 132.9, 130.1, 128.0, 127.8, 118.6, 111.0.

2.4.2. 2-(4′-Cyanobiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3a). A solution of **2a** (1.54 g, 7.48 mmol) 3,4-diaminobenzonitrile (1.0 g, 7.48 mmol) and sodium bisulfite (0.8 g, 7.64 mmol) in 10 mL DMF was allowed to reflux overnight. After cooling, the reaction mixture was poured onto water. The solid was collected by filtration and washed with aqueous sodium bicarbonate (2.5%) and water to furnish **3a** in a 83% yield, mp 311–312 °C (DMF). ^{1}H NMR (DMSO-d_6); δ 7.58 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.96 (m, 6H), 8.13 (s, 1H), 8.32 (d, J = 6.8 Hz, 2H). ^{13}C NMR (DMSO-d_6); δ 154.6, 143.5, 142.5, 140.5, 139.6, 132.8, 130.0, 127.6, 127.5, 127.4, 125.2, 120.6, 120.2, 118.7, 115.8, 110.4, 103.4. HRMS (EI): calcd mass 320.347; obsd mass 320.106. Anal. Calcd for $C_{21}H_{12}N_4 \cdot 0.25H_2O$: C, 77.65; H, 3.85. Found: C, 77.59; H, 3.92.

2.4.3. 2-[4′-(*N*-Hydroxyamidino)-biphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4a). A mixture of hydroxylamine hydrochloride (1.04 g, 15 mmol, 10 equiv) in anhydrous DMSO (8 mL) was cooled to 5 °C under nitrogen and potassium *t*-butoxide (1.68 g, 15 mmol, 10 equiv) was added in portions. The mixture was stirred for 30 min and added to the bis-cyanoderivative **3a** (480 mg, 1.5 mmol, 1 equiv). The reaction mixture was stirred overnight at room temperature and then poured slowly onto ice water (100 mL). The precipitate was filtered and washed with water to afford **4a** in a 94% yield; mp dec. at 340 °C. ^{1}H NMR (DMSO-d_6); δ 5.91 (s, 4H), 7.61 (s, 2H), 7.82 (s, 5H), 7.93 (d, J = 7.2 Hz, 2H), 8.30 (d, J = 7.2 Hz, 2H), 9.62 (s, 1H), 9.75 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 151.7, 150.4, 140.6, 139.4, 132.7, 129.0, 127.5, 127.0, 126.9, 126.2, 125.9, 120.2. Anal Calcd for $C_{21}H_{18}N_6O_2 \cdot 1.5H_2O$: C, 61.00; H, 5.12. Found: C, 60.94; H, 5.12.

2.4.4. 2-(4′-Amidinobiphenyl-4-yl)-1*H*-benzimidazole-5-amidine (5a). To a solution of **4a** (386 mg, 1 mmol) in glacial acetic acid (10 mL), acetic anhydride (0.35 mL; 3.7 mmol) was slowly added. After stirring overnight, TLC indicated complete acylation of the starting material, the solvent was removed under reduced pressure; and 10% palladium on carbon (80 mg) was added to the acetoxy derivative in 40 mL glacial acetic acid/ethanol (1:3). The mixture was placed on Parr hydrogenation apparatus at 50 psi for 4 h at room temperature. The mixture was filtered through hyflo and the filter pad was washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **5a** in a 64% yield, mp 241–242 °C. ^{1}H NMR (DMSO-d_6); δ 1.82 (s, 2.8× CH_3), 7.65 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.4, 1H), 8.00 (m, 7H), 8.13 (s, 1H), 8.36 (d, J = 8.4 Hz, 2H). HRMS as free base: Calcd mass+1 = 355.410; obsd mass, 355.167. Anal Calcd for $C_{21}H_{18}N_6 \cdot 2.8AcOH \cdot 2.5H_2O$: C, 56.31; H, 6.03; N, 14.84. Found: C, 56.29; H, 5.75; N, 15.19.

2.4.5. 4′-Formylbiphenyl-3-carbonitrile (2b). The same procedure described for **2a** was used employing 3-bromobenzonitrile, instead of 4-bromobenzonitrile. Yield: 91%, mp 132–133 °C. ^{1}H NMR (DMSO-d_6); δ 7.28 (t, J = 7.8 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.99–8.05 (m, 4H), 8.13 (d, J = 7.8 Hz, 1H), 8.28 (s, 1H), 10.08 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 143.5, 139.8, 135.6, 132.0, 131.8, 130.7, 130.2, 130.1, 127.6, 118.5, 112.2. MS (*m*/*z*, rel. int.); 207 (M^+, 100), 178 (45), 151 (35). Anal. Calcd for $C_{14}H_9NO$: C, 81.14; H, 4.37. Found: C, 80.89; H, 4.52.

2.4.6. 2-(3′-Cyanobiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3b). The same procedure described for **3a** was used starting with **2b**. Yield: 86%, mp 316–317.5 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.63 (d, J = 8.1 Hz, 1H), 7.70–7.79 (m, 2H), 7.89 (d, J = 8.1 Hz, 1H), 8.01

(d, J = 7.8 Hz, 2H), 8.16 (d, J = 8.1 Hz, 2H), 8.30–8.35 (m, 3H), 13.60 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 153.6, 140.0, 139.7, 131.3, 131.2, 130.1, 130.0, 128.8, 127.4, 127.3, 127.1, 125.5, 119.7, 118.4, 112.1, 104.0. MS (*m*/*z*, rel. int.); 321 (M^++1, 100), 283 (50), 229 (10). Anal. Calcd for $C_{21}H_{12}N_4$: C, 78.73; H, 3.78. Found: C, 78.41; H, 3.89.

2.4.7. 2-[3′-(*N*-Hydroxyamidino)-biphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4b). The same procedure described for **4a** was used starting with **3b**. Yield: 95%, mp 290–293 °C. ^{1}H NMR (DMSO-d_6); δ 5.89 (s, 2H), 5.99 (s, 2H), 7.48–7.53 (m, 4H), 7.73 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.1 Hz, 2H), 8.06 (s, 1H), 8.30 (d, J = 8.1 Hz, 2H), 9.60 (s, 1H), 9.71 (s, 1H), 13.07 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 151.7, 150.7, 141.2, 139.1, 134.0, 129.1, 128.8, 127.2, 127.1, 127.0, 124.9, 123.7. MS (*m*/*z*, rel. int.); 387 (M^++1, 80), 194 (100). Anal. Calcd for $C_{21}H_{18}N_6O_2$·1.0H_2O: C, 62.36; H, 4.98. Found: C, 62.40; H, 4.93.

2.4.8. 2-(3′-Amidinobiphenyl-4-yl)-1*H*-benzimidazole-5-amidine acetate salt (5b). The same procedure described for **5a** was used starting with **4b**. Yield: 77%, mp 211–212.5 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.89 (s, 3× CH_3 of acetate), 7.62–7.75 (m, 4H), 7.80–8.15 (m, 4H), 8.20 (s, 1H), 8.38 (d, J = 8.1 Hz, 2H). MS (*m*/*z*, rel. int.); 355 (M^++1, 20), 339 (60), 322 (100). Anal. Calcd for $C_{21}H_{18}N_6$·3.0AcOH·2.4H_2O: C, 56.12; H, 6.07; N, 14.55. Found: C, 56.09; H, 5.81; N, 14.80.

2.4.9. 3-Fluoro-4′-formylbiphenyl-4-carbonitrile (2c). The same procedure described for **2a** was used employing 4-bromo-2-fluorobenzonitrile, instead of 4-bromobenzonitrile. Yield: 78%, mp 186–187 °C. ^{1}H NMR (DMSO-d_6); δ 7.83–7.87 (m, 1H), 7.99–8.09 (m, 6H), 10.09 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 164.5, 161.1, 146.2, 146.1, 142.4, 136.3, 134.4, 130.1, 128.0, 124.1, 115.1, 114.8, 113.9, 99.8, 99.6 (fluorine splitting). MS (*m*/*z*, rel. int.); 225 (M^+, 75), 224 (100), 195 (25), 169 (20). Anal. Calcd for $C_{14}H_8FNO$: C, 74.66; H, 3.58. Found: C, 74.63; H, 3.55.

2.4.10. 2-(4′-Cyano-3′-fluorobiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3c). The same procedure described for **3a** was used starting with **2c**. Yield: 84%, mp: 302–305 °C. ^{1}H NMR (DMSO-d_6); δ 7.62 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 8.00–8.07 (m, 4H), 8.17 (s, 1H), 8.33 (d, J = 8.1 Hz, 2H), 13.60 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 164.5, 161.2, 146.5, 146.4, 138.7, 134.3, 129.8, 127.9, 127.5, 123.5, 119.9, 115.5, 114.5, 114.3, 114.0, 104.1, 99.2, 99.0 (fluorine splitting). EI-MS (*m*/*z*, rel. int.); 338 (M^+, 100), 222 (5), 195 (5), 169 (10). High resolution mass calcd for $C_{21}H_{11}FN_4$: 338.09677. Obsd: 338.09778.

2.4.11. 2-[3′-Fluoro-4′-(*N*-hydroxyamidino)-biphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4c). The same procedure described for **4a** was used starting with **3c**. Yield: 96%, mp: 281–283 °C. ^{1}H NMR (DMSO-d_6); δ 5.86 (s, 4H), 7.60–7.74 (m, 6H), 7.98 (d, J = 8.4 Hz, 2H), 8.32 (d, J = 8.4 Hz, 2H), 9.60 (s, 1H), 9.74 (s, 1H), 13.09 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 161.7, 158.4, 151.7, 151.5, 148.0, 141.6, 141.5, 139.2, 130.4, 129.6, 127.2, 127.0, 122.2, 121.2, 121.0, 114.2, 113.9. MS (*m*/*z*, rel. int.); 405 (M^++1, 70), 203 (100). Anal. Calcd for $C_{21}H_{17}FN_6O_2$·0.8H_2O: C, 60.17; H, 4.44. Found: C, 60.00; H, 4.37.

2.4.12. 2-(4′-Amidino-3′-fluorobiphenyl-4-yl)-1*H*-benzimidazole-5-amidine acetate salt (5c). The same procedure described for **5a** was used starting with **4c**. Yield: 85%, mp: 210–212 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.78 (s, 3× CH_3), 7.61 (d, J = 8.4 Hz, 1H), 7.84–8.05 (m, 6H), 8.12 (s, 1H), 8.39 (d, J = 8.4 Hz, 2H). MS (*m*/*z*, rel. int.); 373 (M^++1, 8), 357 (100), 340 (40). Anal. Calcd for $C_{21}H_{17}FN_6$·3.0AcOH·1.3H_2O: C, 56.30; H, 5.52; N, 14.59. Found: C, 56.12; H, 5.18; N, 14.21.

2.4.13. 4′-Formyl-2-methylbiphenyl-4-carbonitrile (2d). The same procedure described for **2a** was used employing 4-bromo-3-methylbenzonitrile, instead of 4-bromobenzonitrile. Yield: 71%, mp: 130–130.5 °C. ^{1}H NMR (DMSO-d_6); δ 2.28 (s, 3H), 7.46 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.86 (s, 1H), 8.02 (d, J = 8.1 Hz, 2H), 10.08 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 145.4, 144.9, 136.6, 135.3, 133.9, 130.3, 129.7, 129.6, 129.5, 118.6, 110.7, 19.6. MS (*m*/*z*, rel. int.); 221 (M^+, 100), 203 (5), 192 (40), 177 (20), 165 (25). Anal. Calcd for $C_{15}H_{11}NO$: C, 81.42; H, 5.01. Found: C, 81.50; H, 5.13.

2.4.14. 2-(4′-Cyano-2′-methylbiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3d). The same procedure described for **3a** was used starting with **2d**. Yield: 74%, mp: 269–270 °C. ^{1}H NMR (DMSO-d_6); δ 2.34 (s, 3H), 7.49 (d, J = 7.8 Hz, 1H), 7.60–7.63 (m, 3H), 7.77 (d, J = 7.8 Hz, 2H), 7.85 (s, 1H), 8.16–8.32 (m, 3H), 13.57 (br s, 1H). ^{13}C NMR (DMSO-d_6); 145.1, 141.6, 136.7, 133.8, 130.4, 129.7, 129.5, 128.5, 126.8, 119.9, 118.7, 110.3, 104.0, 19.7. MS (*m*/*z*, rel. int.); 335 (M^++1, 100), 306 (5), 176 (35). Anal. Calcd for $C_{22}H_{14}N_4$: C, 79.02; H, 4.22. Found: C, 78.67; H, 4.39.

2.4.15. 2-[4′-(*N*-Hydroxyamidino)-2′-methylbiphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4d). The same procedure described for **4a** was used starting with **3d**. Yield: 93%, mp: 300–302 °C. ^{1}H NMR (DMSO-d_6); 2.33 (s, 3H), 5.84 (s, 4H), 7.29 (d, J = 7.8 Hz, 1H), 7.53–7.66 (m, 5H), 7.82 (s, 1H), 7.99 (s, 1H), 8.26 (d, J = 7.8 Hz, 2H), 9.59 (s, 1H), 9.66 (s, 1H), 13.02 (br s, 1H). MS (*m*/*z*, rel. int.); 401 (M^++1, 100), 163 (25). Anal. Calcd for $C_{22}H_{20}N_6O_2$·2.0H_2O: C, 60.49; H, 5.50. Found: C, 60.82; H, 5.54.

2.4.16. 2-(4′-Amidino-2′-methylbiphenyl-4-yl)-1*H*-benzimidazole-5-amidine acetate salt (5d). The same procedure described for **5a** was used starting with **4d**. Yield: 82%, mp: 193–195 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.82 (s, 3× CH_3), 2.40 (s, 3H), 7.60–8.00 (m, 7H), 8.20 (s, 1H), 8.25–8.38 (m, 2H). MS (*m*/*z*, rel. int.); 369 (M^++1, 10), 353 (100), 336 (30). Anal. Calcd for $C_{22}H_{20}N_6$·3.0AcOH·0.25H_2O: C, 60.80; H, 5.92; N, 15.19. Found: C, 60.57; H, 5.73; N, 15.33.

2.4.17. 2-Benzyloxy-4′-formylbiphenyl-4-carbonitrile (2e). The same procedure described for **2a** was used employing 3-benzyloxy-4-bromobenzonitrile,[26] instead of 4-bromobenzonitrile. Yield: 69%, mp: 131–132 °C. ^{1}H NMR (DMSO-d_6); δ 5.24 (s, 2H), 7.30–7.39 (m, 5H), 7.54–7.61 (m, 2H), 7.76 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H), 10.04 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 155.3, 142.3, 136.1, 135.3, 133.8, 131.5, 130.0, 129.1, 128.4, 127.9, 127.4, 125.1, 118.5, 116.5, 111.9, 70.2. MS (*m*/*z*, rel. int.); 313 (M^+, 70), 285 (5), 220 (10), 193 (30), 164 (50), 91 (100). Anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82. Found: C, 80.23; H, 4.75.

2.4.18. 2-(2′-Benzyloxy-4′-cyanobiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3e). The same procedure described for **3a** was used starting with **2e**. Yield: 75%, mp: 205–208 °C. ^{1}H NMR (DMSO-d_6); δ 5.24 (s, 2H), 7.31–7.43 (m, 6H), 7.56–7.64 (m, 3H), 7.70–7.81 (m, 4H), 8.23–8.26 (m, 2H), 13.57 (br s, 1H). MS (*m*/*z*, rel. int.); 427 (M^++1, 75), 371 (10), 293 (15), 241 (100). Anal. Calcd for $C_{28}H_{18}N_4O{\cdot}0.5H_2O$: C, 77.22; H, 4.39. Found: C, 77.20; H, 4.24.

2.4.19. 2-[2′-Benzyloxy-4′-(*N*-hydroxyamidino)-biphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4e). The same procedure described for **4a** was used starting with **3e**. Yield: 90%, mp: 250–253 °C. ^{1}H NMR (DMSO-d_6); 5.21 (s, 2H), 5.83 (s, 2H), 5.93 (s, 2H), 7.28–7.43 (m, 7H), 7.48–7.62 (m, 4H), 7.75–7.80 (m, 2H), 8.16–8.21 (m, 2H), 9.59 (s, 1H), 9.73 (s, 1H), 13.00 (br s, 1H). MS (*m*/*z*, rel. int.); 493 (M^++1, 45), 247 (100). Anal. Calcd for $C_{28}H_{24}N_6O_3{\cdot}0.7H_2O$: C, 66.57; H, 5.06. Found: C, 66.59; H, 4.98.

2.4.20. 2-(4′-Amidino-2′-hydroxybiphenyl-4-yl)-1*H*-benzimidazole-5-amidine acetate salt (5e). The same procedure described for **5a** was used starting with **4e**. Yield: 73%, mp: 220–222 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.82 (s, 3× CH_3), 7.30–7.50 (m, 3H), 7.60–7.78 (m, 2H), 7.85 (m, 2H), 8.20–8.38 (m, 3H). MS (*m*/*z*, rel. int.); 371 (M^++1, 80), 186 (100). HRMS calcd for $C_{21}H_{19}N_6O$ MH+ 371.1620. Obsd: 371.1618. Anal. Calcd for $C_{21}H_{18}N_6O{\cdot}3.0AcOH{\cdot}0.4H_2O{\cdot}1.0EtOH$: C, 57.68; H, 6.14; N, 13.91. Found: C, 58.10; H, 5.75; N, 13.50.

2.4.21. 6-(4-Formylphenyl)-nicotinonitrile (2f). The same procedure described for **2a** was used employing 6-chloronicotinonitrile, instead of 4-bromobenzonitrile. Yield: 82%, mp: 200–201 °C. ^{1}H NMR (DMSO-d_6); δ 8.07 (d, J = 8.1 Hz, 2H), 8.33 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 8.1 Hz, 2H), 8.47 (dd, J = 8.4, 2.1 Hz, 1H), 9.16 (d, J = 2.1 Hz, 1H), 10.11 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.8, 157.7, 152.6, 142.0, 141.2, 137.1, 130.0, 127.9, 121.1, 117.0, 108.3. MS (*m*/*z*, rel. int.); 208 (M^+, 85), 207 (100), 179 (55), 152 (15). Anal. Calcd for $C_{13}H_8N_2O$: C, 74.98; H, 3.87. Found: C, 74.81; H, 3.95.

2.4.22. 2-[4-(5-Cyanopyridin-2-yl)-phenyl]-1*H*-benzimidazole-5-carbonitrile (3f). The same procedure described for **3a** was used starting with **2f**. Yield: 91%, mp: 346–347 °C. ^{1}H NMR (DMSO-d_6); δ 7.61 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 8.19 (s, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.33–8.41 (m, 4H), 8.43 (dd, J = 8.4, 2.1 Hz, 1H), 9.17 (d, J = 2.1 Hz, 1H), 13.60 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 158.0, 152.6, 141.1, 138.6, 130.8, 127.8, 127.4, 125.9, 120.5, 119.9, 117.2, 107.8, 104.2. EI-MS (*m*/*z*, rel. int.); 321 (M^+, 100), 293 (5), 204 (5), 160 (10). High resolution mass calcd for $C_{20}H_{11}N_5$: 321.10145. Obsd: 321.10151.

2.4.23. 2-{4-[5-(*N*-Hydroxyamidino)-pyridin-2-yl]-phenyl}-1*H*-benzimidazole-5-*N*-hydroxyamidine (4f). The same procedure described for **4a** was used starting with **3f**. Yield: 96%, mp: 305–308 °C. ^{1}H NMR (DMSO-d_6); 5.82 (s, 2H), 6.07 (s, 2H), 7.50–7.65 (m, 2H), 7.85 (s, 1H), 8.10–8.20 (m, 2H), 8.38 (s, 4H), 9.02 (s, 1H), 9.60 (s, 1H), 9.97 (s, 1H). 13.16 (br s, 1H). MS (*m*/*z*, rel. int.); 388 (M^++1, 100), 194 (40). Anal. Calcd for $C_{20}H_{17}N_7O_2{\cdot}2.5H_2O$: C, 55.54; H, 5.12. Found: C, 55.42; H, 5.17.

2.4.24. 2-[4-(5-Amidinopyridin-2-yl)-phenyl]-1*H*-benzimidazole-5-amidine acetate salt (5f). The same procedure described for **5a** was used starting with **4f**. Yield: 82%, mp: 240–241 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, 2.7× CH_3), 7.58 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 8.28–8.42 (m, 6H), 9.08 (s, 1H). MS (*m*/*z*, rel. int.); 356 (M^++1, 10), 340 (100), 323 (60). Anal. Calcd for $C_{20}H_{17}N_7{\cdot}2.7AcOH{\cdot}1.3H_2O$: C, 56.39; H, 5.66; N, 18.12. Found: C, 56.15; H, 5.49; N, 18.43.

2.4.25. 5-(4-Formylphenyl)pyridine-2-carbonitrile (2g). Adopting the same procedure used for the preparation of **2a**, a Suzuki coupling reaction was performed using 5-bromopyridine-2-carbonitrile, instead of 4-bromobenzonitrile, in the presence of $NaHCO_3$ as a base to yield compound **2g** in 64% yield, mp: 193–194 °C. ^{1}H NMR (DMSO-d_6); δ 8.06 (s, 4H), 8.18 (d, J = 8.4 Hz, 1H), 8.45 (dd, J = 8.4, 2.4 Hz, 1H), 9.17 (d, J = 2.4 Hz, 1H), 10.08 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.7, 149.5, 140.7, 137.6, 136.2, 135.9, 131.9, 130.1, 129.0, 128.1, 117.3. MS (*m*/*z*, rel. int.); 208 (M^+, 95), 207 (100), 179 (30). Anal. Calcd for $C_{13}H_8N_2O$: C, 74.98; H, 3.87. Found: C, 74.66; H, 4.03.

2.4.26. 2-[4-(6-Cyanopyridin-3-yl)-phenyl]-1*H*-benzimidazole-5-carbonitrile (3g). A solution of **2g** (520 mg, 2.5 mmol), 3,4-diaminobenzonitrile (332.5 mg, 2.5 mmol), and 1,4-benzoquinone (270.2 mg, 2.5 mmol) in ethanol (40 mL) was allowed to reflux under nitrogen for overnight. The solvent was distilled under reduced pressure. The residue was triturated with ether and filtered to afford **3g** in 79% yield, mp: >300 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.58–7.75 (m, 2H), 8.04–8.10 (m, 4H), 8.34–8.42 (m, 3H), 9.17 (s, 1H), 13.0 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 153.3, 149.1, 137.7, 136.9, 135.2, 131.4, 129.7, 128.9, 127.8, 127.6, 127.5, 125.6, 119.7, 117.3, 104.1. MS (*m*/*z*, rel. int.); 322 (M^++1, 100), 250 (10), 228 (15). Anal. Calcd for $C_{20}H_{11}N_5$: C, 74.75; H, 3.45. Found: C, 74.40; H, 3.59.

2.4.27. 2-{4-[6-(*N*-Hydroxyamidino)-pyridin-3-yl]-phenyl}-1*H*-benzimidazole-5-*N*-hydroxyamidine (4g). The same procedure described for **4a** was used starting with **3g**.

Yield: 94%, mp: 296–298 °C. ^{1}H NMR (DMSO-d_6); 5.73 (s, 2H), 5.84 (s, 2H), 7.55 (s, 2H), 7.89–7.94 (m, 4H), 8.14 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 7.8 Hz, 2H), 8.93 (s, 1H), 9.80 (s, 2H), 12.90 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 152.2, 151.6, 149.3, 149.2, 146.3, 137.8, 134.7, 134.6, 129.7, 127.8, 127.3, 127.2, 120.4, 119.5. MS (*m*/*z*, rel. int.); 388 (M^++1, 100), 356 (5), 176 (10), 163 (10).

2.4.28. 2-[4-(6-Amidinopyridin-3-yl)-phenyl]-1*H*-benzimidazole-5-amidine acetate salt (5g). The same procedure described for **5a** was used starting with **4g**. Yield: 75%, mp: 217–219 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.84 (s, 3× CH_3), 7.69 (s, 1H), 7.82 (s, 1H), 8.05–8.20 (m, 3H), 8.41 (s, 4H), 9.09 (s, 1H). MS (*m*/*z*, rel. int.); 356 (M^++1, 100), 339 (25), 275 (5), 178 (30). Anal. Calcd for $C_{20}H_{17}N_7 \cdot 3.0AcOH \cdot 2.6H_2O$: C, 53.62; H, 5.87; N, 16.85. Found: C, 53.24; H, 5.51; N, 17.15.

2.4.29. 3′-Fluoro-4′-formylbiphenyl-4-carbonitrile (2h). The same procedure described for **2a** was used employing 4-bromo-2-fluorobenzaldehyde, instead of 4-bromobenzonitrile and using 4-cyanophenylboronic acid. Yield: 66%, mp: 169–170 °C. ^{1}H NMR (DMSO-d_6); δ 7.76–7.87 (m, 3H), 7.90–8.01 (m, 4H), 10.23 (s, 1H). MS (*m*/*z*, rel. int.); 225 (M^+, 70), 224 (100), 195 (10), 169 (10). Anal. Calcd for $C_{14}H_8FNO$: C, 74.66; H, 3.58. Found: C, 74.38; H, 3.59.

2.4.30. 2-(4′-Cyano-3-fluorobiphenyl-4-yl)-1*H*-benzimidazole-5-carbonitrile (3h). The same procedure described for **3a** was used starting with **2h**. Yield: 90%, mp: 268–270 °C. ^{1}H NMR (DMSO-d_6); δ 7.61 (d, J = 8.7 Hz, 1H), 7.79–8.02 (m, 7H), 8.13 (s, 1H), 8.35 (t, J = 8.1 Hz, 1H), 12.60 (br s, 1H). MS (*m*/*z*, rel.int.); 339 (M^++1, 100), 318 (40), 291 (30), 265 (15). Anal. Calcd for $C_{21}H_{11}FN_4$: C, 74.55; H, 3.28. Found: C, 74.17; H, 3.46.

2.4.31. 2-[3-Fluoro-4′-(*N*-hydroxyamidino)-biphenyl-4-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (4h). The same procedure described for **4a** was used starting with **3h**. Yield: 98%, mp: 291–293 °C. ^{1}H NMR (DMSO-d_6); δ 5.90 (s, 2H), 6.05 (s, 2H), 7.60 (d, J = 8.7 Hz, 1H), 7.72–7.91 (m, 8H), 8.28 (t, J = 8.1 Hz, 1H), 9.60 (s, 1H), 9.73 (s, 1H), 12.70 (br s, 1H). MS (*m*/*z*, rel. int.); 405 (M^++1, 75), 203 (100). Anal. Calcd for $C_{21}H_{17}FN_6O_2 \cdot 1.0H_2O$: C, 59.71; H, 4.52. Found: C, 59.61; H, 4.35.

2.4.32. 2-(4′-Amidino-3-fluorobiphenyl-4-yl)-1*H*-benzimidazole-5-amidine acetate salt (5h). The same procedure described for **5a** was used starting with **4h**. Yield: 82%, mp: 237–238 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, 3× CH_3), 7.71 (d, J = 8.7 Hz, 1H), 7.83–7.88 (m, 3H), 7.97–8.05 (m, 4H), 8.23 (s, 1H), 8.39 (t, J = 7.8 Hz, 1H). MS (*m*/*z*, rel. int.); 373 (M^++1, 60), 187 (100). Anal. Calcd for $C_{21}H_{17}FN_6 \cdot 3.0AcOH \cdot 1.25H_2O$: C, 56.39; H, 5.53; N, 14.62. Found: C, 56.16; H, 5.61; N, 14.64.

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References and notes

1. (a) Tidwell, R. R.; Boykin, D. W. Dicationic DNA Minor Groove Binders as Antimicrobial Agents. In Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Small Molecule DNA and RNA Binders; From Synthesis to Nucleic Acid Complexes; Wiley-VCH: New York, 2003; Vol. 2, p 414; (b) Wilson, W. D.; Nguyen, B.; Tanious, F. A.; Mathis, A.; Hall, J. E.; Stephens, C. E.; Boykin, D. W. Dications that target the DNA minor groove: Compound design and preparation, DNA interactions, cellular distribution and biological activity. *Curr. Med. Chem.* **2005**, *5*, 389.
2. Ashley, J. N.; Barber, H. J.; Ewins, A. J.; Newbery, G.; Self, A. D. H. *J. Chem. Soc.* **1942**, 103.
3. Fairlamb, A. H. *Trends Parasitol.* **2003**, *19*, 488.
4. Bouteille, B.; Oukem, O.; Bisser, S.; Dumas, M. *Fundam. Clin. Pharmacol.* **2003**, *17*, 171.
5. 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.
6. Yeramian, P.; Krudsood, S.; Chalermrut, K.; Silachamroon, U.; Allen, J.; Brun, R.; Meshnick, S.; Tidwell, R.; Looareesuwan, S. Efficacy of DB289 in acute uncomplicated *P. falciparum*/*P. vivax* malaria. Presented at the 52nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, December 3–7, 2003, Philadelphia, PA.
7. Dykstra, C. C.; McClernon, D. R.; Elwell, L. P.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1994**, *38*, 1890.
8. Bailly, C.; Dassonneville, L.; Carrascol, C.; Lucasl, D.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *Anti-Cancer Drug Des.* **1999**, *14*, 47.
9. Fitzgerald, D. J.; Anderson, J. N. *J. Biol. Chem.* **1999**, *274*, 27128.
10. Henderson, D.; Hurley, L. H. *Nat. Med.* **1995**, *1*, 525.
11. Matovu, E.; Stewart, M. L.; Geiser, F.; Brun, R.; Maser, P.; Wallace, L. J. M.; Burchmore, R. J.; Enyaru, J. C. K.; Barrett, M. P.; Kaminsky, R.; Seebeck, T.; de Koning, H. P. *Eukaryot. Cell* **2003**, *2*, 1003.
12. (a) Goodsell, D.; Dickerson, R. E. *J. Med. Chem.* **1986**, *29*, 245; (b) Cory, M.; Tidwell, R. R.; Fairley, T. A. *J. Med. Chem.* **1992**, *35*, 431.
13. Czarny, A.; Boykin, D. W.; Wood, A. A.; Nunn, C. M.; Neidle, S.; Zhao, M. R. J.; Wilson, W. D. *J. Am. Chem. Soc.* **1995**, *117*, 4716.
14. Mazur, S.; Tanious, F. A.; Ding, D.; Kumar, A.; Boykin, D. W.; Neidle, S.; Wilson, W. D. *J. Mol. Biol.* **2000**, *300*, 321.
15. Wilson, W. D.; Tanious, F. A.; Ding, D.; Kumar, A.; Boykin, D. W.; Colson, P.; Houssier, C.; Bailey, C. *J. Am. Chem. Soc.* **1998**, *120*, 10310.
16. Shaikh, S. A.; Ahmed, S. R.; Jayaram, B. A. *Arch. Biochem. Biophys.* **2004**, *429*, 81.
17. Boykin, D. W.; Kumar, A.; Xiao, G.; Wilson, W. D.; Bender, B. K.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R. *J. Med. Chem.* **1998**, *41*, 124.
18. Boykin, D. W.; Kumar, A.; Spychala, J.; Zhou, M.; Lombardy, R. L.; 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.
19. Ismail, M. A.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2004**, *12*, 5405, and references cited therein.
20. Brun, R.; Buhler, Y.; Sandmeier, U.; Kaminsky, R.; Bacchi, C. J.; Rattendi, D.; Lane, S.; Croft, S. L.; Snowdon, D.; Yardley, Caravatti, G.; Frei, J.; Stanek, J.; Mett, H. *Antimicrob. Agents Chemother.* **1996**, *40*, 1442.
21. Nguyen, B.; Lee, M. P. H.; Hamelberg, D.; Joubert, A.; Bailly, C.; Brun, R.; Neidle, S.; Wilson, D. W. *J. Am. Chem. Soc.* **2002**, *124*, 13680.
22. Nguyen, B.; Hamelberg, D.; Bailly, C.; Colson, P.; Stanek, J.; Brun, R.; Neidle, S.; Wilson, D. W. *Biophys. J.* **2004**, *86*, 1028.
23. Kumar, A.; Rhodes, R. A.; Spychala, J.; Wilson, W. D.; Boykin, D. W.; Tidwell, R. R.; Dykstra, C. C.; Hall, J. E. , Jones, S. K.; Schinazi, R. F. *Eur. J. Med. Chem.* **1995**, *30*, 99.
24. Ismail, M. A.; Brun, R.; Easterbrook, J. D.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2003**, *46*, 4761.
25. Raz, B. M.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139.
26. Ismail, M. A.; Anbazhagan, M.; Stephens, C. E.; Boykin, D. W. *Synth. Commun.* **2004**, *34*, 751.