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Bioorganic & Medicinal Chemistry 12 (2004) 5405–5413
Bioorganic & Medicinal Chemistry

Dicationic biphenyl benzimidazole derivatives as antiprotozoal agents

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Received 7 April 2004; accepted 23 July 2004
Available online 23 August 2004

Abstract—A series of biphenyl benzimidazoles diamidines **6a**–**i** were synthesized from their respective diamidoximes, through the bis-*O*-acetoxyamidoxime followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd–C. The target compounds contain hydroxy and/or methoxy substituted 1,3-phenyl groups as the central spacer between the two amidino bearing aryl groups. All of the diamidines showed strong DNA affinities as judged by high ΔT_m values with poly(dA·dT)$_2$, which varied with structure and is discussed. Seven of the nine new diamidines gave in vitro IC_{50} values of approximately 30 nM or less versus *Trypanosoma brucei rhodesiense* (*T.b.r.*). Generally the diamidines were less active versus *Plasmodium falciparum* (*P.f.*), however one compound exhibited excellent activity with an IC_{50} value of 2.1 nM. Five of the nine diamidines exhibited excellent in vivo activity in the trypanosomal STIB900 mouse model giving 3/4 or 4/4 cures at dosage of 20 mg/kg ip and three showed similar efficacy at dosage of 10 mg/kg or lower.

1. Introduction

It has been well documented that aromatic diamidines exhibit broad-spectrum antimicrobial activity including effectiveness against the protozoan diseases caused by *Trypanosoma* sp. and *Plasmodium* sp.[1] Despite the broad activity of the aromatic diamidines, pentamidine (**I**) is the only compound of this class to be used extensively in the clinic.[1] A prodrug, 2,5-bis[4-(methoxyamidino)phenyl]furan, of furamidine, (**IIa**), is an effective antiprotozoan compound, which is currently entered into two different Phase II clinical trials as an oral drug versus human African trypanosomiasis and malaria.[1] Many of the active aromatic diamidines for various structural classes have been shown to bind to the minor groove of DNA at AT rich sites.[2–8] It has been hypothesized that the minor groove binding of these type compounds leads to inhibition of one or more DNA dependant enzymes, which gives rise to the anti-microbial effect.[9–11] Recently, we have made compounds in which the phenyl group(s) of furamidine have been replaced with pyridyl group(s) (**IIb**). Several of these aza-analogues show in vivo activity, which is superior to that of furamidine.[12] Benzimidazole units are often key structural elements in the aromatic frame work for the more effective diamidines.[13–16] The benzimidazole analogue **III** has been found to bind to DNA in a unique stacked dimer array, which has potential for development of new gene regulation molecules.[17–20] Compound **III** has shown some activity in an immunosupressed rat model for *Pneumocystis carinii pneumonia*[17] and in the STIB900 mouse model for acute stage African trypanosomiasis (this manuscript) (Fig. 1).

Given the promising properties of the various benzimidazole aromatic diamidines and the unusual DNA binding properties of **III** we decided to study the effect of replacing the central furan ring with a 1,3-phenyl group and we have included hydroxy and/or methoxy substituted analogues to aid in water solubility. In this work we also replaced the terminal phenyl group with a pyridyl group. Such alterations in structure offer the potential to change the base pair recognition on DNA binding and to yield different absorption and distribution profiles. We report the synthesis of novel diamidino

Keywords: Diamidines; Benzimidazoles; 1,3-Phenylene; Antiprotozoal agents; Suzuki coupling.
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doi:10.1016/j.bmc.2004.07.056

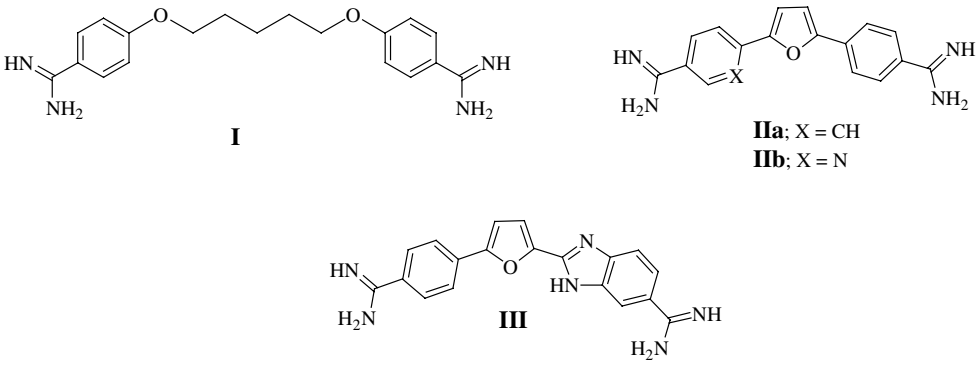


Figure 1.

biphenyl benzimidazole derivatives and their evaluation versus *Trypanosoma b. rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*).

2. Results and discussion

2.1. Chemistry

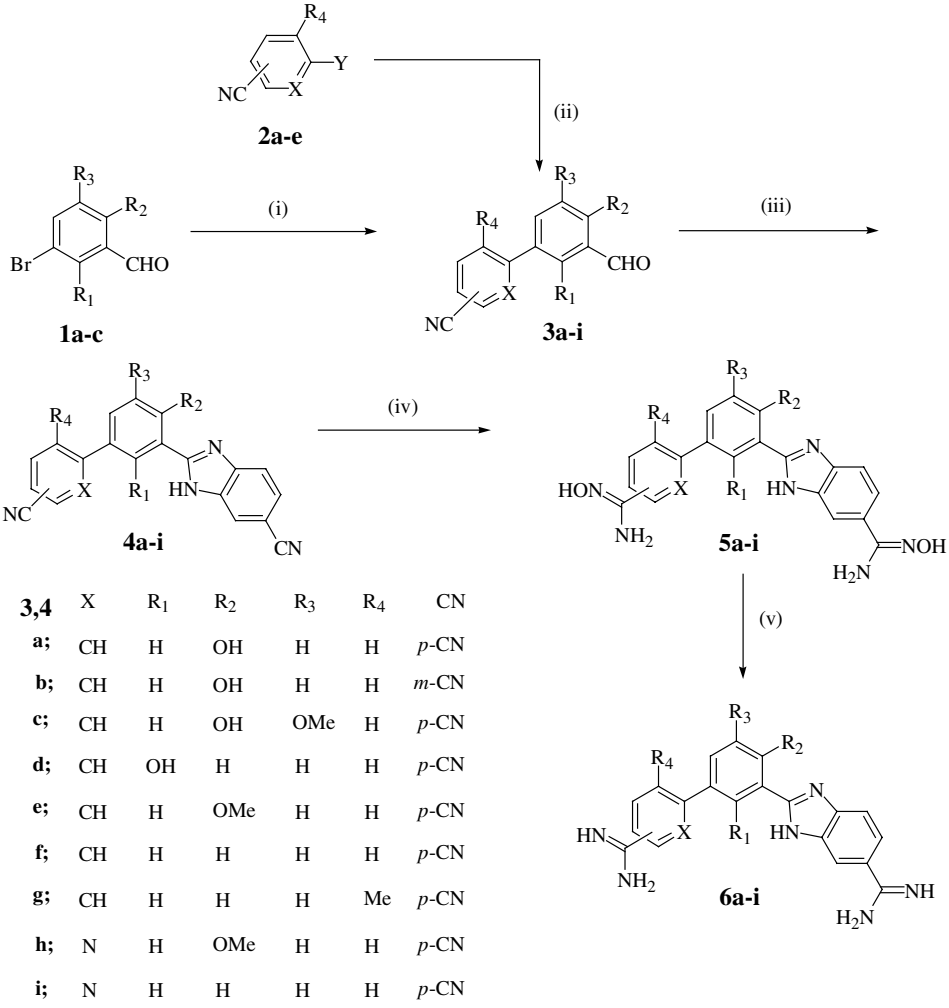
Acetate salts of a series of benzimidazole aromatic diamidines **6a**–**i** were synthesized from their respective diamidoximes **5a**–**i**, through the corresponding bis-*O*-acetoxyamidoximes followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd–C (Scheme 1). Compounds **5a**–**i** were obtained in three steps starting with the Suzuki coupling reaction of the appropriate bromobenzaldehyde **1a**–**c** or haloaryl/hetaryl carbonitriles **2a**–**e** with cyanophenylboronic acids or substituted 3-formylphenylboronic acids to form the anticipated 3-formylbiphenyl carbonitrile analogues **3a**–**i**. Subsequent condensation of the formyl derivatives **3a**–**i** with 3,4-diaminobenzonitrile in the presence of equimolar ratio of 1,4-benzoquinone gave the desired dinitriles **4a**–**i**, which were allowed to react at room temperature for 24 h with a mixture of hydroxylamine hydrochloride and potassium *tert*-butoxide in DMSO solution to furnish **5a**–**i** in excellent yields. The hydrochloride salt of the amidoxime **5a** was made by passing hydrogen chloride gas into ethanolic solution of its free base.

2.2. Biology

Table 1 contains the results of DNA binding studies and in vitro evaluation against *Trypanosoma b. rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*) for the diamidino biphenyl benzimidazoles. The DNA affinities for the biphenyl benzimidazoles as reflected by ΔT_m values for poly(dA·dT)$_2$ are shown in Table 1. Replacement of the furan ring of **III** by a 1,3-phenylene unit does not significantly alter the DNA affinity (cf. values for **III** and **6f**). However, introduction of a methyl or a methoxy group *ortho* to the triaryl ring junctions (**6e**, **6g**, **6h**) results in lowering of the ΔT_m values. This reduction in affinity is presumably due to torsion angle rotation of the triaryl system, which would result in poorer van der Waals interactions between the aryl rings and the walls of the minor groove. In contrast, substitution of an *ortho* hydroxy group does not cause a reduction in ΔT_m values as can be seen from the values for **6a** and **6d**. This may be due to the smaller size of the hydroxyl group and/or intramolecular hydrogen bonding between it and a benzimidazole nitrogen atom. The multisubstituted hydroxyl analogue **6c** displays a somewhat lowered ΔT_m but, this may be a consequence of the buttressing effect of the methoxy group yielding greater torsion angle twist. The presence of a *meta*-amidine unit (**6b**) lowers the ΔT_m value relative to its *para* isomer. This effect has been noted previously in other systems and has been attributed to a reduction in complementarity of the curvature of the diamidine compared to the minor groove.[21] Replacement of the terminal phenyl group with a pyridyl one does not appreciably alter the DNA affinity (cf. the values for **6f** with **6i** and that of **6e** with **6h**). Consistent with previous studies, there is no direct correlation between DNA affinity and antiparasitic activity.[1] The strong affinities and excellent activities observed are consistent with the hypothesis that a threshold level of DNA affinity is required for antiparasitic activity.[1]

The new diamidines, except the meta analogue **6b**, are more effective in vitro versus *T.b.r.* and all except **6c**, **6d** and **6h** are more effective against *P.f.* than the prototype molecule **III**. Consequently, replacement of the furan ring of **III** by a 1,3-phenyl is clearly advantageous for increased activity of the benzimidazole types versus these two protozoan organisms. All of the diamidines except **6b** and **6e** exhibit anti-trypanosomal IC_{50} values of approximately 30 nM or less. Four (**6d**, **6f**, **6h**, and **6i**) gave IC_{50} values of approximately 20 nM or less. These benzimidazole diamidines were generally not as active in vitro against *P.f.*, however **6f** shows good activity with an IC_{50} value of 27.5 nM and **6a** shows excellent activity with an IC_{50} value of 2.1 nM. In general, there is good selectivity for the parasitic organisms by these new diamidines as judged from their cellular cytotoxicity (Table 1). The selectivity indices range from over 100 to over 1000, except for compound **6h**.

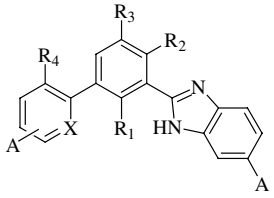
The results for the in vivo evaluation of the diamidines versus *T.b.r.* are shown in Table 2. Five (**6c**, **6d**, **6f**, **6h**, and **6i**) of the eight diamidines tested in vivo showed excellent activity curing three or more of the four animals used in each test at an ip dosage of 20 mg/kg or less.



3,4	X	R_1	R_2	R_3	R_4	CN
a;	CH	H	OH	H	H	*p*-CN
b;	CH	H	OH	H	H	*m*-CN
c;	CH	H	OH	OMe	H	*p*-CN
d;	CH	OH	H	H	H	*p*-CN
e;	CH	H	OMe	H	H	*p*-CN
f;	CH	H	H	H	H	*p*-CN
g;	CH	H	H	H	Me	*p*-CN
h;	N	H	OMe	H	H	*p*-CN
i;	N	H	H	H	H	*p*-CN

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

Table 1. DNA affinities and in vitro anti-protozoan data for biphenyl benzimidazole analogues



Code	X	R_1	R_2	R_3	R_4	A	ΔT_m	IC_{50} *T.b.r.*[a,b] nM	IC_{50} *P.f.*[a,b] nM	TD_{50} L6Cells[c] μM
I	NA	NA	NA	NA	NA	*p*-(C=NH)NH_2	12.6	2.8	64.2	11.4
IIa	CH	NA	NA	NA	NA	*p*-(C=NH)NH_2	25	4.5	15.5	6.4
III	CH	NA	NA	NA	NA	*p*-(C=NH)NH_2	24.6	122	96	10.1
6a	CH	H	OH	H	H	*p*-(C=NH)NH_2	25.3	27	2.1	31.7
6b	CH	H	OH	H	H	*m*-(C=NH)NH_2	18.9	214	34.3	117
6c	CH	H	OH	OMe	H	*p*-(C=NH)NH_2	20.4	31	131	19.4
6d	CH	OH	H	H	H	*p*-(C=NH)NH_2	25.3	17	131	37
6e	CH	H	OMe	H	H	*p*-(C=NH)NH_2	13.7	54	40	19.3
6f	CH	H	H	H	H	*p*-(C=NH)NH_2	25.6	4.4	27.5	23
6g	CH	H	H	H	Me	*p*-(C=NH)NH_2	17.1	23	65	13.5
6h	N	H	OMe	H	H	*p*-(C=NH)NH_2	13.1	14	364	23.3
6i	N	H	H	H	H	*p*-(C=NH)NH_2	24.4	15	96.4	117

[a] The *T.b.r.* strain used was STIB 900 and the *P.f.* strain was K1; See Refs. 12 and 22 for details.
[b] Average of duplicate determinations. DNA: polydA·polydT; buffer: MES10; Ratio (compound/DNA): 0.3.
[c] Cytotoxicity was evaluated using cultured L-6 rat myoblast cells using an Alamar Blue assay.

Table 2. In vivo anti-trypanosomal activity of biphenyl benzimidazole analogues in the STIB900 mouse model[a,b]

Compound	Dosage[c] (mg/kg)	Cures[d]	Survival (days)[e]
Pentamidine	20	0/4	42.75
Furamidine (**IIa**)	20	0/4	52.5
III	20	1/4	>27.75
6a	20	0/4	35.25
6c	20	3/4	>58
6d	20	4/4	>60
	5	2/4	>46.5
6e	20	2/4	>56.5
6f	20	4/4	>60
	5	3/4	>49
6g	20	2/4	>47.5
6h	10	3/4	>53
	5	3/4	>60
6i	20	4/4	>60
	10	4/4	>60

[a] See Ref. 12 for details of STIB900 model.
[b] IC_{50} value for **6b** did not meet criteria for entry into animal studies.
[c] Dosage was intraperitoneal.
[d] Number of mice that survive and are parasite free for 60 days.
[e] Average days of survival; untreated control animals expire between day 7 and 8 post infection.

Three of the dications (**6f**, **6h**, and **6i**) provided cures of three of four or four of four mice at lower dosage (5 or 10 mg/kg). These compounds show significant improvement of in vivo efficacy over the prototype dications furamidine (**IIa**) and **III**. Clearly, these compounds merit further evaluation in other animal models.

3. Experimental section

3.1. Biology

In vitro assays with *Trypanosoma b. rhodesiense* STIB 900 and *Plasmodium falciparum* K1 strain as well as efficacy study in an acute mouse model for *T.b. rhodesiense* STIB 900 were carried out as described elsewhere.[12,22]

3.2. 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. ^1H and ^{13}C NMR spectra were recorded for all compounds expect **6a** and **6h** employing a Varian Unity Plus 300 spectrometer, a Varian GX400 was used for **6a** and **6h**. 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 EI, unless otherwise stated). Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ±0.4 of the theoretical values (Table 3). 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 Co., Fisher Scientific, Frontier, or Lancaster.

3.2.1. 3′-Formyl-4′-hydroxybiphenyl-4-carbonitrile (3a). To a stirred solution of 5-bromosalicyaldehyde (804 mg, 4 mmol), and tetrakis(triphenylphosphine) palladium (230 mg) in toluene (8 mL) under a nitrogen atmosphere was added 4 mL of a 2 M aqueous solution of Na_2CO_3 followed by 4-cyanophenylboronic acid (657 mg, 4.8 mmol) in 4 mL of methanol. The vigorously stirred mixture was warmed to 80 °C for 12 h. The solvent was evaporated, the precipitate was partitioned between methylene chloride (150 mL) and 2 M aqueous Na_2CO_3 (12 mL) containing 2 mL of concentrated ammonia. The organic layer was dried (Na_2SO_4), and then concentrated to dryness under reduced pressure to afford **3a** in 62% yield; mp 143.5–144 °C (EtOH). ^1H NMR (DMSO-d_6); δ 7.13 (d, J = 8.7 Hz, 1H), 7.83–7.91 (m, 4H), 7.94 (d, J = 8.7 Hz, 1H), 8.02 (s, 1H), 10.30 (s, 1H) 11.00 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 191.0, 161.1, 143.3, 134.7, 132.8, 129.3, 127.3, 126.8, 122.6, 118.8, 118.2, 109.5. MS (*m*/*z*, rel int.); 223 (M^+, 100), 204 (10), 177 (15), 164 (10), 140 (15). HRMS calcd for $C_{14}H_9NO_2$ ms 223.06333. Observed 223.06219. Anal. ($C_{14}H_9NO_2$) C, H, N.

3.2.2. 2-(4′-Cyano-4-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4a). A solution of **3a** (557.5 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 reaction mixture was distilled off under reduced pressure. The residue was triturated with ether and filtered off to afford **4a** in 90% yield, mp >340 °C (EtOH). ^1H NMR (DMSO-d_6); δ 7.20 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.86–7.98 (m, 6H), 8.28 (s, 1H), 8.57 (s, 1H), 12.80 (br s, 1H), 13.65 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 158.4, 153.9 143.5, 132.9, 131.0, 129.3, 126.7, 125.7, 119.7, 118.9, 118.1, 112.8, 109.4, 104.5. MS (*m*/*z*, rel int.); 336 (M^+, 100), 307 (25), 280 (5), 164 (10). HRMS calcd for $C_{21}H_{12}N_4O$ ms 336.10111. Observed 336.10189. Anal. ($C_{21}H_{12}N_4O{\cdot}0.25H_2O$) C, H, N.

3.2.3. 2-[4-Hydroxy-4′-(*N*-hydroxyamidino)-biphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine hydrochloride salt (5a). 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. This mixture was added to the bis cyanoderivative **4a** (1.5 mmol, 1 equiv). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then poured slowly onto ice-water (100 mL). The precipitate was filtered and washed with water to afford **5a** (free base) in 94% yield; mp 319–322 °C. ^1H NMR (DMSO-d_6); δ 5.87 (s, 4H), 7.13 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.69–8.02 (m, 7H), 8.45 (s, 1H), 9.60 (s, 1H), 9.63 (s, 1H), 13.20 (br s, 1H), 13.41 (br s, 1H). (**5a** hydrochoride salt), mp 301–303 °C. Anal. ($C_{21}H_{18}N_6O_3{\cdot}3.0HCl{\cdot}2.8H_2O$) C, H, N.

3.2.4. 2-(4′-Amidino-4-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6a). To a solution of **5a** (402 mg, 1 mmol) in glacial acetic acid (10 mL) was

Table 3. Elemental analysis data

Compound	Calculated			Found		
	C	H	N	C	H	N
3a ($C_{14}H_9NO_2$)	75.32	4.06	6.27	75.00	4.28	5.93
4a ($C_{21}H_{12}N_4O{\cdot}0.25H_2O$)	73.99	3.69	16.43	73.97	3.66	16.48
5a ($C_{21}H_{18}N_6O_3{\cdot}3.0HCl{\cdot}2.8H_2O$)	44.86	4.76	14.94	44.66	4.51	14.60
6a ($C_{21}H_{18}N_6O{\cdot}3.0AcOH{\cdot}1.8H_2O$)	55.62	5.79	14.41	55.30	5.59	14.42
3b ($C_{14}H_9NO_2$)	75.32	4.06		75.17	4.01	
4b ($C_{21}H_{12}N_4O{\cdot}0.3H_2O$)	73.80	3.71		73.46	3.62	
5b ($C_{21}H_{18}N_6O_3{\cdot}0.75H_2O$)	60.64	4.72		60.60	4.58	
6b ($C_{21}H_{18}N_6O{\cdot}3.0AcOH{\cdot}1.0H_2O$)	57.03	5.67	14.78	56.82	5.50	14.99
3c ($C_{15}H_{11}NO_3$)	71.13	4.37		70.91	4.48	
4c ($C_{22}H_{14}N_4O_2$)	72.12	3.85		72.30	3.67	
5c ($C_{22}H_{20}N_6O_4{\cdot}0.5H_2O$)	59.85	4.79		59.59	4.93	
6c ($C_{22}H_{20}N_6O_2{\cdot}3.0AcOH{\cdot}2.1H_2O$)	54.38	5.89	13.58	54.00	5.75	13.31
3d ($C_{14}H_9NO_2$)	75.32	4.06		75.21	4.07	
4d ($C_{21}H_{12}N_4O$)	74.98	3.59	16.65	74.63	3.68	16.52
5d ($C_{21}H_{18}N_6O_3{\cdot}1.5H_2O$)	58.73	4.92		58.60	4.77	
6d ($C_{21}H_{18}N_6O{\cdot}2.8AcOH{\cdot}0.8H_2O{\cdot}0.5EtOH$)	57.55	5.91	14.59	57.44	5.67	14.30
3e ($C_{15}H_{11}NO_2$)	75.94	4.67		75.81	4.70	
4e ($C_{22}H_{14}N_4O$)	75.42	4.03		75.50	4.09	
5e ($C_{22}H_{20}N_6O_3{\cdot}1.6H_2O$)	59.34	5.25		59.08	5.14	
6e ($C_{22}H_{20}N_6O{\cdot}2.6AcOH{\cdot}2.0H_2O$)	56.65	6.00	14.58	56.30	5.76	14.90
3f ($C_{14}H_9NO$)	81.14	4.37		80.84	4.43	
5f ($C_{21}H_{18}N_6O_2{\cdot}1.0H_2O$)	62.36	4.98		62.03	5.07	
6f ($C_{21}H_{18}N_6{\cdot}3.0AcOH{\cdot}2.0H_2O$)	56.83	6.00	14.72	56.60	5.78	14.75
3g ($C_{15}H_{11}NO$)	81.42	5.01		81.30	5.02	
4g ($C_{22}H_{14}N_4{\cdot}0.5H_2O$)	76.95	4.40		76.75	4.34	
5g ($C_{22}H_{20}N_6O_2{\cdot}1.1H_2O$)	62.87	5.32		62.59	5.10	
6g ($C_{22}H_{20}N_6{\cdot}2.8AcOH{\cdot}2.3H_2O$)	57.35	6.24	14.54	57.08	6.00	14.80
4h ($C_{21}H_{13}N_5O$)	71.78	3.72	19.93	71.51	3.72	19.86
5h ($C_{21}H_{19}N_7O_3{\cdot}1.25H_2O$)	57.33	4.92		57.12	4.93	
6h ($C_{21}H_{19}N_7O{\cdot}3.0AcOH{\cdot}1.5H_2O$)	54.56	5.79	16.49	54.78	5.90	16.33
3i ($C_{13}H_8N_2O$)	74.98	3.87		74.80	3.90	
4i ($C_{20}H_{11}N_5$)	74.75	3.45		74.48	3.67	
5i ($C_{20}H_{17}N_7O_2{\cdot}2.5H_2O$)	55.54	5.12	22.67	55.75	5.14	22.29
6i ($C_{20}H_{17}N_7{\cdot}2.8AcOH{\cdot}1.0H_2O$)	56.78	5.62	18.10	56.96	5.64	17.87

slowly added acetic anhydride (0.35 mL). After stirring for overnight TLC indicated complete acylation of the starting material, the solvent was removed under reduced pressure, then to the acetoxy derivative in 40 mL glacial acetic acid/ethanol (1:3) was added 10% palladium on carbon (80 mg). 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 washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **6a** in 78.5% yield, mp 223–224 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, $3\times CH_3$), 7.00 (d, $J = 8.4$ Hz, 1H), 7.48 (d, $J = 8.4$ Hz, 1H), 7.64–7.67 (m, 2H), 7.90 (s, 4H), 8.08 (s, 1H), 8.62 (s, 1H). Anal. ($C_{21}H_{18}N_6O{\cdot}3.0AcOH{\cdot}1.8H_2O$) C, H, N.

3.2.5. 3′-Formyl-4′-hydroxybiphenyl-3-carbonitrile (3b). The same procedure described for **3a** was used employing 3-cyanophenylboronic acid instead of 4-cyanophenylboronic acid. Yield 70%; mp 139–140 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.12 (d, $J = 8.7$ Hz, 1H), 7.62–7.81 (m, 2H), 7.92–8.02 (m, 3H), 8.14 (s, 1H), 10.32 (s, 1H) 11.00 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 191.3, 160.7, 140.0, 134.6, 130.8, 130.5, 130.1, 129.5, 129.2, 127.4, 122.5, 118.7, 118.0, 112.0. MS (*m*/*z*, rel int.); 223 (M^+, 100), 204 (10), 193 (5), 177 (20), 166 (15), 140 (15). Anal. ($C_{14}H_9NO_2$) C, H.

3.2.6. 2-(3′-Cyano-4-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4b). The same procedure described for **4a** was used starting with **3b**. Yield 89%, mp >340 °C (EtOH). ^{1}H NMR (DMSO-d_6); δ 7.20 (d, $J = 8.4$ Hz, 1H), 7.67–7.75 (m, 2H), 7.82–7.88 (m, 3H), 8.10 (d, $J = 8.4$ Hz, 1H), 8.21 (s, 1H), 8.26 (s, 1H), 8.55 (s, 1H), 13.20 (br s, 2H). ^{13}C NMR; δ 158.2, 154.0, 140.2, 130.8, 130.7, 130.5, 130.2, 129.4, 129.1, 126.3, 125.4, 119.7, 118.8, 118.0, 112.7, 112.1, 104.7. MS (*m*/*z*, rel int.); 336 (M^+, 100), 307 (20), 306 (12), 168 (5), 140 (5). HRMS calcd for $C_{21}H_{12}N_4O$: 336.10111. Observed 336.10247. Anal. ($C_{21}H_{12}N_4O$–$0.3H_2O$) C, H.

3.2.7. 2-[4-Hydroxy-3′-(*N*-hydroxyamidino)-biphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5b). The same procedure described for **5a** was used starting with **4b**. Yield 97%, mp >340 °C. ^{1}H NMR (DMSO-d_6); δ 5.89 (s, 4H), 7.20 (d, $J = 8.4$ Hz, 1H), 7.58 (d, $J = 8.4$ Hz, 1H), 7.63–8.90 (m, 6H), 8.09 (s, 1H), 8.50 (s, 1H), 9.70 (s, 2H), 13.20 (br s, 1H), 13.44 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 157.7, 152.4, 151.2, 150.9,

139.3, 134.1, 131.2, 130.2, 128.7, 126.7, 124.5, 124.1, 123.4, 117.8, 115.0, 112.8, 108.6. Anal. ($C_{21}H_{18}N_6O_3 \cdot 0.75H_2O$) C, H.

3.2.8. 2-(3′-Amidino-4-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6b). The same procedure described for **6a** was used starting with **5b**. Yield 80%, mp 208–209 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, $3 \times CH_3$), 7.02 (d, $J = 8.4$ Hz, 1H), 7.47 (d, $J = 8.4$ Hz, 1H), 7.62–7.72 (m, 4H), 8.00 (d, $J = 7.2$ Hz, 1H), 8.07 (s, 1H), 8.11 (s, 1H), 8.66 (s, 1H). Anal. ($C_{21}H_{18}N_6O \cdot 3.0AcOH \cdot 1.0H_2O$) C, H, N.

3.2.9. 5′-Formyl-4′-hydroxy-3′-methoxybiphenyl-4-carbonitrile (3c). The same procedure described for **3a** was used employing 5-bromo-2-hydroxy-3-methoxybenzaldehyde instead of 5-bromosalicyaldehyde. Yield 57%; mp 177–177.5 °C. ^{1}H NMR (DMSO-d_6); δ 3.97 (s, 3H), 7.61 (s, 2H), 7.90 (s, 4H), 10.33 (s, 1H), 10.57 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 191.3, 151.2, 149.0, 143.7, 132.7, 129.2, 127.1, 122.6, 118.9, 118.3, 115.7, 109.5, 56.3. MS (*m*/*z*, rel int.); 253 (M^+, 100), 210 (8), 207 (10), 177 (10), 154 (15), 127 (18). HRMS calcd for $C_{15}H_{11}NO_3$: 253.07389. Observed 253.07181. Anal. ($C_{15}H_{11}NO_3$) C, H.

3.2.10. 2-(4′-Cyano-4-hydroxy-5-methoxybiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4c). The same procedure described for **4a** was used starting with **3c**. Yield 79%, mp 334–335 °C. ^{1}H NMR (DMSO-d_6); δ 3.98 (s, 3H), 7.49 (s, 1H), 7.68 (d, $J = 8.4$ Hz, 1H), 7.84 (d, $J = 8.4$ Hz, 1H), 7.94–8.00 (m, 4H), 8.13 (s, 1H), 8.25 (s, 1H), 13.2 (br s, 2H). ^{13}C NMR; δ 154.2, 149.1, 148.9, 143.8, 132.7, 129.0, 126.9, 126.8, 119.6, 118.9, 116.8, 112.8, 112.3, 109.4, 104.7, 99.4, 56.0. MS (*m*/*z*, rel int.); 366 (M^+, 100), 348 (42), 337 (20), 323 (30), 307 (10). HRMS calcd for $C_{22}H_{14}N_4O_2$: 366.11168. Observed 366.11188. Anal. ($C_{22}H_{14}N_4O_2$) C, H.

3.2.11. 2-[4-Hydroxy-4′-(*N*-hydroxyamidino)-5-methoxybiphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5c). The same procedure described for **5a** was used starting with **4c**. Yield 99%, mp 319–321 °C. ^{1}H NMR (DMSO-d_6); δ 4.00 (s, 3H), 5.89 (s, 4H), 7.43 (s, 1H), 7.59–7.74 (m, 2H), 7.82–7.88 (m, 4H), 8.05 (s, 1H), 8.09 (s, 1H), 9.61 (s, 1H), 9.69 (s, 1H), 13.40 (br s, 2H). Anal. ($C_{22}H_{20}N_6O_4 \cdot 0.5H_2O$) C, H.

3.2.12. 2-(4′-Amidino-4-hydroxy-5-methoxybiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6c). The same procedure described for **6a** was used starting with **5c**. Yield 75%, mp 230–231 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, $3 \times CH_3$), 3.93 (s, 3H), 7.27 (s, 1H), 7.47 (d, $J = 8.4$ Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.87–7.97 (m, 4H), 8.07 (s, 1H), 8.24 (s, 1H). MS (*m*/*z*, rel int.); 383 ($M^+ - NH_3$, 10), 366 (50), 351 (10), 336 (100). Anal. ($C_{22}H_{20}N_6O_2 \cdot 3.0AcOH \cdot 2.1H_2O$) C, H, N.

3.2.13. 3′-Formyl-2′-hydroxybiphenyl-4-carbonitrile (3d). The same procedure described for **3a** was used employing 3-bromosalicyaldehyde[23] instead of 5-bromosalicyaldehyde. Yield 58%; mp 120–121 °C (hexanes/ether). ^{1}H NMR ($CDCl_3$); δ 7.25 (t, $J = 7.8$ Hz, 1H), 7.60–7.70 (m, 2H), 7.73–7.80 (m, 4H), 9.97 (s, 1H), 11.64 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 197.4. 157.5, 141.0, 137.4, 134.2, 132.1, 130.0, 128.0, 121.5, 120.4, 118.8, 110.0. MS (*m*/*z*, rel int.); 223 (M^+, 100), 204 (20), 195 (25), 177 (25), 140 (20). HRMS calcd for $C_{14}H_9NO_2$: 223.06333. Observed 223.06256. Anal. ($C_{14}H_9NO_2$) C, H.

3.2.14. 2-(4′-Cyano-2-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4d). The same procedure described for **4a** was used starting with **3d**. Yield 84%, mp 318–320 °C. ^{1}H NMR (DMSO-d_6); δ 7.18 (t, $J = 7.5$ Hz, 1H), 7.56 (d, $J = 7.5$ Hz, 1H), 7.71 (d, $J = 8.4$ Hz, 1H), 7.78 (d, $J = 7.5$ Hz, 1H), 7.84 (d, $J = 8.1$ Hz, 2H), 7.91 (d, $J = 8.1$ Hz, 2H), 8.14 (d, $J = 8.4$ Hz, 1H), 8.28 (s, 1H), 13.70 (br s, 1H), 13.90 (br s, 1H). MS (*m*/*z*, rel int.); 337 ($M^+ + 1$, 68), 309 (100), 293 (40). Anal. ($C_{21}H_{12}N_4O$) C, H, N.

3.2.15. 2-[2-Hydroxy-4′-(*N*-hydroxyamidino)-biphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5d). The same procedure described for **5a** was used starting with **4d**. Yield 100%, mp 322–325 °C. ^{1}H NMR (DMSO-d_6); δ 6.00 (s, 4H), 7.13 (t, $J = 7.8$ Hz, 1H), 7.49 (d, $J = 7.2$ Hz, 1H), 7.61–7.77 (m, 6H), 7.88–8.08 (m, 2H), 9.70 (s, 2H), 13.40 (br s, 1H), 13.90 (br s, 1H). Anal. ($C_{21}H_{18}N_6O_3 \cdot 1.5H_2O$) C, H.

3.2.16. 2-(4′-Amidino-2-hydroxybiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6d). The same procedure described for **6a** was used starting with **5d**. Yield 90%, mp 228–229.5 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, $2.8 \times CH_3$), 6.97 (t, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 7.5$ Hz, 1H), 7.45 (d, $J = 7.5$ Hz, 1H), 7.60 (d, $J = 7.8$ Hz, 1H), 7.86 (d, $J = 8.4$ Hz, 2H), 7.93 (d, $J = 8.4$ Hz, 2H), 8.05 (s, 1H), 8.27 (d, $J = 7.8$ Hz, 1H). MS (*m*/*z*, rel int.); 371 ($M^+ + 1$, 5), 354 (5), 337 (100), 336 (50), 307 (10). Anal. ($C_{21}H_{18}N_6O \cdot 2.8AcOH \cdot 0.8H_2O \cdot 0.5EtOH$) C, H, N.

3.2.17. 3′-Formyl-4′-methoxybiphenyl-4-carbonitrile (3e). To a stirred solution of 4-bromobenzonitrile (910 mg, 5 mmol), and tetrakis(triphenylphosphine) palladium (288 mg) in toluene (10 mL) under a nitrogen atmosphere was added 5 mL of a 2 M aqueous solution of Na_2CO_3 followed by 3-formyl-4-methoxyphenylboronic acid (1080 mg, 6 mmol) in 5 mL of methanol. The vigorously stirred mixture was warmed to 80 °C for 12 h. The solvent was evaporated, the precipitate was partitioned between methylene chloride (200 mL) and 2 M aqueous Na_2CO_3 (15 mL) containing 3 mL of concentrated ammonia. The organic layer was dried (Na_2SO_4), and then concentrated to dryness under reduced pressure to afford **3e** in 70% yield; mp 146–147 °C (SiO_2, hexanes/EtOAc, 90:10). ^{1}H NMR (DMSO-d_6); δ 4.00 (s, 3H), 7.39 (d, $J = 9.0$ Hz, 1H), 7.88–7.94 (m, 4H), 8.03 (d, $J = 2.7$ Hz, 1H), 8.09 (dd, $J = 9.0$, 2.7 Hz, 1H), 10.40 (s, 1H). ^{13}C NMR; δ 188.9, 161.7, 143.1, 134.7, 132.8, 130.5, 127.0, 126.2, 124.4, 118.8, 113.7, 109.8, 56.2. MS (*m*/*z*, rel int.); 237 (M^+, 100), 220 (20), 208 (10), 191 (25), 177 (35), 140 (20). Anal. ($C_{15}H_{11}NO_2$) C, H.

3.2.18. 2-(4′-Cyano-4-methoxybiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4e). The same procedure described for **4a** was used starting with **3e**. Yield 88%, mp 289–290 °C. ^{1}H NMR (DMSO-d_6); δ 4.09 (s, 3H), 7.43 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.81 (s, 1H), 7.94–8.22 (m, 6H), 8.67 (s, 1H), 12.63 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 157.5, 151.1, 143.5, 142.1, 137.9, 132.9, 130.9, 130.7, 128.3, 127.1, 125.7, 125.6, 123.4, 120.1, 118.9, 117.7, 113.3, 113.2, 109.6, 103.8, 56.3. MS (*m*/*z*, rel int.); 350 (M^+, 100), 321 (40), 306 (12), 144 (50). HRMS calcd for $C_{22}H_{14}N_4O$: 350.11676. Observed 350.11569. Anal. ($C_{22}H_{14}N_4O$) C, H.

3.2.19. 2-[4′-(*N*-Hydroxyamidino)-4-methoxybiphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5e). The same procedure described for **5a** was used starting with **4e**. Yield 95%, mp >340 °C. ^{1}H NMR (DMSO-d_6); δ 4.09 (s, 3H), 6.09 (s, 2H), 6.71 (s, 2H), 7.38 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.87 (dd, J = 8.4, 2.4 Hz, 1H), 7.99 (s, 1H), 8.64 (d, J = 2.4 Hz, 1H), 9.80 (s, 1H), 10.0 (s, 1H), 12.55 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 156.6, 151.0, 150.0, 139.7, 137.9, 132.1, 131.5, 129.6, 127.6, 126.1, 125.9, 120.4, 118.0, 112.9, 56.1. FABMS (*m*/*z*, rel int.); 417 (M^+ + 1, 100), 401 (58), 394 (30), 368 (20), 350 (10). HRMS calcd for $C_{22}H_{21}N_6O_3$: 417.16751. Observed 417.16760. Anal. ($C_{22}H_{20}N_6O_3 \cdot 1.6H_2O$) C, H.

3.2.20. 2-(4′-Amidino-4-methoxybiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6e). The same procedure described for **6a** was used starting with **5e**. Yield 62%, mp 220–221 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.78 (s, 2.6 × CH_3), 4.11 (s, 3H), 7.43 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.87–7.96 (m, 4H), 8.15 (dd, J = 8.4, 2.4 Hz, 1H), 8.31 (s, 1H), 8.69 (d, J = 2.4 Hz, 1H). Anal. ($C_{22}H_{20}N_6O \cdot 2.6AcOH \cdot 2.0H_2O$) C, H, N.

3.2.21. 3′-Formylbiphenyl-4-carbonitrile (3f). The same procedure described for **3e** was used employing 3-formylphenylboronic acid instead of 3-formyl-4-methoxyphenylboronic acid. Yield 80%, mp 122–123 °C. ^{1}H NMR (DMSO-d_6); δ 7.72–7.78 (m, 1H), 7.98–8.03 (m, 5H), 8.11 (m, 1H), 8.29 (s, 1H), 10.13 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 193.0, 143.3, 139.0, 136.9, 132.98, 132.95, 130.0, 129.0, 128.5, 127.7, 118.7, 110.6. Anal. ($C_{14}H_9NO$) C, H.

3.2.22. 2-(4′-Cyanobiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4f). The same procedure described for **4a** was used starting with **3f**. Yield 81%, mp 303–304 °C. ^{1}H NMR (DMSO-d_6); δ 7.63 (d, J = 8.4 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.00–8.06 (m, 4H), 8.19 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.57 (s, 1H), 13.60 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 153.9, 143.7, 139.0, 132.9, 130.07, 130.01, 129.2, 127.7, 127.1, 125.8, 125.4, 119.9, 118.7, 110.5, 104.1. MS (*m*/*z*, rel int.); 320 (M^+, 100), 291 (5), 204 (5), 177 (8), 151 (3). HRMS calcd for $C_{21}H_{12}N_4$: 320.10620. Observed 320.10644.

3.2.23. 2-[4′-(*N*-Hydroxyamidino)biphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5f). The same procedure described for **5a** was used starting with **4f**. Yield 93%, mp 317–319 °C. ^{1}H NMR (DMSO-d_6); δ 5.89 (s, 2H), 5.92 (s, 2H), 7.60–7.70 (m, 3H), 7.80–7.98 (m, 5H), 8.00 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.52 (s, 1H), 9.60 (s, 1H), 9.74 (s, 1H), 13.11 (br s, 1H). Anal. ($C_{21}H_{18}N_6O_2 \cdot 1.0H_2O$) C, H.

3.2.24. 2-(4′-Amidinobiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6f). The same procedure described for **6a** was used starting with **5f**. Yield 72%, mp 211–212 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.78 (s, 3 × CH_3), 7.60 (d, J = 8.4 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.96 (d, J = 8.7 Hz, 2H), 8.03 (d, J = 8.7 Hz, 2H), 8.12 (s, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.65 (s, 1H). Anal. ($C_{21}H_{18}N_6 \cdot 3.0AcOH \cdot 2.0H_2O$) C, H, N.

3.2.25. 3′-Formyl-2-methylbiphenyl-4-carbonitrile (3g). The same procedure described for **3f** was used employing 4-bromo-3-methylbenzonitrile instead of 4-bromobenzonitrile. Yield 82%, mp 86–86.5 °C. ^{1}H NMR (DMSO-d_6); δ 2.27 (s, 3H), 7.47 (d, J = 7.8 Hz, 1H), 7.71–7.79 (m, 3H), 7.85 (s, 1H), 7.90 (s, 1H), 7.95–7.98 (m, 1H), 10.08 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 193.0, 144.8, 140.3, 136.8, 136.3, 134.7, 133.9, 130.5, 130.0, 129.8, 129.4, 128.4, 118.7, 110.6, 19.7. Anal. ($C_{15}H_{11}NO$) C, H.

3.2.26. 2-(4′-Cyano-2′-methylbiphenyl-3-yl)-1*H*-benzimidazole-5-carbonitrile (4g). The same procedure described for **4a** was used starting with **3g**. Yield 75%, mp 247–250 °C. ^{1}H NMR (DMSO-d_6); δ 2.26 (s, 3H), 7.50–7.63 (m, 3H), 7.67–7.80 (m, 3H), 7.87 (s, 1H), 8.17 (d, J = 7.8 Hz, 2H), 8.28 (d, J = 7.8 Hz, 1H), 13.40 (br s, 1H). MS (*m*/*z*, rel int.); 334 (M^+, 100), 215 (5), 190 (20), 167 (6). HRMS calcd for $C_{22}H_{14}N_4$: 334.12185. Observed 334.12142. Anal. ($C_{22}H_{14}N_4 \cdot 0.5H_2O$) C, H.

3.2.27. 2-[4′-(*N*-Hydroxyamidino)-2′-methylbiphenyl-3-yl]-1*H*-benzimidazole-5-*N*-hydroxyamidine (5g). The same procedure described for **5a** was used starting with **4g**. Yield 99%, mp 295–297 °C. ^{1}H NMR (DMSO-d_6); δ 2.30 (s, 3H), 5.87 (s, 4H), 7.32 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.61–7.97 (m, 6H), 8.15 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 9.59 (s, 1H), 9.68 (s, 1H), 13.02 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 151.5, 150.5, 141.4, 141.0, 134.5, 132.5, 130.3, 130.0, 129.3, 128.9, 127.3, 127.2, 126.7, 125.2, 123.0, 119.8, 118.2, 115.9, 110.7, 108.4, 20.2. FABMS (*m*/*z*, rel int.); 401 (M^+ + 1, 100), 386 (55), 368 (30), 352 (15), 335 (10). HRMS calcd for $C_{22}H_{21}N_6O_2$: 401.17260. Observed 401.17287. Anal. ($C_{22}H_{20}N_6O_2 \cdot 1.1H_2O$) C, H.

3.2.28. 2-(4′-Amidino-2′-methylbiphenyl-3-yl)-1*H*-benzimidazole-5-amidine acetate salt (6g). The same procedure described for **6a** was used starting with **5g**. Yield 83%, mp 201–203 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, 2.8 × CH_3), 2.33 (s, 3H), 7.50–7.59 (m, 2H), 7.62–7.78 (m, 3H), 7.82–7.95 (m, 2H), 8.12 (s, 1H), 8.21–8.34 (m, 2H). Anal. ($C_{22}H_{20}N_6 \cdot 2.8AcOH \cdot 2.3H_2O$) C, H, N.

3.2.29. 6-(3-Formyl-4-methoxyphenyl)-nicotinonitrile (3h). The same procedure described for **3e** was used employing 6-chloronicotinonitrile[24] instead of 4-bromobenzonitrile. Yield 66.5%; mp 197–198 °C (EtOH). ^{1}H NMR ($CDCl_3$); δ 4.00 (s, 3H), 7.16 (d, $J = 8.4$ Hz, 1H), 7.87 (d, $J = 8.4$ Hz, 1H), 8.00 (dd, $J = 8.4$, 2.1 Hz, 1H), 8.41 (dd, $J = 8.4$, 2.1 Hz, 1H), 8.46 (d, $J = 2.1$ Hz, 1H), 8.92 (d, $J = 2.1$ Hz, 1H), 10.57 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 188.5, 162.7, 157.5, 152.0, 140.4, 134.3, 129.1, 126.7, 124.3, 119.2, 116.8, 113.1, 106.7, 56.1. MS (*m*/*z*, rel int.); 238 (M^+, 100), 221 (32), 209 (15), 192 (25), 178 (25), 166 (20). HRMS calcd for $C_{14}H_{10}N_2O_2$: 238.07423. Observed 238.07486.

3.2.30. 2-[5-(5-Cyanopyridin-2-yl)-2-methoxyphenyl]-1*H*-benzimidazole-5-carbonitrile (4h). The same procedure described for **4a** was used starting with **3h**. Yield 92%, mp 316.5–319 °C. ^{1}H NMR (DMSO-d_6); δ 4.07 (s, 3H), 7.44 (d, $J = 8.4$ Hz, 1H), 7.58–7.87 (m, 2H), 8.03–8.36 (m, 4H), 9.09 (s, 1H), 9.20 (d, $J = 2.1$ Hz, 1H), 12.60 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 158.7, 157.9, 152.4, 151.4, 140.8, 130.8, 129.6, 129.1, 125.4, 119.9, 119.4, 117.5, 117.2, 112.9, 106.8, 103.8, 56.3. MS (*m*/*z*, rel int.); 351 (M^+, 100), 322 (40), 293 (10), 143 (35). HRMS calcd for $C_{21}H_{13}N_5O$: 351.11201. Observed 351.11067. Anal. ($C_{21}H_{13}N_5O$) C, H, N.

3.2.31. 2-{5-[5-(*N*-Hydroxyamidino)-pyridin-2-yl]-2-methoxyphenyl}-1*H*-benzimidazole-5-*N*-hydroxyamidine (5h). The same procedure described for **5a** was used starting with **4h**. Yield 99%, mp 276–279 °C. ^{1}H NMR (DMSO-d_6); δ 4.08 (s, 3H), 5.83 (s, 2H), 6.02 (s, 2H), 7.37 (d, $J = 8.4$ Hz, 1H), 7.58–7.68 (m, 2H), 7.95 (s, 1H), 8.02 (d, $J = 8.4$ Hz, 1H), 8.11 (d, $J = 8.4$ Hz, 1H), 8.22 (dd, $J = 8.4$, 2.1 Hz, 1H), 8.95 (s, 1H), 9.13 (d, $J = 2.1$ Hz, 1H), 9.57 (s, 1H), 9.87 (s, 1H), 12.30 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 157.6, 155.2, 152.1, 151.9, 149.7, 148.8, 146.5, 133.9, 130.9, 129.4, 128.0, 127.2, 118.8, 118.2, 112.6, 56.1. FABMS (*m*/*z*, rel int.); 418 ($M^+ + 1$, 70), 401 (40), 385 (23), 327 (50), 237 (100). HRMS calcd for $C_{21}H_{20}N_7O_3$: 418.16276. Observed 418.16178. Anal. ($C_{21}H_{19}N_7O_3 \cdot 1.25H_2O$) C, H.

3.2.32. 2-[5-(5-Amidinopyridin-2-yl)-2-methoxyphenyl]-1*H*-benzimidazole-5-amidine acetate salt (6h). The same procedure described for **6a** was used starting with **5h**. Yield 80%, mp 231–232 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.83 (s, $3 \times CH_3$), 4.18 (s, 3H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.78–7.88 (m, 2H), 8.10–8.33 (m, 3H), 9.00 (s, 1H), 9.17 (s, 1H). MS (*m*/*z*, rel int.); 385 (M^+, 4), 351 (100), 337 (75). Anal. ($C_{21}H_{19}N_7O \cdot 3.0AcOH \cdot 1.5H_2O$) C, H, N.

3.2.33. 6-(3-Formylphenyl)-nicotinonitrile (3i). The same procedure described for **3h** was used employing 3-formylphenylboronic acid instead of 3-formyl-4-methoxyphenylboronic acid. Yield 58%; mp 182–183 °C. ^{1}H NMR (DMSO-d_6); δ 7.77 (t, $J = 7.8$ Hz, 1H), 8.04 (d, $J = 7.8$ Hz, 1H), 8.29 (d, $J = 8.4$ Hz, 1H), 8.43 (dd, $J = 8.4$, 2.1 Hz, 1H), 8.48 (d, $J = 7.8$ Hz, 1H), 8.69 (s, 1H), 9.13 (d, $J = 2.1$ Hz, 1H), 10.13 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 192.8, 157.7, 152.4, 141.1, 137.6, 136.8, 132.7, 130.7, 129.8, 128.3, 120.4, 116.9, 107.9. MS (*m*/*z*, rel int.); 208 (M^+, 100), 179 (70), 152 (15), 125 (5). HRMS calcd for $C_{13}H_8N_2O$: 208.06366. Observed 208.06066. Anal. ($C_{13}H_8N_2O$) C, H.

3.2.34. 2-[3-(5-Cyanopyridin-2-yl)-phenyl]-1*H*-benzimidazole-5-carbonitrile (4i). The same procedure described for **4a** was used starting with **3i**. Yield 83.5%, mp 281–282 °C. ^{1}H NMR (DMSO-d_6); δ 7.63 (d, $J = 7.8$ Hz, 1H), 7.76 (t, $J = 7.8$ Hz, 1H), 7.81–7.88 (m, 1H), 8.24–8.38 (m, 4H), 8.49 (d, $J = 7.8$ Hz, 1H), 9.03 (s, 1H), 9.18 (s, 1H), 13.61 (br s, 1H). ^{13}C NMR (DMSO-d_6); δ 158.2, 152.5, 141.1, 137.6, 129.8, 129.2, 128.7, 126.0, 125.5, 124.0, 120.3, 119.9, 117.1, 112.9, 107.8, 104.0. MS (*m*/*z*, rel int.); 321 (M^+, 100), 293 (12), 268 (5). HRMS calcd for $C_{20}H_{11}N_5$: 321.10145. Observed 321.10069. Anal. ($C_{20}H_{11}N_5$) C, H.

3.2.35. 2-{3-[5-(*N*-Hydroxyamidino)-pyridin-2-yl]-phenyl}-1*H*-benzimidazole-5-*N*-hydroxyamidine (5i). The same procedure described for **5a** was used starting with **4i**. Yield 97%, mp 295–297 °C. ^{1}H NMR (DMSO-d_6); 5.82 (s, 2H), 6.08 (s, 2H), 7.51–7.72 (m, 3H), 8.00 (s, 1H), 8.11–8.28 (m, 4H), 8.95 (s, 1H), 9.03 (s, 1H), 9.61 (s, 1H), 9.93 (s, 1H). 13.18 (br s, 1H). Anal. ($C_{20}H_{17}N_7O_2 \cdot 2.5H_2O$) C, H, N.

3.2.36. 2-[3-(5-Amidinopyridin-2-yl)-phenyl]-1*H*-benzimidazole-5-amidine acetate salt (6i). The same procedure described for **6a** was used starting with **5i**. Yield 73%, mp 198–200 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, $2.8 \times CH_3$), 7.64 (d, $J = 8.1$ Hz, 1H), 7.72–7.81 (m, 2H), 8.14 (s, 1H), 8.24–8.40 (m, 4H), 9.08 (s, 1H), 9.12 (s, 1H). MS (*m*/*z*, rel int.); 356 ($M^+ + 1$, 5), 322 (100), 297 (5). Anal. ($C_{20}H_{17}N_7 \cdot 2.8AcOH \cdot 1.0H_2O$) C, H, N.

Acknowledgements

This work was supported by NIH Grant GM-61587, an award from the Bill and Melinda Gates Foundation and M.A.I. was awarded a Fellowship by ICSC-World Laboratory.

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