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Exploration of larger central ring linkers in furamidine analogues: Synthesis and evaluation of their DNA binding, antiparasitic and fluorescence properties

Abdelbasset A. Farahat [a,b], Ekaterina Paliakov [a], Arvind Kumar [a], Alaa-Eldin M. Barghash [b], Fatma E. Goda [b], Hassan M. Eisa [b], Tanja Wenzler [c,d], Reto Brun [c,d], Yang Liu [a], W. David Wilson [a], David W. Boykin [a,*]

[a] Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA
[b] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[c] Swiss Tropical and Public Health Institute, Basel CH-4002, Switzerland
[d] University of Basel, Basel CH-4002, Switzerland

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ABSTRACT

The effects of replacing the central furan ring of furamidine with indole and benzimidazole on their DNA binding affinity, antiparasitic activity and fluorescence are reported. The bis-cyanophenylindoles required to make the corresponding amidines were prepared by sequential Stille and/or Suzuki coupling reactions. The bis-cyanophenylbenzimidazoles were obtained by coupling 4-cyanobenzaldehydes with the appropriate cyano substituted phenylenediamine. The bis-nitriles were converted to the diamidines by reaction with $LiN[Si(CH_3)_3]_2$ or by Pinner methodology. Specifically, we have prepared new series of 2,6- and 2,5-diaryl indoles (**6a**,**b**, **12** and **17a**–**d**) and the related benzimidazoles (**24**, **30** and **35**). The new compounds bind in the DNA minor groove in DNA AT base pair sequences and eight of the ten new analogues exhibit ΔT_m values comparable to or higher than that of furamidine. Six of ten of the new compounds exhibit lower IC_{50} values against *Trypanosoma brucei rhodesiense* (*T. b. r.*) and eight of ten exhibit lower IC_{50} values against *Plasmodium falciparum* (*P. f.*) than furamidine. Four of the ten show greater efficacy than furamidine in the rigorous *T. b. r.* STIB900 mouse model for African trypanosomiasis. Generally, the fluorescence properties of the new analogues are similar to that of DAPI.

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1. Introduction

The antimicrobial activity of aromatic diamidines was first reported in the 1930s.[1] Numerous dicationic systems have been subsequently investigated, with the primary objective of discovering useful therapeutic agents, however pentamidine (**I**, Fig. 1) is the only compound of this class which has seen significant human use.[2] Furamidine (**IIa**, Fig. 1), was found to be more potent and less toxic than pentamidine in murine models of Trypanosomiasis.[3] An orally effective prodrug of furamidine (**IIb**, pafuramidine, Fig. 1), showed promising results in Phase II clinical trials against both African sleeping sickness and malaria.[2,3] Unfortunately, in an additional safety study of pafuramidine paralleling Phase III trials, liver and kidney toxicities in some volunteers were found and the development of pafuramidine was terminated.[3] A number of spacers between the amidinophenyl moieties have been investigated in search for effective compounds including; thiophene,[4] pyrrole,[5] *N*-methyl pyrrole,[4] phenyl,[6] selenophene,[7] isoxazole[8] triazole[9] and thiazole.[10] Only the triazole spacer was found to be more effective than the furan linker. DAPI (**III**, 4′,6-diamidino-2-phenylindole, Fig. 1), was developed as an antitrypanosomal agent related to diminazene and stilbamidine.[10] DAPI showed a variety of biological effects, including antifungal, antibacterial, antitrypanosomal and antiviral activities.[11,12] DAPI, an important tool in molecular biology, is a fluorescent dye which exhibits several binding modes to DNA[13] and it has been frequently utilized as a DNA specific probe for flow cytometry, chromosome staining, DNA visualization and quantitation.[14] The near linear molecules, DB921 (**IV**)[15] and DB1883 (**V**)[16] (Fig. 1) are active antiparasitic agents, that have a new sequence of aryl rings, a biphenyl at 2-position of benzimidazole or indole, respectively. Here we report the replacement of the furan linker of IIa with the larger indole and benizimidazole rings (new curved isomers of **IV** and **V**) in anticipation of finding enhanced DNA binding and improved physiochemical properties. The new compounds may be viewed as DAPI analogues with increased length and the effects of these modifications on DNA binding and antiprotozoal activity are evaluated. Specifically, we have prepared new series of 2,6 and 2,5-diaryl indoles (**6a**,**b**, **12** and **17a**–**d**) and the related benzimidazoles (**24**, **30** and **35**) all as hydrochloride salts.

* Corresponding author. Tel.: +1 404 413 5498; fax: +1 404 413 5505.
E-mail address: dboykin@gsu.edu (D.W. Boykin).

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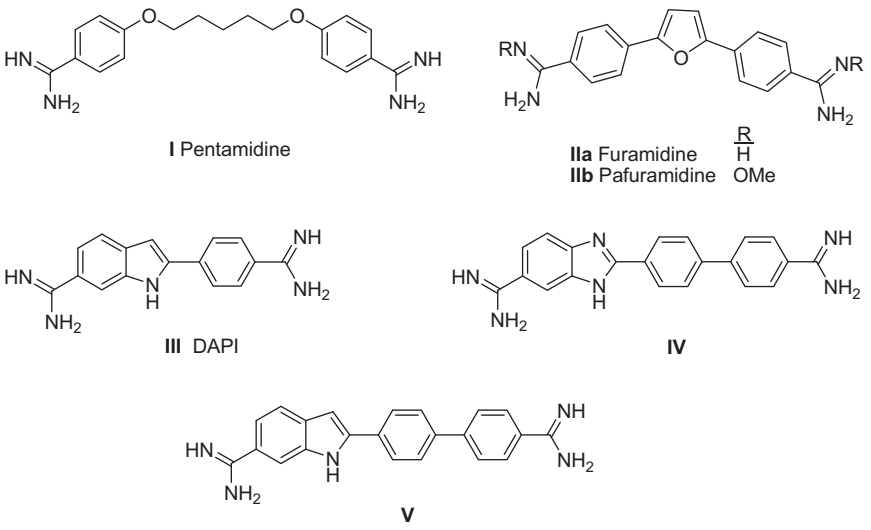
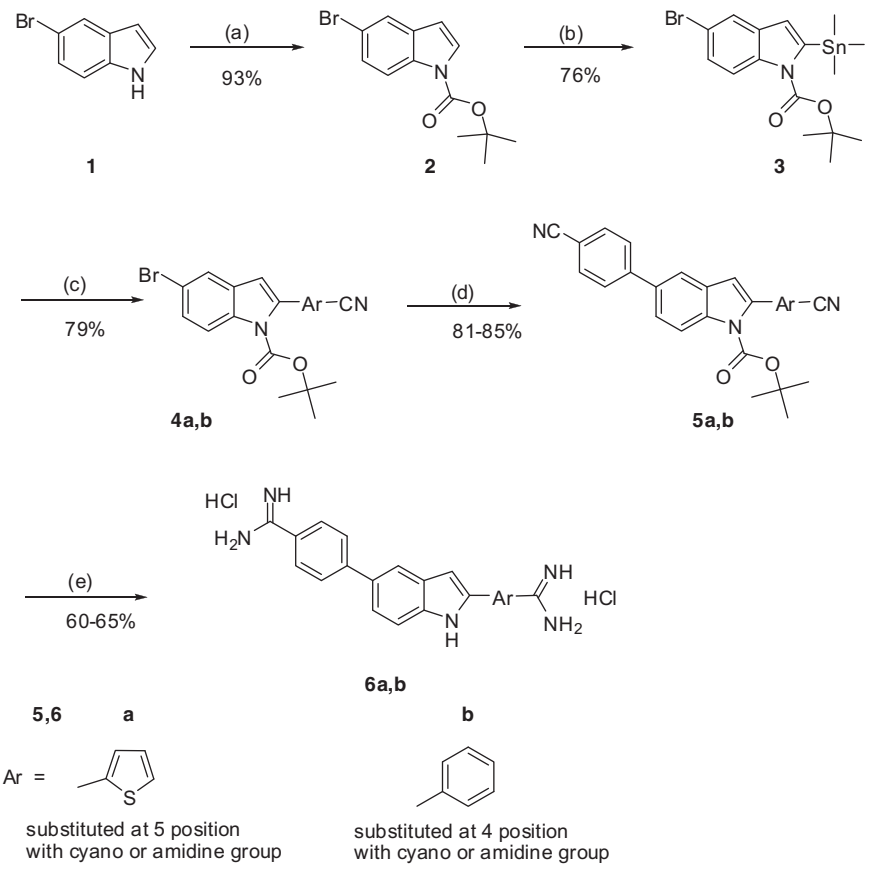


Figure 1.

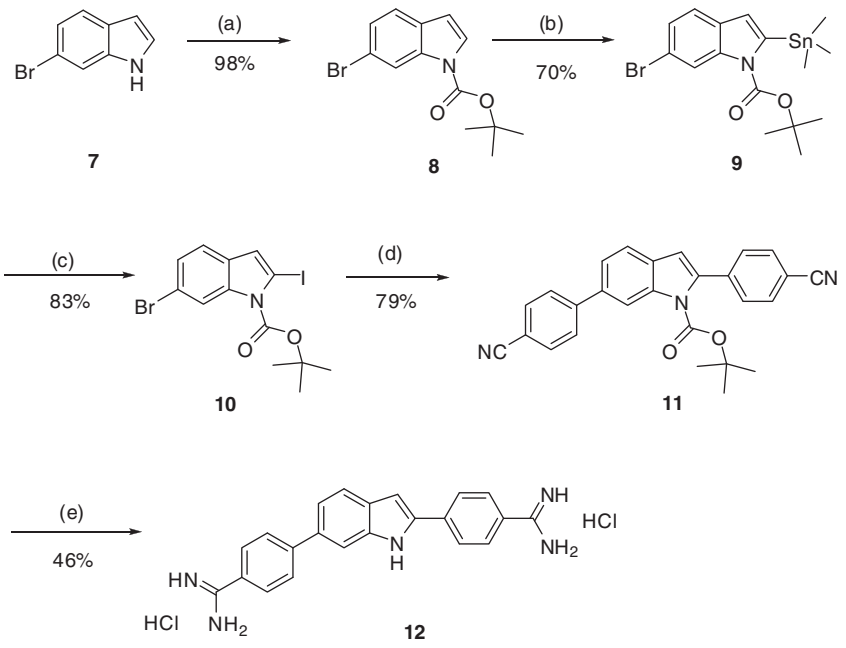


Scheme 1. Reagents and conditions: (a) $(BOC)_2O$, DMAP,CH_2Cl_2; (b) $ClSn(CH_3)_3$, LDA/THF; (c) Br-Ar-CN, $Pd(PPh_3)_4$, dioxane; (d) *p*-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene; (e) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines our approach to the synthesis of the hydrochloride salts of 2,5-diaryl indole diamidines **6a**,**b**. The Boc-protected indole **2** and the indole stannane **3** were prepared in high yield by employing a procedure we have used previously.[17] A Stille coupling reaction[18] between the commercially available haloaryl nitriles and the indole stannane **3** using 5 mol % $Pd(PPh_3)_4$ and 1,4-dioxane as the solvent afforded **4a**,**b** in good yield. The dinitriles **5a**,**b** were prepared in good yield by employing a Suzuki coupling reaction[19] between *p*-cyanophenylboronic acid and the 5-bromoindole derivatives **4a**,**b** in the presence of 5 mol % $Pd(PPh_3)_4$. The dinitriles **5a**,**b** were allowed to react with lithium bis(trimethylsilyl)amide in THF,[20] followed by deprotection of



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, DMAP,CH_2Cl_2; (b) $ClSn(CH_3)_3$, LDA/THF; (c) I_2/THF; (d) *p*-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene; (e) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.

the silylated amidines with ethanolic HCl to furnish the hydrochloride salts of **6a**,**b**.

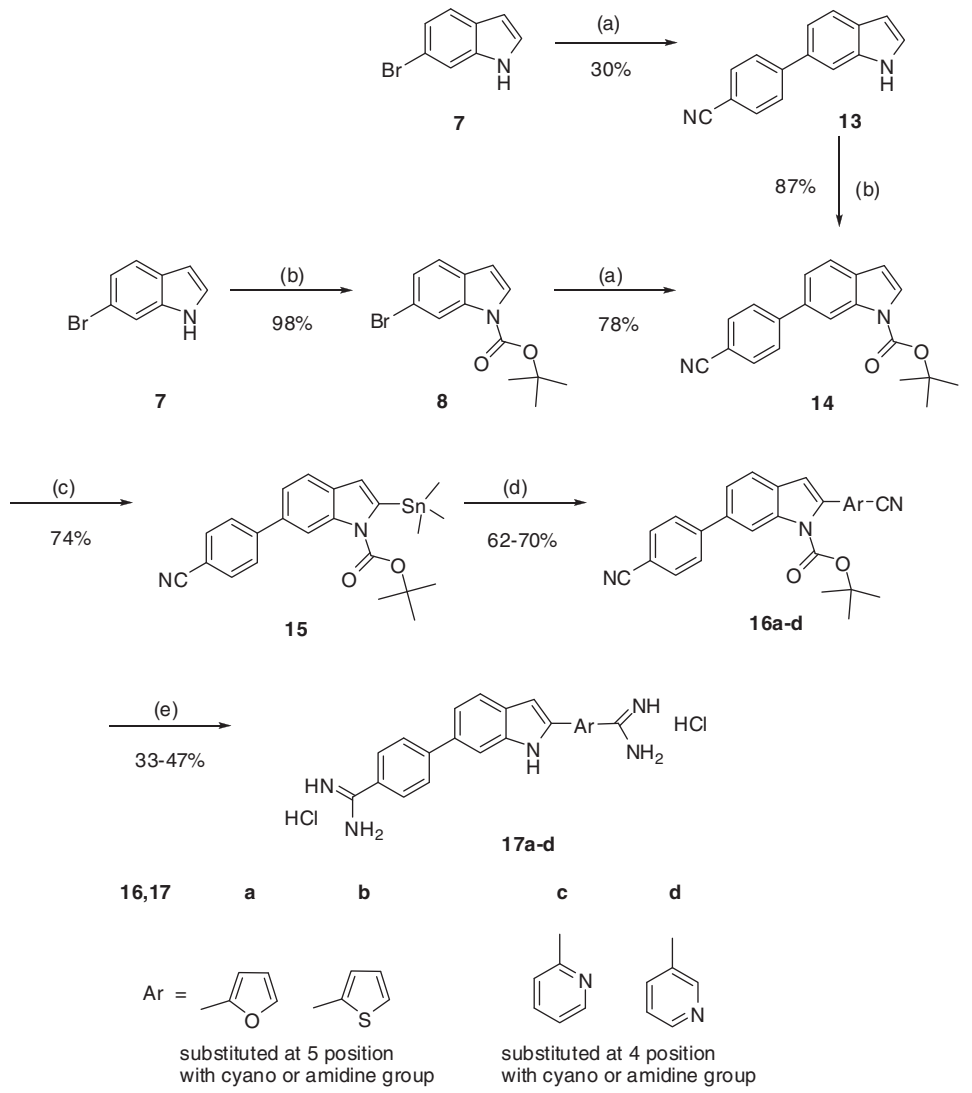
Scheme 2 outlines the synthesis of the hydrochloride salt of the diamidine **12**. Compound **8** was prepared by the same approach as the indole **2**.[17] As previously reported,[16] we tried a number of iodination methods to prepare the 2-iodoindole derivative **10**, however we were unable to obtain **10** in a reasonable yield until we reacted the indole stannane **9**[17] with molecular iodine at room temperature to give the iodoindole **10** in good yield (83%). We note that the introduction of the iodo group at 2-position of indole was recently reported also by employing an indirect route starting with the analogous boronic acid.[21] The dinitrile **11** was prepared in good yield by employing a Suzuki coupling reaction[19] between two equivalents of *p*-cyanophenylboronic acid and the dihaloindole **10** in the presence of 5 mol % $Pd(PPh_3)_4$. The diamidine **12**, as a hydrochloride salt, was obtained by the lithium bis(trimethylsilyl)amide method discussed before.[20]

After preparation of the symmetric 2,6-diphenylindole diamidine **12**, we decided to prepare dissymmetric analogues by introduction of heterocyclic rings at position 2 of the indole. The synthesis of the diamidines **17a–d** was achieved as outlined in Scheme 3. 6-Bromoindole **7** was allowed to react with *p*-cyanophenylboronic acid under Suzuki coupling conditions[19] in the presence of 5 mol % $Pd(PPh_3)_4$, however this reaction gave **13** in low yield (<20%). Therefore, we decided to attempt the reaction with the Boc-protected bromoindole **8**, which was more soluble than **7**. This approach provided compound **14** in good yield (87%), by using the standard Suzuki coupling reaction.[19] As before, the indole stannane **15** was obtained in a similar manner as **3**. A Stille coupling reaction[18] between the commercially available haloaryl nitriles and the indole stannane **15** using 5 mol % $Pd(PPh_3)_4$ and 1,4-dioxane as the solvent afforded the dinitriles **16a–d** in good yield. The hydrochloride salts of the diamidines **17a–d** were obtained by the lithium bis(trimethylsilyl)amide method.[20]

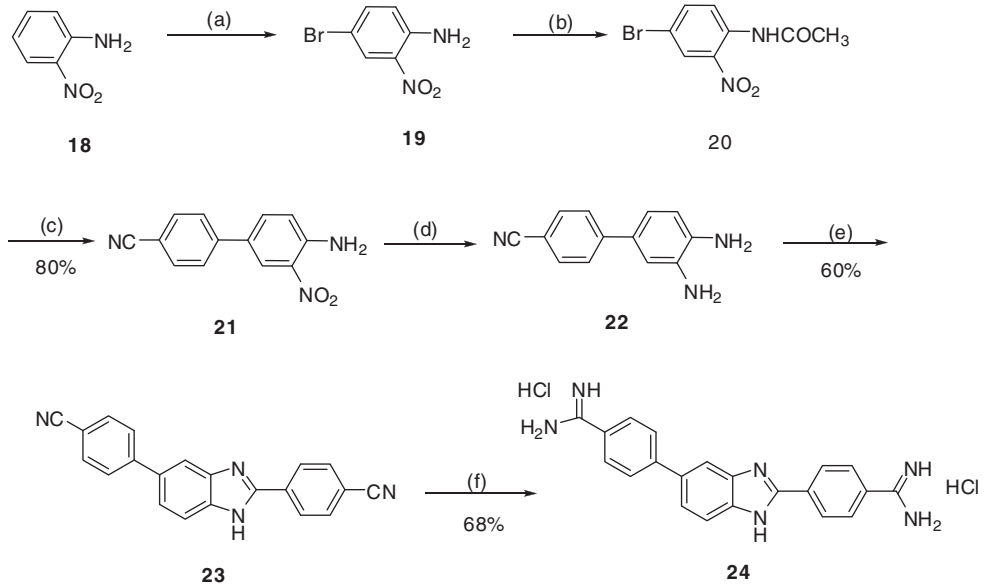
Scheme 4 outlines the synthesis of the hydrochloride salt of 2,5-diamidinobenzimidazole **24**. Bromination of the starting material **18** with hydrogen peroxide mediated HBr afforded the bromo derivative **19** in good yield.[22] Compound **19** was protected by acetylation after heating in acetic anhydride at 100 °C to yield **20**.[23] Compound **20** was allowed to react with *p*-cyanophenylboronic acid according to a published procedure[23] in the presence of DAPCY (diacetylpalladium (II)bis(dicyclohexylamine)) and potassium phosphate to afford **21**. The nitro compound **21** was hydrogenated at 50 psi using 10% Pd/C to afford the diamine **22**.[24] A solution of the diamine **22** in DMF and *p*-cyanobenzaldehyde in the presence of a catalytic amount of nitrobenzene was heated at reflux to afford the diphenyl benzimidazole **23**.[15] Stirring the dinitrile **23** in ethanolic HCl according to Pinner methodology[25] afforded the corresponding bis-imidate ester hydrochloride which was converted to the hydrochloride salt of the diamidine **24** by stirring with ethanol saturated with ammonia gas.

Scheme 5 outlines the synthesis of the hydrochloride salt of 1-methyl-2.5-diamidinobenzimidazole **30** starting with the nitro activated fluoro derivative **25** which on reaction with methylamine in dichloromethane in the presence of potassium carbonate afforded the *N*-methylaniline analogue **26**.[26] The bromo derivative **26** was allowed to react with *p*-cyanophenylboronic acid under Suzuki coupling conditions in the presence of DAPCy and potassium phosphate to afford **27**.[27] The nitro compound **27** was hydrogenated under 50 psi using 10% Pd/C to afford **28** in high yield (90%). Heating a DMF solution of the diamine **28** with *p*-cyanobenzaldehyde in the presence of sodium bisulphite afforded the diphenyl benzimidazole **29**.[15] The hydrochloride salt of the diamidine **30** was obtained by the lithium bis(trimethylsilyl)amide method.[20]

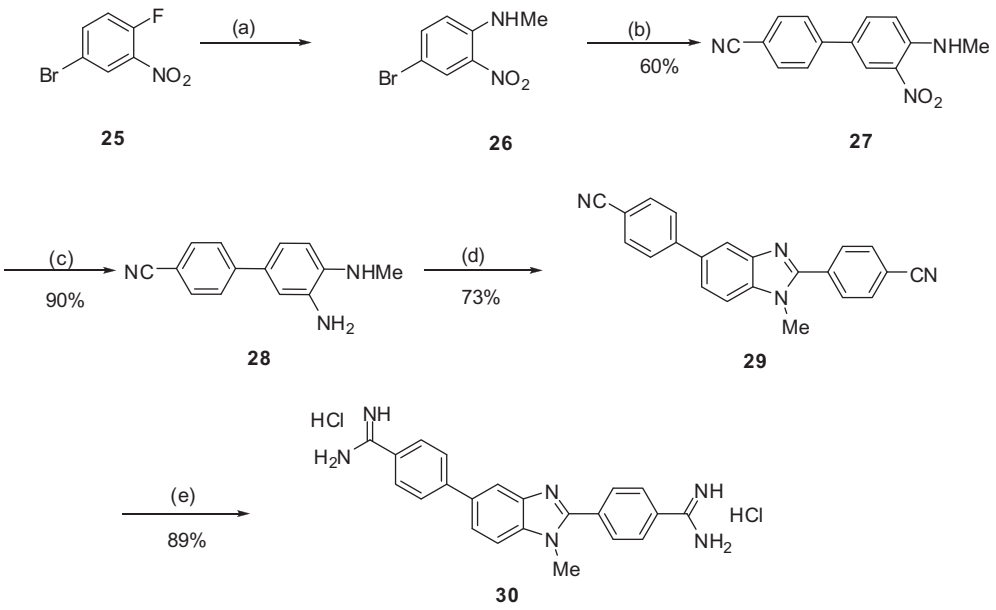
Scheme 6 outlines the synthesis of the hydrochloride salt of 1-hydroxy-2,5-diamidinobenzimidazole **35** starting with the nitro activated fluoro derivative **25** which on reaction with *p*-cyanobenzylamine **31** in dioxane in the presence of potassium carbonate gave **32**. The bromo derivative **32** was allowed to react with *p*-cyanophenylboronic acid under Suzuki coupling conditions in the presence of DAPCy and potassium phosphate to give **33**.[27] The dicyano derivative **33** was cyclized by heating in a sodium methoxide/methanol solution to afford the *N*-hydroxybenzimidazole **34**. The hydrochloride salt of the diamidine **35** was obtained by applying Pinner methodology as discussed above.[25]



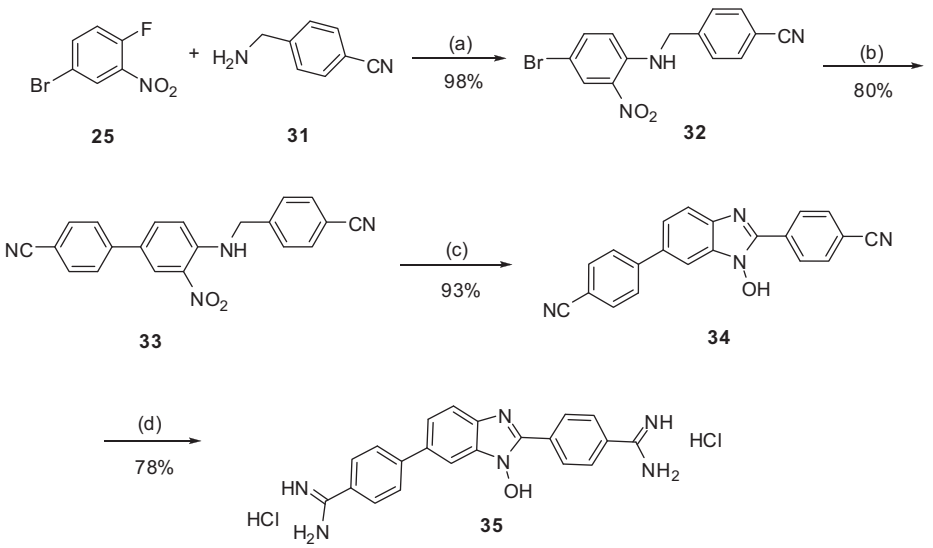
Scheme 3. Reagents and conditions: (a) *p*-cyanophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene; (b) $(Boc)_2O$, DMAP, CH_2Cl_2; (c) $ClSn(CH_3)_3$, LDA/THF; (d) Br-Ar-CN, $Pd(PPh_3)_4$, dioxane; (e) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.



Scheme 4. Reagents and conditions: (a) 49% HBr, MeOH, 30% H_2O_2; (b) Ac_2O, 100 °C; (c) *p*-cyanophenylboronic acid, DAPCy, K_3PO_4; (i) PrOH; (d) EtOAc, Pd/C, H_2; (e) 4-cyanobenzaldehyde, *p*-nitrobenzene, DMF, 145 °C; (f) (i) HCl gas/EtOH; (ii) NH_3 gas/EtOH.



Scheme 5. Reagents and conditions: (a) 40% $MeNH_2$, K_2CO_3, CH_2Cl_2; (b) 4-cyanophenylboronic acid, DAPCy, K_3PO_4, EtOH; (c) EtOH, Pd/C, H_2; (d) (i) 4-cyanobenzaldehyde, $NaHSO_3$, EtOH; (ii) DMF; (e) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.



Scheme 6. Reagents and conditions: (a) K_2CO_3, dioxane; (b) *p*-cyanophenylboronic acid, DAPCy, K_3PO_4, EtOH; (c) (i) MeOH, NaOMe; (ii) HCl; (d) (i) HCl gas/EtOH; (ii) NH_3 gas/EtOH.

2.2. Biology

Table 1 contains the DNA binding affinities for the new diamidines with central ring indoles and benzimidazoles as well as the in vitro activities for these compounds against *T. b. r.* and *P. f.* For comparative purposes, similar data for DAPI, furamidine, pentamidine and the two near linear analogues **IV** and **V** are included. We continue to use the thermal melting increase ΔT_m (T_m of complex − T_m of free DNA) as a rapid and reliable method for ranking binding affinities for a large number of diverse classes of aryldiamidines.[16] The ΔT_m values for the complexes between poly(dA-dT) and the linker modified bis-benzamidines are quite high ranging from 23 to >27 °C for all the diamidines except for **30** and **35**. The compounds with high DNA affinity (**6a**, **6b**, **12**, **17a**, **17b**, **17c**, **17d** and **24**) exhibit ΔT_m values comparable to or higher than that of furamidine. Since the complexes of five of these compounds do not melt under the conditions of the ΔT_m measurements, ranking and analysis of structural effects must await detailed biophysical studies performed under conditions for which affinities can be directly compared. Nevertheless, it is clear that, in general, replacement of the central furan ring of furamidine with an indole or benzimidazole ring (but not N-substituted benzimidazole **30** and **35**) leads to compounds with quite high affinity for AT rich DNA minor grooves.

Generally, heterocyclic diamidines have been found to bind in the DNA minor groove in AT base pair sequences of DNA.[28] Circular dichroism (CD) spectroscopy has proven to be a valuable technique for characterizing minor groove interactions. In order to determine if these new analogues bind in the DNA minor groove, we observed the CD spectroscopy of the complex formation. Figure 2 shows the CD data for the interaction between DNA and **12** and **24** along with that of DAPI as a reference compound. Minor groove binders yield a

Table 1
DNA binding and in vitro antiparasitic data for centrally linked indoles and benzimidazoles

	ΔT_m[a] (°C)	Cytoxicity[b] (μM)	*T. b. r*[c] (nM)	SI_T[d]	*P. f*[e] (nM)	SI_P[f]
DAPI (III)	>27	0.86	18	48	3	287
Furamidine (IIa)	25	6.4	4.5	1422	15.5	413
Pentamidine (I)	12.6	46	2.2	20,909	46.4	991
V	>27	10.5	2	5250	3.3	3182
IV	>27	17	7.7	2207	0.5	34,000
6a	25.5	9.9	2	4950	7.1	1394
6b	23.7	6.7	3	2333	5.7	1175
12	>27	3.4	3	1133	5.2	653
17a	24.9	9.2	17	541	12.5	736
17b	>27	2.2	4	550	11.1	198
17c	>27	11.6	5	2320	18	644
17d	>27	2.6	4	650	9.8	265
24	>27	62.4	4	15,600	3.7	16,864
30	11.2	>169	15	>11,266	1.3	>130,000
35	12.2	124	484	256	18	6,888

[a] Increase in thermal melting of poly(dA-dT)$_n$ in °C.[32]
[b] Cytotoxicity (IC_{50}) was evaluated using cultured L6 rat myoblast cells.[9]
[c] IC_{50} values obtained against the STIB900 strain of *T. b. r.* The IC_{50} values are the mean of two independent assays. Individual values differed by less than 50% of the mean value.[9]
[d] Selectivity index for *T. b. r.* expressed as the ratio: IC_{50} (L6)/IC_{50} (*T. b. r.*).
[e] IC_{50} values obtained against the K1 strain of *P. f.* The IC_{50} values are the mean of two independent assays. Individual values differed by less than 50% of the mean value.[9]
[f] Selectivity index for *P. f.* expressed as the ratio: IC_{50} (L6)/IC_{50} (*P. f.*).

large positive induced CD signal on binding to AT sequences and only exhibit small changes in the CD pattern of the DNA spectrum. The induced CD spectrum of DAPI is shown as a reference in Figure 2a. The expected positive signal in the compound absorption region with relatively small changes in the DNA region are observed. Figure 2 also shows the CD results for the interaction between DNA and **12** and **24**. In agreement with their strong binding to DNA, both of these compounds exhibit large induced CD spectra in their absorption regions that is approximately twice the DAPI value. They are clearly binding in the DNA minor groove. Interestingly, both of these compounds cause larger changes in the DNA CD spectral region than DAPI. It seems likely that their shapes and H-bond donor groups do not initially match the DNA groove shape and H-bond acceptors as well as DAPI. In this case they induce complementary changes in DNA that yield a very favorable final complex with high Tm values.

DAPI and a number of analogues have exhibited excellent in vitro activity against *T. b. r.* and *P. f.*[16] Six of the seven new indole linked analogues (**6a**, **6b**, **12**, **17b**, **17c** and **17d**) give IC_{50} values against *T. b. r.* ranging between 2 and 5 nM. The remaining indole **17a** give an IC_{50} value of 17 nM. The in vitro antitrypanosomal activity of the six indoles compares favorably with that of the reference compounds. The indole analogues are somewhat less active against *P. f.* compared to that against *T. b. r.*, with only three compounds (**6a**, **6b** and **12**) giving IC_{50} values ranging between 5 and 7 nM and the remaining four gave values between 9 and 18 nM. None of the indole compounds achieve better antimalarial activity than DAPI, **IV** and **V**, several show higher activity than furamidine and all are superior to that of pentamidine. Generally, the indoles show acceptable selectivity for both parasites, SI values range from 541 to 4950 against *T. b. r.* and from 198 to 1394 versus *P. f.* The activity against *T. b. r.* for two of the three benzimidazole compounds (**24**, **30**; IC_{50} values 4 and 15 nM, respectively) was comparable to that of the indoles. The same two compounds were moderately more active against *P. f.* (IC_{50} values 3.7 and 1.3 nM, respectively) than the indoles. The benzimidazoles generally show greater selectivity than the indoles; SI values against *T. b. r* range from 256 to 15,600 and against *P. f.* from 6880 to >130,000.

The in vitro activity and selectivity of these new diamidines lead us to evaluate them in the rigorous *T. b. r.* STIB900 mouse model for the acute phase of African trypanosomiasis and the results are presented in Table 2. The compounds were screened by daily intraperitoneal dosage of 5 mg/kg for four consecutive days. Four of the seven indole compounds (**6a**, **17b**, **17c** and **17d**) gave at least 2 of 4 cures. No cures were obtained with the three benzimidazoles (**24**, **30** and **35**) which may reflect differences in PK properties from that of the indoles. The in vivo results for four compounds (**6a**, **17b**, **17c** and **17d**) compare favorably with that for pentamidine and is superior to that of furamidine in this mouse model and merit further evaluation.

The widespread use of DAPI in molecular biology is largely due to its fluorescence properties. During the study of these new indole diamidines we observed that several of the compounds were fluorescent in the visible region. Therefore, we decided to record the fluorescence data for the new indole and benzimidazole compounds and compare the results with that of DAPI. These data are presented in Table 3. The absorption maximum for the new indoles ranges from 341 to 380 nm; that for DAPI is 343 nm. The absorption maximum for the new benzimidazoles is a small range of 280–286 nm. The emission maximum ranges from 508 to 579 nm for the indoles and from 468 to 503 nm for the benzimidazoles; that for DAPI is 458 nm. A qualitative comparison of quantum yield of emission for the indoles shows a 2.5–30-fold decline from that of DAPI; whereas the benzimidazole **24** shows a modest 20% increase in emission intensity over that of DAPI. The other two benzimidazoles showed a decline in emission intensity similar to the decline noted for the indoles.

DAPI on binding to DNA exhibits an approximately 20-fold enhancement in its fluorescence, an attribute which significantly

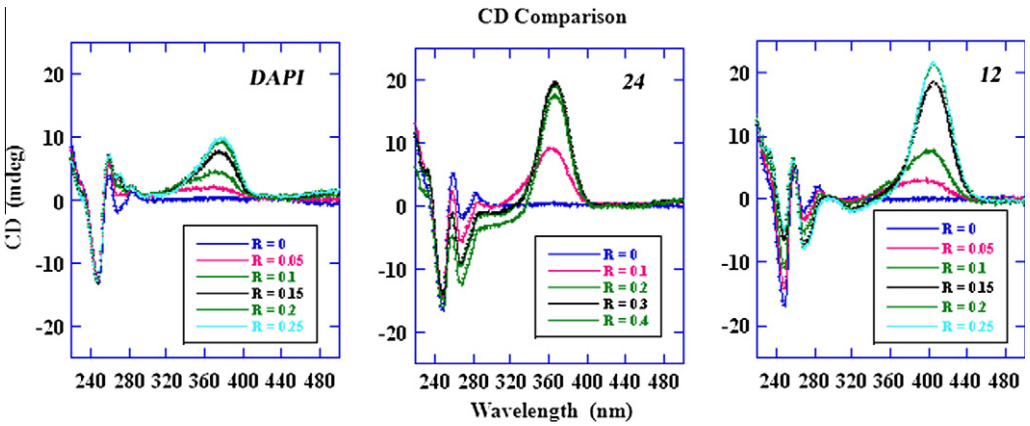


Figure 2. The concentration of polydA·polydT is 30 μM. The ratio of compound to DNA is shown in the inset.

Table 2
Antitrypanosomal evaluation of indole and benzimidazole analogues in the acute STIB900 mouse model[a]

Compound	In vitro IC_{50}[b] (nM)	Cures[c]	MRD[d]
DAPI (III)	18	2/4	17
Pentamidine(I)	2.2	2/4	20
Furamidine (IIa)	4.5	1/4	24
6a	2	2/4	17
6b	3	1/4	19.7
12	3	0/4	20
17a	17	0/4	17.75
17b	4	2/4	17
17c	5	3/4	24
17d	4	2/4	24
24	4	0/4	18
30	15	0/4	11
35	484	0/4	7.75

[a] See Ref. 31 for details of STIB900 model; dosage at 5 mg/kg/day for four consecutive days by intraperitoneal administration.
[b] IC_{50} values obtained against the STIB900 strain of *T. b. r.* The values are the average of duplicate determinations.[9]
[c] Number of mice that survive and are parasite free on day 60.
[d] Mean relapse day of parasitemia.

Table 3
Fluorescence data for new indole and benzimidazole diamidines

Compound	λ_{ex}[a,b] (nm)	λ_{em}[a,b] (nm)	Em intensity[c]
DAPI	343	458	821
6a	368	519	177
6b	341	517	42
12	358	508	32
17a	370	508	146
17b	380	532	358
17c	369	515	55
17d	368	579	27
24	280	496	986
30	286	503	106
35	284	468	51

[a] Wavelengths (λ) are indicated for excitation (ex) and emission (em).
[b] Measurements were made with 0.001 M solutions in distilled water.
[c] Emission intensities were measured at excitation slit width of 5 and emission slit width of 20.

contributes to its utility for molecular biology studies.[29] To further access the possible utility of the new compounds we have examined the effect of DNA binding on the fluorescence of **12** and **24** as shown in Figure 2. While the emission intensity of **24** is approximately 30 fold greater than that of **12**, both show an approximately 5-fold enhancement on binding to DNA (Fig. 3). DAPI shows a 4-fold greater enhancement of emission on binding to DNA. Nevertheless, in special circumstances **12** or **24** may be of utility since their emission maxima are approximately 50 nm greater than that of DAPI.

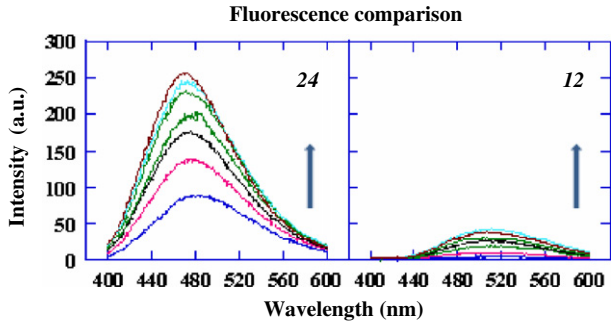


Figure 3. The free compound concentrations were 0.5 μM, polydA·polydT was titrated into the cell at 0.025 μM.

3. Conclusions

Replacement of the central furan ring of furamidine with indole or benzimidazole (but not N-substituted benzimidazoles) yields compounds that bind in the DNA minor groove with high affinity and exhibit high in vitro antiparasitic activity. In general, the DNA affinities and the in vitro antiparasitic activities are comparable to or better than that of furamidine. The DNA affinities of the indole analogues are also comparable to those of the structurally related bis-benzamdinoimidazo[1,2-*a*]pyridines, however the in vitro activity of the indoles is generally superior to that of the imidazo[1,2-*a*]pyridines.[30] In vivo efficacies of four indoles (**6a**, **17b**, **17c** and **17d**) are comparable to that of pentamidine and superior to that of furamidine. Generally, the fluorescence properties of the new analogues are similar to that of DAPI. The two compounds **12** and **24** show emission enhancement on binding to DNA, albeit 4-fold less than DAPI, however their longer emission wavelength maybe beneficial in certain circumstances.

4. Experimental

4.1. Biology

4.1.1. Efficacy studies

The in vitro assays[9] with *T. b. r.* STIB 900 and *P. f.* K1 strain as well as the efficacy studies in an acute mouse model for *T. b. r.* STIB 900[31] were carried out, using the hydrochloride salts, as previously reported.

4.1.2. T_m measurements

Thermal melting experiments were conducted with a Cary 300 spectrophotometer. Cuvettes for the experiment were mounted in a thermal block and the solution temperatures monitored by a thermistor in the reference cuvette. Temperatures were maintained under computer control and 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 its absorbance at 260 nm. A ratio of 0.3 moles compound per mole of DNA was used for the complex and DNA alone was used as a control.[32] ΔT_m values were determined by the peak in first derivative curves (dA/dT).

4.1.3. Circular dichroism (CD)

CD spectra were collected employing 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 the desired wavelength range. DAPI, **12** and **24**, at increasing ratios, were titrated into the same cuvette and the complexes rescanned under the same conditions.[33]

4.2. Chemistry

All commercial reagents were used without purification. Melting points were determined on a Mel-Temp 3.0 melting point apparatus, and are uncorrected. TLC analysis was carried out on Silica Gel 60 F254 precoated aluminum sheets using UV light for detection. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer (except as noted) using the indicated solvents. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Compounds reported as salts often analyzed correctly for solvates of water and/or other solvents, in each case the solvates were detected by ^1H NMR. Elemental analysis was performed by Atlantic Microlab Inc., Norcross, GA.

4.2.1. General procedure for 4a,b

Tetrakistriphenylphosphine palladium (0.288 g, 0.25 mmol) was added to a stirred mixture of the indole stannane **3** (2.29 g, 5 mmol) and the halo arylnitriles (5-bromothiophene-2-carbonitrile and 4-bromobenzonitrile) (5 mmol) in deaireated dioxane (20 mL) under a nitrogen atmosphere. The mixture was warmed to 90–100 °C and stirred for 24 h. The solvent was evaporated under reduced pressure. The resultant solid was partitioned between ethyl acetate (200 mL) containing 5 mL of concentrated ammonia to destroy the palladium complex, washed with water, passed through celite, dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (80:20, v/v) as the eluent.

4.2.1.1. 5-Bromo-1-(*tert*-butoxycarbonyl)-2-(5-cyanothien-2-yl)-1*H*-indole (4a). White solid (1.56 g, 79%). Mp 152–153 °C; ^{1}H NMR ($CDCl_3$): δ 8.08 (d, 1H, J = 8.2 Hz), 7.70 (s, 1H), 7.60 (d, 1H, J = 3.6 Hz), 7.47 (d, 1H, J = 8.2 Hz), 7.15 (d, 1H, J = 3.6 Hz), 6.71 (s, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 149.2, 142.3, 136.9, 136.3, 130.9, 130, 128.6, 128.1, 123.5, 117.1, 116.7, 113.9, 112.7, 110, 85.1, 27.8. Anal. Calcd for $C_{18}H_{15}BrN_2O_2S$: C, 53.61; H, 3.75; N, 6.95. Found: C, 53.58; H, 3.84; N, 6.63.

4.2.1.2. 5-Bromo-1-(*tert*-butoxycarbonyl)-2-(4-cyanophenyl)-1*H*-indole (4b). White solid (1.59 g, 79%). Mp 188–188.5 °C ; ^{1}H NMR (DMSO-d_6): δ 8.07 (d, 1H, J = 9.2 Hz), 7.94 (d, 2H, J = 8 Hz), 7.86 (d, 1H, J = 1.2 Hz), 7.7 (d, 2H, J = 8 Hz), 7.53 (dd, 1H, J = 9.2, 1.2 Hz), 6.71 (s, 1H), 1.49 (s, 9H); ^{13}C NMR (DMSO-d_6): δ 149.4, 140.1, 138.8, 136.6, 132.2, 131.1, 130.1, 127.9, 123.8, 118.9, 117.2, 116.1, 111.2, 110.7, 85.2, 27.7. Anal. Calcd for $C_{20}H_{17}BrN_2O_2$: C, 60.47; H, 4.31; N, 7.05. Found: C, 60.55; H, 4.33; N, 6.92.

4.2.2. General procedure for 5a,b

An aqueous solution of Na_2CO_3 (5 mL 2 M, deaireated) and 4-cyanophenyl boronic acid (0.73 g, 5 mmol) in 5 mL deaireated methanol were added to a stirred solution of the bromoindole derivatives **4a**,**b** (5 mmol), and tetrakistriphenylphosphine palladium (0.288 g, 0.25 mmol) in deaireated toluene (20 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 80 °C for 24 h. The solvent was evaporated under reduced pressure, the solid was partitioned between ethyl acetate (200 mL) and 2 M aqueous Na_2CO_3 (25 mL) containing 5 mL of concentrated ammonia, to destroy the palladium complex, washed with water, passed through celite to remove the catalyst, dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (80:20, v/v) as the eluent.

4.2.2.1. 1-(*tert*-Butoxycarbonyl)-5-(4-cyanophenyl)-2-(5-cyanothien-2-yl)-1*H*-indole (5a). Yellow solid (1.72 g, 81%). Mp 237–237.5 °C ; ^{1}H NMR ($CDCl_3$): δ 8.30 (d, 1H, J = 8.8 Hz), 7.81 (br s, 1H), 7.76 (br s, 4H), 7.63 (d, 1H, J = 8.8 Hz), 7.52 (d, 1H, J = 4 Hz), 7.18 (d, 1H, J = 4 Hz), 6.86 (s, 1H), 1.52 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.3, 145.6, 142.5, 137.7, 136.8, 134.7, 132.6, 130.9, 129, 128, 127.8, 125, 119.6, 118.9, 116.3, 113.9, 113.8, 110.7, 109.2, 85.1, 27.8; ESI-MS: m/z calcd for $C_{25}H_{19}N_3O_2S$: 425.50, found: 426.2 (M^++1). Anal. Calcd for $C_{25}H_{19}N_3O_2S$: C, 70.57; H, 4.50; N, 9.88. Found: C, 70.24; H, 4.51; N, 9.72.

4.2.2.2. 1-(*tert*-Butoxycarbonyl)-2,5-bis(4-cyanophenyl)-1*H*-indole (5b). White solid (1.78 g, 85%). Mp 222–222.5 °C; ^{1}H NMR ($CDCl_3$): δ 8.32 (d, 1H, J = 8.8 Hz)), 7.82 (d, 1H, J = 1.6 Hz), 7.76 (br s, 4H), 7.75 (d, 2H, J = 8.4 Hz), 7.64 (dd, 1H, J = 1.6 Hz, J = 8.8 Hz), 7.58 (d, 2H, J = 8.4 Hz), 6.72 (s, 1H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.2, 145.9, 139.4, 139.1, 137.8, 134.6, 132.6, 131.7, 129.6, 129.3, 127.8, 124, 119.4, 119, 118.7, 116.2, 111.6, 111.4, 110.7, 84.7, 27.8; ESI-MS: m/z calcd for $C_{27}H_{21}N_3O_2$: 419.47, found: 420.3 (M^++1). Anal. Calcd for $C_{27}H_{21}N_3O_2$: C, 77.31; H, 5.05; N, 10.02. Found: C, 77.68; H, 5.22; N, 9.97.

4.2.3. General procedure for 6a,b

The dinitriles **5a**,**b** (0.66 mmol), were suspended in freshly distilled tetrahydrofuran (5 mL), and treated with lithium bis(trimethylsilyl)amide 1 M solution in tetrahydrofuran (4 mL, 3.98 mmol), and stirred for three days at room temperature. The reaction mixture was cooled to 0 °C to which was added HCl saturated ethanol (2 mL). The mixture was stirred for two days, diluted with ether and the resultant solid was collected by filtration. The diamidine was purified by neutralization with 1 N sodium hydroxide followed by filtration of the resultant solid, washed with water and dried. Finally, the free base was stirred with ethanolic HCl for one week to ensure that the Boc groups were completely removed, diluted with ether, and the solid formed was filtered and dried to give the diamidine salt.

4.2.3.1. Dihydrochloride salt of 5-(4-amidinophenyl)-2-(5-amidinothien-2-yl)-1*H*-indole (6a). Yellow solid (0.179 g, 60%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (s, 1H), 9.49 (s, 2H), 9.46 (s, 2H), 9.24 (s, 2H), 9.21 (s, 2H), 8.17 (d, 1H, J = 4 Hz), 8.07 (d, 1H, J = 4 Hz), 7.96 (br s, 4H), 7.82 (d, 1H, J = 4 Hz), 7.64 (dd, 1H, J = 1.6 Hz, J = 8.4 Hz), 7.57 (d, 1H, J = 8.4 Hz), 7.01 (d, 1H, J = 1.6 Hz); ^{13}C NMR (DMSO-d_6) δ 165.8, 163.9, 147, 143.2, 138.2, 135.9, 132, 131, 129.2, 127.2, 127, 126, 125.3, 123, 119.7, 112.8, 102.4; ESI-MS: m/z calcd for $C_{20}H_{17}N_5S$: 359.45, found: 360.50 (amidine base M^++1). Anal. Calcd for $C_{20}H_{17}N_5S{\cdot}2HCl{\cdot}1.25H_2O$: C, 52.80; H, 4.76; N, 15.39. Found: C, 52.80; H, 4.72; N, 15.22.

4.2.3.2. Dihydrochloride salt of 2,5-bis(4-amidinophenyl)-1*H*-indole (6b). Yellow solid (0.19 g, 65%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.31 (s, 1H), 9.55 (s, 2H), 9.52 (s, 2H), 9.35 (s, 2H), 9.33 (s, 2H), 8.24 (d, 2H, J = 8.8 Hz), 8.06 (d, 2H, J = 8.4 Hz), 7.99–7.92 (m, 4H), 7.67 (d, 2H, J = 8.4 Hz), 7.61 (s, 1H), 7.19 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 165.8, 165.5, 147.2, 138.4, 137.5, 130.7, 129.4, 129.3, 129.2, 127, 126.5, 125.8, 125.3, 122.4, 119.8, 112.8, 102.2; ESI-MS: m/z calcd for $C_{22}H_{19}N_5$: 353.42, found: 354.30 (amidine base M^++1). Anal. Calcd for $C_{22}H_{19}N_5$. $2HCl{\cdot}0.90H_2O$: C, 59.70; H, 5.19; N, 15.82. Found: C, 60.05; H, 5.12; N, 15.57.

4.2.4. 6-Bromo-1-(*tert*-butoxycarbonyl)-2-(iodo)-1*H*-indole (10)

A molecular iodine (25 g, 98.76 mmol) solution in dry tetrahydrofuran (100 mL) was added slowly to a solution of the indole stannane **9** (22.61 g, 49.38 mmol) in dry tetrahydrofuran (100 mL). The mixture was stirred at room temperature for 6 h, the solvent was removed under reduced pressure, the residue dissolved in ethyl acetate (200 mL), washed with sodium thiosulfate solution (10%) to remove any excess iodine, with water the organic phase was dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (97:3, v/v) as the eluent. White solid (17.21 g, 83%). Mp 48–48.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H), 7.34–7.30 (m, 2H), 6.96 (s, 1H), 1.74 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 148.8, 138, 129.8, 126.1, 121.5, 120.2, 118.6, 85.9, 75.3, 28.2; ESI-MS: m/z calcd for $C_{13}H_{13}BrINO_2$: 420.92, found: 422.2 (M^++1). Anal. Calcd for $C_{13}H_{13}BrINO_2$: C, 36.99; H, 3.10; N, 3.32. Found: C, 37.21; H, 3.08; N, 3.30.

4.2.5. 1-(*tert*-Butoxycarbonyl)-2,6-bis(4-cyanophenyl)-1*H*-indole (11)

An aqueous solution of Na_2CO_3 (10 mL 2 M, deaireated) and 4-cyanophenyl boronic acid (1.46 g, 10 mmol) in 10 mL deaireated methanol were added to a stirred solution of the dihalo indole **10** (2.11 g, 5 mmol), and tetrakistriphenylphosphine palladium

(0.57 g, 0.5 mmol) in deaireated toluene (30 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 80 °C for 24 h. The solvent was evaporated under reduced pressure, the solid was partitioned between ethyl acetate (200 mL) and 2 M aqueous Na_2CO_3 (25 mL) containing 5 mL of concentrated ammonia, to destroy the palladium complex, washed with water, passed through celite to remove the catalyst, dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (85:15, v/v) as the eluent. White solid (1.65 g, 79%). Mp 230–230.5 °C ; ^{1}H NMR (DMSO-d_6): δ 8.47 (s, 1H), 7.98–7.90 (m, 6H), 7.79 (d, 1H, J = 8 Hz), 7.73 (d, 2H, J = 8 Hz), 7.70 (d, 1H, J = 8 Hz), 6.96 (s, 1H), 1.17 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 154.6, 145.8, 139.4, 139.1, 137.8, 134.5, 132.6, 131.6, 129.6, 129.3, 127.8, 124.4, 119.5, 119, 118.6, 116.1, 111.8, 111.4, 110.6, 84.7, 27.7; ESI-MS: m/z calcd for $C_{27}H_{21}N_3O_2$: 419.47, found: 420.3 (M^++1). Anal. Calcd for $C_{27}H_{21}N_3O_2$: C, 77.31; H, 5.05; N, 10.02. Found: C, 77.45; H, 5.39; N, 9.89.

4.2.6. Dihydrochloride salt of 2,6-bis(4-amidinophenyl)-1*H*-indole (12)

This compound was prepared by following the experimental procedure used for compounds **6a**,**b**. Yellow solid, yield (0.140 g, 46%), mp >300 °C; ^{1}H NMR (DMSO-d_6): 12.28 (s, 1H), 9.46 (s, 4H), 9.22 (s, 4H), 8.19 (d, 2H, J = 8.4 Hz), 7.98 (d, 2H, J = 8.4 Hz), 7.97 (br s, 4H), 7.81 (s, 1H), 7.73 (d, 1H, J = 8.4 Hz), 7.48 (dd, 1H, J = 1.2 Hz, J = 8.4 Hz), 7.25 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 165.7, 165.4, 147, 138.6, 137.8, 137.3, 133.3, 129.3, 129.4, 127.9, 126.6, 126.3, 125.6, 125.2, 121.7, 119.8, 110.5, 101.7; ESI-MS: m/z calcd for $C_{22}H_{19}N_5$: 353.42, found: 354.3 (amidine base M^++1). Anal. Calcd for $C_{22}H_{19}N_5{\cdot}2HCl{\cdot}2H_2O$: C, 57.14; H, 5.44; N, 15.14. Found: C, 57.44; H, 5.62; N, 14.88.

4.2.7. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-1*H*-indole (14)

An aqueous solution of Na_2CO_3 (50 mL 2 M, deaireated) and 4-cyanophenyl boronic acid (7.3 g, 50 mmol) in 50 ml deaireated methanol were added to a stirred solution of indole derivative **8** (14.75 g, 50 mmol), and tetrakistriphenyl-phosphine palladium (2.31 g, 2 mmol) in deaireated toluene (150 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 80 °C for 24 h. After evaporation of the solvent under reduced pressure, the solid was partitioned between ethyl acetate (250 mL) and 2 M aqueous Na_2CO_3 (50 mL) containing 20 mL of concentrated ammonia, to destroy the palladium complex, then washing with water, passed through celite to remove the catalyst, dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (90:10, v/v) as the eluent. White solid (12.4 g, 78%). Mp 123–124 °C; ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 1H), 7.79 (d, 2H, J = 7.2 Hz), 7.74 (d, 2H, J = 7.2 Hz), 7.69–7.62 (m, 2H), 7.49 (d, 1H, J = 8 Hz), 6.63 (s, 1H), 1.71 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.6, 146.4, 135.9, 135.4, 128.7, 127.9, 127.2, 122, 121.5, 119.1, 114.2, 110.4, 107, 84, 28.2; ESI-MS: m/z calcd for $C_{20}H_{18}N_2O_2$: 318.37, found: 319.2 (M^++1). Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.49; H, 5.74; N, 8.80.

4.2.8. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-2-(trimethylstannyl)-1*H*-indole (15)

A 2.0 M solution of LDA in heptane-tetrahydrofuran-ethylbenzene (22.63 mL, 45.27 mmol) was added slowly to a solution of the indole derivative **14** (12 g, 37.73 mmol) and trimethyltin chloride (14.5 g, 42.76 mmol) in dry tetrahydrofuran (100 mL) at −20 °C. The reaction mixture was left to warm to room temperature with stirring for 48 h, quenched with water (50 mL), the solvent was removed under reduced pressure, the residue was extracted with ether (2 × 200 ml), the organic phase was washed with potassium fluoride solution (10%) to remove any excess stannane, with water and the combined extract was dried over sodium sulfate and the solvent was evaporated. Crystallization from hexanes/ethanol (4:1) mixture gave a give yellow solid (13.43 g, 74%), mp 118–118.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 7.72–7.58 (m, 4H), 7.63 (d, 1H, J = 8 Hz), 7.47 (d, 1H, J = 8 Hz), 6.80 (s, 1H), 1.71 (s, 9H), 0.39 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 151.9, 146.9, 145.5, 138, 134.5, 132.5, 127.8, 121.6, 120.6, 118.9, 117.8, 114.3, 110.4, 108.3, 84.4, 28.2, −0.1; ESI-MS: m/z calcd for $C_{23}H_{26}N_2O_2Sn$: 481.17, found: 482.1 (M^++1). Anal. Calcd for $C_{23}H_{26}N_2O_2Sn$: C, 57.41; H, 5.45; N, 5.82. Found: C, 57.72; H, 5.63; N, 5.53.

4.2.9. General procedure for 16a–d

These series were prepared by following the experimental procedure used for compounds **4a**,**b**.

4.2.9.1. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-2-(5-cyanofur-2-yl)-1*H*-indole (16a). White solid (1.38 g, 68%), mp 178–179 °C; ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 1H, J = 1.6 Hz), 7.81 (d, 2H, J = 6.8 Hz), 7.77 (d, 2H, J = 6.8 Hz), 7.71 (d, 1H, J = 8 Hz), 7.55 (dd, 1H, J = 1.6 Hz, J = 8 Hz), 7.22 (d, 1H, J = 3.6 Hz), 6.93 (s, 1H), 6.73 (d, 1H, J = 3.6 Hz), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 150, 145.7, 142.6, 137.7, 136.9, 134.7, 132.6, 131.2, 129, 128.2, 127.8, 125, 119.6, 119, 116.6, 113.6, 113.5, 110.7, 110, 85.2, 28.2; ESI-MS: m/z calcd for $C_{25}H_{19}N_3O_3$: 409.44, found: 410.20 (M^++1). Anal. Calcd for $C_{25}H_{19}N_3O_3$: C, 73.34; H, 4.68; N, 10.26. Found: C, 73.17; H, 4.67; N, 10.22.

4.2.9.2. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-2-(5-cyanothien-2-yl)-1*H*-indole (16b). Yellow solid (1.48 g, 70%), mp 186–187 °C; ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 1H, J = 1.2 Hz), 7.80 (d, 2H, J = 8.4 Hz), 7.77 (d, 2H, J = 8.4 Hz), 7.69 (d, 1H, J = 8 Hz), 7.63 (d, 1H, J = 4 Hz), 7.55 (dd, 1H, J = 2 Hz, J = 8 Hz), 7.17 (d, 1H, J = 4 Hz), 6.83 (s, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.3, 145.7, 142.5, 137.7, 136.9, 134.7, 132.6, 131, 129, 128, 127.8, 125, 119.6, 119, 116.3, 114, 113.8, 110.7, 110, 85.1, 27.8; ESI-MS: m/z calcd for $C_{25}H_{19}N_3O_2S$: 425.12, found: 426.2 (M^++1). Anal. Calcd for $C_{25}H_{19}N_3O_2S$: C, 70.56; H, 4.50; N, 9.88. Found: C, 70.61; H, 4.52; N, 9.66.

4.2.9.3. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-2-(5-cyanopyridin-2-yl)-1*H*-indole (16c). White solid (1.36 g, 65%), mp 193–195 °C; ^{1}H NMR ($CDCl_3$): δ 8.96 (d, 1H, J = 2 Hz), 8.48 (d, 1H, J = 1.6 Hz), 8.05 (dd, 1H, J = 2 Hz, J = 8 Hz), 7.81 (d, 2H, J = 6.8 Hz), 7.78 (d, 2H, J = 6.8 Hz), 7.69 (d, 2H, J = 8 Hz), 7.56 (dd, 1H, J = 1.6 Hz, J = 8 Hz), 6.97 (s, 1H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 150.8, 149.5, 146, 138.4, 136.9, 136.3, 136, 134, 132.7, 132.2, 129, 128, 127.6, 123, 121.7, 119, 117.2, 114.8, 112.7, 110.8, 85.4, 27.8; ESI-MS: m/z calcd for $C_{26}H_{20}N_4O_2$: 420.16, found: 421.2 (M^++1). Anal. Calcd for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.13; H, 5.03; N, 13.19.

4.2.9.4. 1-(*tert*-Butoxycarbonyl)-6-(4-cyanophenyl)-2-(6-cyanopyridin-3-yl)-1*H*-indole (16d). Yellowish white solid (1.29 g, 62%), mp 172–174 °C. ^{1}H NMR ($CDCl_3$): δ 8.83 (d, 1H, J = 2 Hz), 8.55 (s, 1H), 7.92 (dd, 1H, J = 2 Hz, J = 8 Hz), 7.82 (d, 2H, J = 6.8 Hz), 7.80 (d, 2H, J = 6.8 Hz), 7.79 (d, 1H, J = 8 Hz), 7.72 (d, 1H, J = 8 Hz), 7.58 (dd, 1H, J = 2 Hz, J = 8 Hz), 6.78 (s, 1H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 150.8, 149.5, 146, 138.4, 136.8, 136, 136.1, 134, 132.6, 132.2, 129, 128, 127.6, 123.5, 121.7, 118, 117.3, 114.7, 113, 110.4, 85.4, 27.2; ESI-MS: m/z calcd for $C_{26}H_{20}N_4O_2$: 420.1, found: 421.2 (M^++1). Anal. Calcd for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.21; H, 4.94; N, 13.07.

4.2.10. General procedure for 17a–d

These series was prepared by following the experimental procedure used for compounds **6a**,**b**.

4.2.10.1. Dihydrochloride salt of 6-(4-amidinophenyl)-2-(5-amidinofur-2-yl)-1*H*-indole (17a). Brown solid (0.138 g, 47%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.58 (s, 1H), 9.72 (s, 2H), 9.58 (s, 2H), 9.25 (s, 2H), 9.17 (s, 2H), 7.99–7.95 (m, 5H), 7.77 (s, 1H), 7.74 (s, 1H), 7.49 (d, 1H, J = 8.4 Hz), 7.30 (d, 1H, J = 3.2 Hz), 7.15 (s, 1H).; ^{13}C NMR (DMSO-d_6) δ 165.3, 160.4, 151.8, 146.2, 139.3, 137.5, 133.2, 128.6, 128.5, 128.2, 126.8, 125.7, 121.4, 121, 119.4, 109.8, 108.6, 101.2; ESI-MS: m/z calcd for $C_{20}H_{17}N_5O$: 343.38, found: 344.30 (amidine base M$^+$+1). Anal. Calcd for $C_{20}H_{17}N_5O{\cdot}2HCl{\cdot}1.75H_2O$: C, 53.63; H, 5.06; N, 15.63. Found: C, 53.81; H, 5.17; N, 15.31.

4.2.10.2. Dihydrochloride salt of 6-(4-amidinophenyl)-2-(5-amidinothien-2-yl)-1*H*-indole (17b). Yellow solid (0.136 g, 45%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.38 (s, 1H), 9.44 (br s, 4H), 9.18 (s, 2H), 9.13 (s, 2H), 8.14 (d, 1H, J = 3.6 Hz), 7.99 (br s, 4H), 7.83 (d, 1H, J = 3.6 Hz), 7.78 (s, 1H), 7.71 (d, 1H, J = 8.4 Hz), 7.49 (d, 1H, J = 8.4 Hz), 6.99 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 165.6, 160.9, 147.5, 144, 138.2, 135.9, 132, 130.8, 129.2, 127, 126.9, 125.9, 125.1, 123.1, 119, 112.2, 101.8; ESI-MS: m/z calcd for $C_{20}H_{17}N_5S$: 359.45, found: 361.50 (amidine base M$^+$+2). Anal. Calcd for $C_{20}H_{17}N_5S{\cdot}2HCl{\cdot}0.75H_2O{\cdot}0.2Et_2O$: C, 54.22; H, 4.93; N, 15.20. Found: C, 54.57; H, 4.53; N, 15.30.

4.2.10.3. Trihydrochloride salt of 6-(4-amidinophenyl)-2-(5-amidinopyridin-2-yl)-1*H*-indole (17c). Yellow solid (0.103 g, 33%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.18 (s, 1H), 9.61 (s, 2H), 9.43 (s, 2H), 9.30 (s, 2H), 9.15 (br s, 2H), 9.08 (s, 1H), 8.32–8.30 (m, 2H), 7.97 (d, 2H, J = 8.4 Hz), 7.94 (d, 2H, J = 8.4 Hz), 7.85 (s, 1H), 7.78 (d, 1H, J = 8.4 Hz), 7.49 (d, 1H, J = 8.4 Hz), 7.47 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 165.8, 164, 149.4, 146.9, 138.8, 137.6, 137.5, 134.2, 129.3, 129, 127.4, 126.5, 122.5, 122.4, 120, 119.9, 111.2, 104, 103.7; ESI-MS: m/z calcd for $C_{21}H_{18}N_6$: 354.16, found: 355.3 (amidine base M$^+$+1). Anal. Calcd for $C_{21}H_{18}N_6{\cdot}3HCl{\cdot}0.75H_2O$: C, 52.84; H, 4.75; N, 17.60. Found: C, 52.96; H, 5.01; N, 17.31.

4.2.10.4. Dihydrochloride salt of 6-(4-amidinophenyl)-2-(6-amidinopyridin-3-yl)-1*H*-indole (17d). Yellow solid (0.118 g, 37%), mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.49 (s, 1H)), 9.73 (s, 2H), 9.43 (s, 2H), 9.41 (s, 2H), 9.17 (s, 2H), 8.68 (d, 1H, J = 8.8 Hz), 8.45 (d, 1H, J = 8.8 Hz), 7.99 (s, 1H), 7.98 (br s, 4H), 7.84 (s, 1H), 7.77 (d, 1H, J = 8 Hz), 7.50 (d, 1H, J = 8 Hz), 7.39 (s, 1H); ^{13}C NMR (DMSO-d_6); δ 165.8, 164.1, 149.4, 146.1, 138.7, 137.9, 137, 134.5, 129.3, 129, 127.5, 126.5, 122.5, 122.4, 120, 119.9, 111.3, 104.4, 102.3; ESI-MS: m/z calcd for $C_{21}H_{18}N_6$: 354.16, found: 355.3 (amidine base M$^+$+1). Anal. Calcd for $C_{21}H_{18}N_6{\cdot}2HCl{\cdot}2H_2O$: C, 54.43; H, 5.22; N, 18.13. Found: C, 54.52; H, 5.11; N, 17.98.

4.2.11. 4-(4-Amino-3-nitrophenyl)-benzonitrile (21)

DAPCy (0.435 g, 0.6 mmol %) and K_3PO_4 (6.3 g, 30 mmol) were added to a solution of **20** (3.88 g, 15 mmol) and *p*-cyanophenylboronic acid (2.64 g, 18 mmol) in deaireated *iso*-propanol (60 mL). The vigorously stirred mixture was warmed to 80 °C for 24 h under a nitrogen atmosphere. The solvent was concentrated under reduced pressure, the crystals which formed were filtered, dissolved in dichloromethane and passed through celite and evaporated under reduced pressure to give **21** (2.9 g, 80%). Mp 204.4–205.4 °C.; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.66 (s, 1H), 7.73 (d, 2H, J = 7.2 Hz), 7.57 (d, 2H, J = 7.2 Hz), 7.43 (d, 2H, J = 9.0 Hz), 5.77 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 145.9, 142.5, 133.7, 132.5, 130.6, 126.2, 125.0, 123.2, 119.9, 118.6, 109.1; HRMS (ESI): Calcd for $C_{13}H_8N_3O_2$ m/z: 238.0617 (M$^+$+1); Found m/z: 238.0612.

4.2.12. Trihydrochloride salt of 2,5-bis(4-amidinophenyl)-1*H*-benzimidazole (24)

p-Nitrobenzene (0.5 mL, 4.5 mmol) was added to a stirred mixture of **22** (310 mg, 1.5 mmol) and 4-cyanobenzaldehyde (195 mg, 1.5 mmol), in dry dimethylformamide. The vigorously stirred mixture was warmed to 145 °C for 48 h under a nitrogen atmosphere. Ethyl acetate was added, and the precipitated crystals were filtered and recrystalized from ethanol to give 2,5 (6)-bis(4-cyanophenyl)-1*H*-benzimidazole **23** (0.288 g, 60%), mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 13.22 (s, 1H), 8.36 (d, 2H, J = 8.1 Hz), 8.02 (d, 2H, J = 8.1 Hz), 7.91 (m, 5H), 7.74 (d, 1H, J = 8.1 Hz), 7.63 (d, 1H, J = 8.4 Hz); HRMS (ESI): calcd for $C_{21}H_{13}N_4$ m/z: 321.1140 (M$^+$+1); Found m/z: 321.1154. Compound **23** was used in the next step without further characterization. The dinitrile **23** (0.16 g, 0.5 mmol) was dissolved in saturated ethanolic HCl (5 mL) and stirred at room temperature for 1 week, isolated from air and moisture. Dry ether was added, the crystals were filtered, dried under vacuum for 1 h and dissolved in absolute ethanol (10 mL), ammonia gas was passed for 30 min while cooling and the resulting solution was stirred for 4 days. Dry ether was added, the precipitated crystals (HCl salt) were filtered. The diamidine salt was purified by neutralization with 1 N sodium hydroxide solution followed by filtration of the resultant solid and washing with water and dried. Finally, the free base was stirred with ethanolic HCl (5 mL) for 2 days, diluted with ether, and the solid formed was filtered and dried to give the diamidine HCl salt (0.12 g, 68%), mp >300 °C dec.; ^{1}H NMR (DMSO-d_6) δ 9.50 (s, 2H), 9.39 (s, 2H), 9.31 (s, 2H), 9.20 (s, 2H), 8.54 (d, 2H, J = 8.4 Hz), 8.08 (d, 2H, J = 8.4 Hz), 8.06 (s, 1H), 7.99 (s, 4H), 7.84 (d, 1H, J = 8.4 Hz), 7.74 (dd, 1H, J = 1.5 Hz, J = 8.4). ^{13}C NMR (DMSO-d_6) δ 165.2, 164.8, 149.7, 145.5, 137.0, 136.6, 135.0, 131.6, 130.1, 129.2, 129.0, 127.7, 127.5, 126.4, 124.0, 115.7, 113.4. ESI-MS: m/z calcd for $C_{21}H_{18}N_6$: 354.4, found: 355.1 (amidine base M$^+$+1). Anal. Calcd for $C_{21}H_{18}N_6{\cdot}3HCl{\cdot}2H_2O$: C, 50.46; H, 5.04; N, 16.81. Found: C, 50.53; H, 4.76; N, 16.49.

4.2.13. 2,5-Bis(4-cyanophenyl)-1-*N*-methyl-1*H*-benzimidazole (29)

DAPCy (0.3 g, 0.44 mmol %) and K_3PO_4 (3.8 g, 17.8 mmol) were added to a solution of **26** (2.5 g, 8.9 mmol) and *p*-cyanophenylboronic acid (1.55 g, 10.6 mmol) in deaireated absolute ethanol (60 mL). The vigorously stirred mixture was warmed to 80 °C for 24 h under a nitrogen atmosphere. The solvent was concentrated under reduced pressure; the crystals which formed were filtered, dissolved in dichloromethane and passed through celite. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (70:30, v/v) as the eluent gave 4-(4-*N*-methylamino-3-nitrophenyl)-benzonitrile **(27)** (1.35 g, 60%). Mp 194.4–195.4 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.39 (d, 1H, J = 2.4 Hz), 8.14 (br s, 1H), 7.95 (dd, 1H, J = 2.4, J = 9.0 Hz), 7.83 (m, 4H), 7.12 (d, 1H, J = 9.3 Hz), 3.01 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 145.9, 142.7, 134.8, 132.9, 131.5, 126.6, 124.7, 124.2, 119.0, 115.4, 109.4, 29.7. Compound **27** was used in the next step without further characterization. Pd/C (10%) (0.5 g) was added to a solution of the nitro compound **27** (4 g, 15.6 mmol) in ethanol: ethyl acetate (60 mL: 20 mL) mixture. Shaking in a Parr hydrogenator at 50 psi was continued until the uptake of hydrogen ceased; the solution was passed through celite to remove the catalyst, and concentrated under reduced pressure. Purification by crystallization from acetone gave 4-(3-amino-4-*N*-methylaminophenyl)-benzonitrile (**28**) (3.2 g, 90%). Mp 215–216 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.63 (m, 4H), 7.13 (dd, 1H, J = 2.1, J = 8.1 Hz), 6.98 (d, 1H, J = 1.8 Hz), 6.72 (d, 1H, J = 8.4 Hz), 3.40 (br s, 2H), 2.92 (s, 3H). ESI-MS: m/z calcd for $C_{14}H_{13}N_3$: 223.27, found: 224.0 (M$^+$+1). Compound **28** was used in the next step without further characterization. 4-Cyanobenzaldehyde (0.7 g, 5.6 mmol) and **28** (1.4 g, 5.4 mmol) were stirred in anhydrous dimethylformamide for 1 h, then sodium

bisulfite (0.6 g, 5.6 mmol) was added and the resulting mixture was stirred at 130 °C for 48 h, cooled to room temperature, poured into water and the resulting crystals were filtered and recrystalized from ethanol to give **29** (1.4 g, 73% yield). Mp 257–258 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.10 (m, 3H), 8.03 (d, 2H, J = 8.7 Hz), 7.95 (d, 2H, J = 8.7 Hz), 7.89 (d, 2H, J = 8.7 Hz), 7.80 (d, 1H, J = 8.7 Hz), 7.72 (dd, 1H, J = 1.5, J = 8.4 Hz), 3.95 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.2, 145.2, 142.9, 137, 134.1, 132.7, 132.5, 132.3, 129.8, 127.4, 122.2, 118.6, 118.1, 117.7, 112.1, 111.2, 109.2, 31.7. ESI-MS: m/z calcd for $C_{22}H_{14}N_4$: 334.12, found: 335.1 (M^++1). Anal. Calcd for $C_{22}H_{14}N_4{\cdot}0.2H_2O$: C, 78.18; H, 4.29; N, 16.58. Found: C, 78.28; H, 4.15; N, 16.88.

4.2.14. Trihydrochloride salt of 2,5-bis(4-amidinophenyl)-1-*N*-methyl-1*H*-benzimidazole (30)

This compound was prepared by following the experimental procedure used for compounds **6a**,**b**. Yellow solid (0.183 g, 89%). Mp >300 °C dec. ^{1}H NMR (DMSO-d_6) δ 9.68 (s, 2H), 9.52 (s, 2H), 9.45 (s, 2H), 9.29 (s, 2H), 8.18 (d, 2H, J = 9.0 Hz), 8.13 (d, 2H, J = 8.4 Hz), 8.02 (s, 3H), 7.95 (d, 2H, J = 8.7 Hz), 7.89 (d, 2H, J = 8.4 Hz), 4.02 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.4, 165.9, 141, 138.7, 136.7, 136.6, 136.1, 133.2, 131.2, 130.3, 129.6, 127.5, 126.5, 126.3, 123.5, 121, 21.4; ESI-MS: m/z calcd for $C_{22}H_{20}N_6$: 368.43, found: 368.40 (amidine base M^+). Anal. Calcd for $C_{22}H_{20}N_6{\cdot}3HCl{\cdot}2.95H_2O$: C, 49.76; H, 5.49; N, 15.83. Found: C, 50.13; H, 5.30; N, 15.44.

4.2.15. 2,5-Bis(4-cyanophenyl)-1-*N*-hydroxy-1*H*-benzimidazole (34)

3-Bromo-6-fluoronitrobenzene **25** (1.7 mL, 13.6 mmol) was added to a solution of 4-cyanobenzylamine **31** (3.4 g, 20.3 mmol) and K_2CO_3 (5.6 g, 40.5 mmol) in dioxane (150 mL). The resulting solution was heated to reflux under nitrogen for 24 h. The inorganics were filtered from the mixture, the solution was concentrated, hexane was added and the product, orange crystals were filtered and recrystalized from ethyl acetate to give 4.4 g of 4-(*N*-4-cyanobenzylamino)-3-nitro-bromobenzene **32** in 98% yield, mp 153–154 °C. Compound **32** was used in the next step without further characterization. DAPCy (0.2 g, 0.37 mmol %) and K_3PO_4 (3.1 g, 15 mmol) were added to a solution of **32** (2.65 g, 7.5 mmol) and *p*-cyanophenylboronic acid (1.1 g, 7.5 mmol) in deaireated absolute ethanol (60 mL). The vigorously stirred mixture was warmed to 80 °C for 72 h under a nitrogen atmosphere. The precipitated product was filtered. The solid was dissolved in acetone, filtered through celite and recrystalized from EtOAc/MeOH to give 4-(4-cyanobenzylamino-3-nitrophenyl)-benzonitrile (**33**) (2.1 g, 80% yield), mp 181–182 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.86 (br s, 1H), 8.43 (d, 1H, J = 2.1 Hz), 7.85 (m, 7 H), 7.57 (d, 2H, J = 8.1 Hz), 6.96 (d, 1H, J = 9.0 Hz), 4.80 (d, 2H, J = 6.3 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 144.4, 144.3, 142.4, 134.5, 132.8, 132.0, 132.1, 127.8, 126.5, 125.5, 124.3, 118.7, 118.6, 115.6, 109.9, 109.5, 45.4. ESI-MS: m/z calcd for $C_{21}H_{14}N_4O_2$: 354.36, found: 355.1 (M^++1). Compound **33** was used in the next step without further characterization. A solution of sodium methoxide (0.3 g, 5.6 mmol) and **33** (1 g, 2.8 mmol) in 30 mL dry methanol was heated at reflux for 24 h under nitrogen atmosphere. The reaction mixture was cooled to room temperature, brought to pH 7 with 20% HCl, the resulting yellow crystals were filtered and dried to give **34** (0.9 g, 93%). Mp 244.4–244.5 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.46 (d, 2H, J = 8.7 Hz), 7.94 (d, 2H, J = 8.1 Hz), 7.90 (d, 2H, J = 5.7 Hz), 7.86 (m, 2H), 7.81 (m, 1H), 7.75 (d, 1H, J = 8.7 Hz), 7.61 (dd, 1H, J = 1.5, J = 8.1 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 144.9, 138.4, 134.2, 132.8, 132.7, 132.6, 128.7, 128.5, 127.8, 122.3, 120.3, 118.8, 118.4, 112.3, 109.7, 109.9, 107.9. HRMS (ESI) calcd for $C_{21}H_{13}N_4O$ m/z: 337.1090 (M^++1); found m/z: 337.1173.

4.2.16. Trihydrochloride salt of 2,5-bis(4-amidinophenyl)-1-*N*-hydroxy-1*H*-benzimidazole (35)

This compound was prepared by following the experimental procedure used for compound **24**. Yellow solid (0.118 g, 78%), mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 9.63 (br s, 2H), 9.52 (br s, 2H), 9.39 (br s, 2H), 9.28 (br s, 2H), 8.54 (d, 2H, J = 8.0 Hz), 8.05 (t, 4H, J = 7.2 Hz), 7.98 (d, 3H, J = 8.8 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.80 (d, 1H, J = 8.4 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.1, 146.3, 145.4, 137.7, 134.0, 133.8, 132.8, 129.0, 128.6, 128.3, 127.0, 126.2, 122.1, 119.7, 119.7, 108.0. ESI-MS: m/z calcd for $C_{21}H_{18}N_6O$: 370.40, found: 370.4 (amidine base M^+). Anal. Calcd for $C_{21}H_{18}N_6O{\cdot}3HCl{\cdot}0.5H_2O{\cdot}0.4EtOH$: C, 51.62; H, 4.84; N, 16.56. Found: C, 51.62; H, 4.61; N, 16.24.

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