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Synthesis, DNA binding, fluorescence measurements and antiparasitic activity of DAPI related diamidines

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ABSTRACT

A novel series of extended DAPI analogues were prepared by insertion of either a carbon–carbon triple bond (**16a–d**) or a phenyl group (**21a**,**b** and **24**) at position-2. The new amidines were evaluated in vitro against both *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*). Five compounds (**16a**, **16b**, **16d**, **21a**, **21b**) exhibited IC_{50} values against *T. b. r.* of 9 nM or less which is two to nine folds more effective than DAPI. The same five compounds exhibited IC_{50} values against *P. f.* of 5.9 nM or less which is comparable to that of DAPI. The fluorescence properties of these new molecules were recorded, however; they do not offer any advantage over those of DAPI.

1. Introduction

Eukaryotic parasitic diseases, such as sleeping sickness and malaria are caused by ancient, unusual, and often quite deadly microorganisms.[1] *Trypanosoma brucei*, which causes sleeping sickness, limits the health and economic hopes of 50,000–70,000 people/year, and if not effectively treated, is fatal.[2] Dicationic systems that bind in the DNA minor groove are promising agents against these diseases. Pentamidine (Fig. 1) is the only one of this class which has seen significant human clinical use.[3] Furamidine (Fig. 1), a diphenyl furan diamidine analogue, has been shown to be more potent and less toxic than pentamidine in murine models of trypanosomiasis.[4] The orally effective prodrug of furamidine (pafuramidine) (Fig. 1), showed promising results in Phase I and II clinical trials against both sleeping sickness and malaria.[3,4] Unfortunately, in an additional safety study of pafuramidine parallelling the Phase III trials, liver and kidney toxicities in some volunteers were found and the development of pafuramidine was suspended.[4] DAPI (4′,6-diamidino-2-phenylindole) (Fig. 1), was developed as a compound related to diminazene and stilbamidine, to be used as an antitrypanosomal agent.[5] DAPI, however showed a variety of biological effects, including antifungal, antibacterial, antitrypanosomal and antiviral activities.[5,6] Also, DAPI is a fluorescent dye which exhibits several binding modes to DNA[7] and so it has been frequently utilized as a DNA specific probe for flow cytometry, chromosome staining, DNA visualization and quantitation,[8] and it is now an important tool in molecular biology. The antiparasitic mode of action of these diamidines is thought to involve binding to the minor groove of DNA at AT rich regions in the nucleus or kinetoplast.[9] For decades, strong minor groove binding was thought to require a curved molecule (crescent shape) that complemented the shape of the DNA minor groove. Furamidine appears to work in this way as it binds strongly to the parasite DNA and causes rapid destruction of the mitochondrial kinetoplast.[10] In contrast to curved compounds, linear molecules have not been significantly investigated. The classical model for minor groove binding emphasizes complementary curvature between DNA and the binding compound.[11] This model indicates that compounds, which are linear or have a large radius of curvature, should bind weakly to the minor groove. DB921 (Fig. 1), a near linear heterocyclic diamidine, however, binds quite strongly to the DNA minor groove, even more strongly than related curved compounds.[12] DB921 was found to simulate the curved structure of DNA minor groove by incorporation of a water molecule into the recognition complex with DNA.[12,13] Based on both the discovery of this new binding mode

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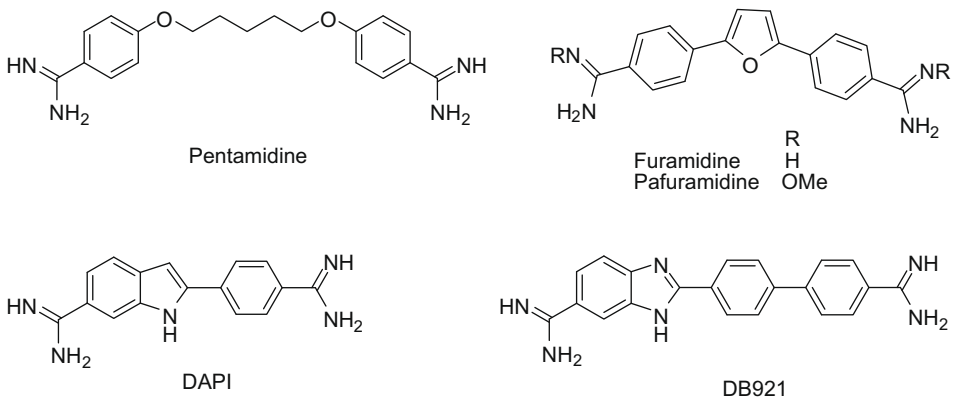


Figure 1. Active antiparasitic agents.

and the favorable biological activities and fluorescence of DAPI, we prepared new series of near linear cationic indole derivatives (**16a–d**, **21a–b**, **24**) which retain many of the features of both DAPI and DB921.

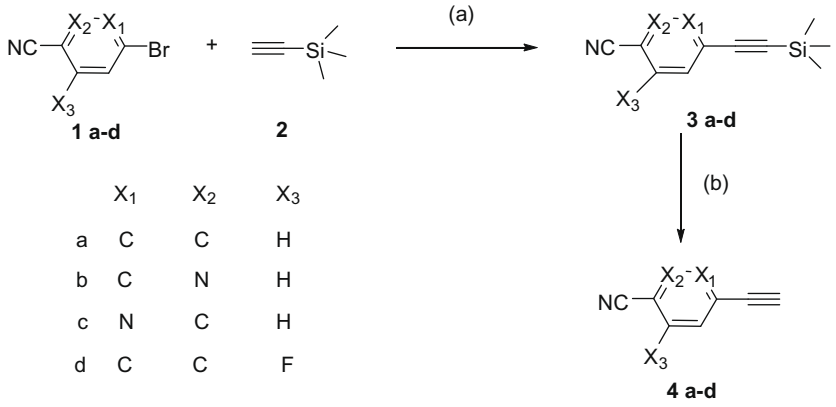
2. Results and discussion

2.1. Chemistry

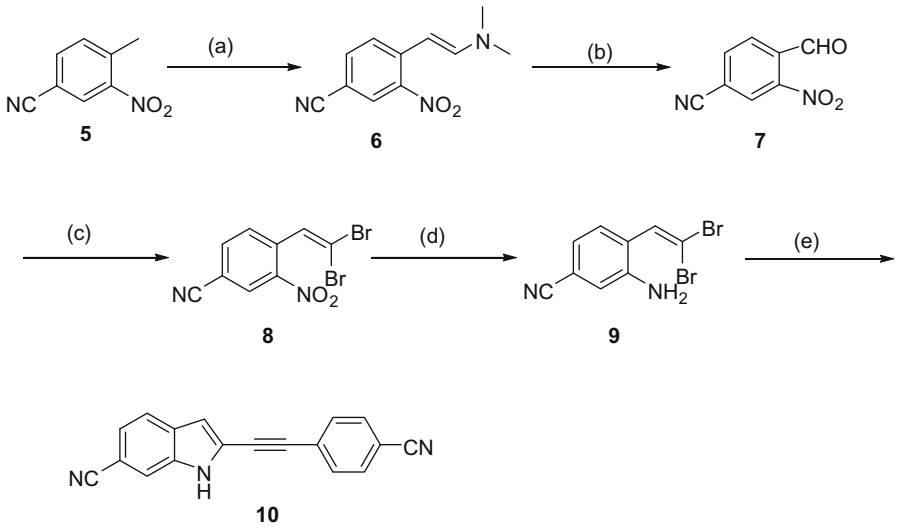
Scheme 1 outlines our approach to the synthesis of new ethynyl benzonitrile derivatives which will be used in the synthesis of the target compounds **10**, **15a–d**. We employed Sonogashira-type[14] cross coupling reactions of bromo benzonitrile derivatives with trimethylsilylacetylene to give the acetylenes **3a–d** which were desilylated[14] by stirring with tetrabutylammonium fluoride in tetrahydrofuran-methylene chloride mixture to afford the ethynyl benzonitrile derivatives **4a–d.**

Scheme 2 outlines the synthesis of indole acetylene **10**, starting with **5** which contains a methyl group *ortho* to the strong electron withdrawing nitro group that can be easily functionalized by condensation with *N*,*N*-dimethylformamide dimethyl acetal to provide the corresponding *N*,*N*-dimethylamino alkene **6**.[15] Oxidative cleavage of **6** using sodium periodate in tetrahydrofuran-water mixture afforded the aldehyde **7**[15,16] Since the Ramirez olefination is sensitive to amine or amide functional groups, so we decided to start with the nitro compound **7** which we planned to reduce later. The dibromoalkene **8** was prepared efficiently with the Ramirez (Wittig-type) dibromoolefination of the corresponding aldehyde **7** using carbon tetrabromide as a reagent and triphenylphosphine as a catalyst.[17] Recently, it was discovered that performing this reaction in the presence of zinc powder, a bromine scavenger, gave higher yields.[18] The reduction of the nitro compound **8** is a potential challenge due to practical drawbacks regarding the use of traditional reducing agents such as tin or iron metal, including large amount of metal oxide waste and difficulties with workup.[19] Also palladium catalyzed hydrogenation could affect the bromo groups or the double bond. Due to these potential problems, we sought a more promising method for reduction. Vanadium-doped palladium on carbon catalyst reported by Baumister appeared to be useful.[20] The presence of vanadium is thought to minimize contamination by undesired hydroxylamines formed in the reduction process. Using this approach, the amine **9** was obtained in excellent yield using a mixture of toluene and ethyl acetate (3:1) as the hydrogenation solvent. This amine was allowed to undergo cross coupling with the terminal acetylene **4a** according to the method of Lautens and co-workers[19] This process involves Ullman cyclization to form 2-bromoindoles which undergoes Sonogashira coupling with terminal alkynes giving the desired 2-alkynylindole **10**. In our hands, a yield of 15% was obtained. The relatively poor yield lead us to search for another method for preparation of the indole acetylenes. We decided to attempt Sonogashira coupling between the unreported 2-iodoindole **14** and the ethynyl benzonitrile derivatives **4a–d**.

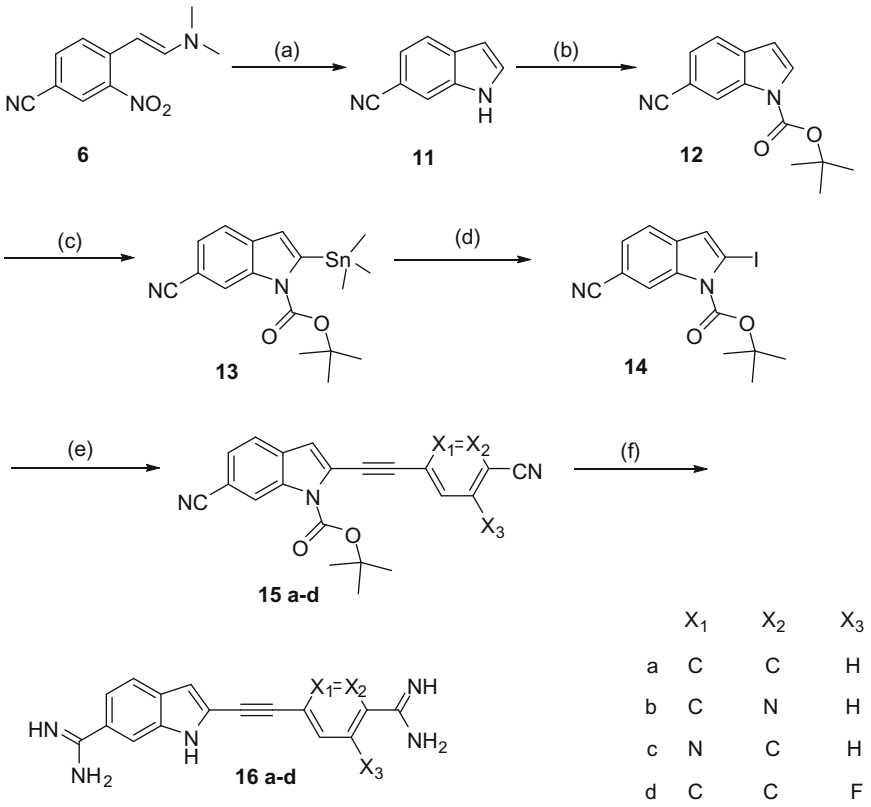
Scheme 3 outlines the preparation of the indole acetylenes **15a–d** in high yield. The enamine **6** undergoes catalytic reductive cyclization using Pd/C in tetrahydrofuran to form 1*H*-indole-6-carbonitrile.[21] The Boc-protected indole **12** was prepared in high yield by employing a procedure we have used previously[22] employing di-*tert*-butyldicarbonate (Boc_2O) and 4-(dimethylamino) pyridine (DMAP) in dichloromethane as the solvent. We tried a number of iodination methods to prepare the 2-iodoindole derivative **14**, including the use of diiodoethane in presence of butyl lithium,[23]



Scheme 1. Reagents: (a) $PdCl_2(PPh_3)_2$, CuI, TEA, THF; (b) TBAF, THF, CH_2Cl_2.



Scheme 2. Reagents: (a) DMF–DMA, DMF; (b) $NaIO_4$, THF/H_2O; (c) CBr_4, $P(Ph)_3$, CH_2Cl_2; (d) H_2/Pd(C), toluene/EtOAc; (e) Pd(C), $P(4\text{-}OCH_3Ph)_3$, CuI, toluene, 4-ethynylbenzonitrile.



Scheme 3. Reagents: (a) H_2/Pd(C), THF; (b) BOC_2O, DMAP, CH_2Cl_2; (c) $ClSn(CH_3)_3$, LDA/THF; (d) I_2/THF; (e) $PdCl_2(PPh_3)_2$, CuI, TEA, THF, ethynylbenzonitrile derivatives; (f) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.

iodine monochloride-pyridine complex in acetic acid,[24] molecular iodine with butyl lithium in tetrahydrofuran,[25] *N*-iodosuccinimide with butyl lithium in tetrahydrofuran.[26] However we were unable to obtain **14** in a reasonable yield using these methods, therefore we decided to introduce a trimethylstannyl group at the 2-position of indole which has been reported in other systems to react with iodine to form the corresponding iodo compound.[27] Lithiation of the Boc-protected indole **12** was readily achieved with lithium diisopropylamide (LDA) in anhydrous tetrahydrofuran at −20 °C. Subsequent reaction of the lithioindole intermediate with trimethyltin chloride gave the indole stannane **13** in good yield.[22] Stirring the indole stannane **13** with molecular iodine in dry tetrahydrofuran at room temperature give the iodoindole **14** in high yield (88%). We note that the preparation of the 2-iodoindole **14** was recently reported also employing an indirect route starting with the analogous boronic acid.[28] The alkynylindole derivatives **15a–d** were prepared through Sonogashira coupling of iodoindole **14** with the terminal alkynes **4a–d** using $PdCl_2(PPh_3)_2$ as the

catalyst, in anhydrous tetrahydrofuran as the solvent under nitrogen atmosphere. The dinitriles **15a–d** were allowed to react with lithium bis(trimethylsilyl)amide[29] in THF, followed by deprotection of the silylated amidines with ethanolic HCl to furnish hydrochloride salts of the diamidines **16a–d**.

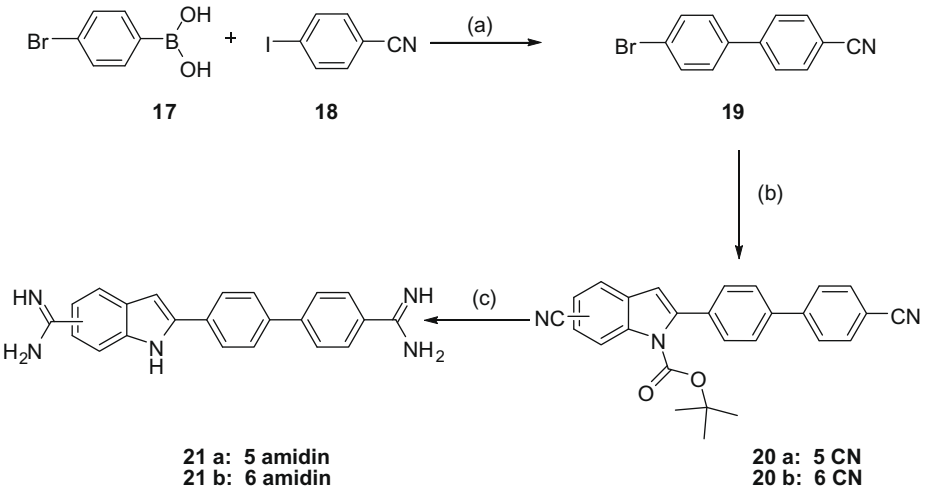
Scheme 4 outlines the synthesis of the linear diphenyl indole diamidines **21a**,**b**. 4,4′-Bromobiphenyl benzonitrile **19** was prepared in good yield by employing a Suzuki coupling reaction[30] between the boronic acid **17** and the iodobenzonitrile **18** in the presence of 5 mol % $Pd(PPh_3)_4$. A Stille coupling reaction[31] between **19** and 1-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)-1*H*-indole-5 or 6-carbonitrile[22] using 5 mol % $Pd(PPh_3)$ and 1,4-dioxane as the solvent afforded the dinitriles **20a**,**b** in good yield. These dinitriles were converted to the diamidines **21a**,**b** utilizing the lithium bis(trimethylsilyl)amide method.

Scheme 5 outlines the synthesis of the monoamidine **24**. The Stille coupling reaction between **13** and the commercially available **22** gave the mononitrile **23** which was converted to the amidine **24** utilizing the lithium bis(trimethylsilyl)amide method.

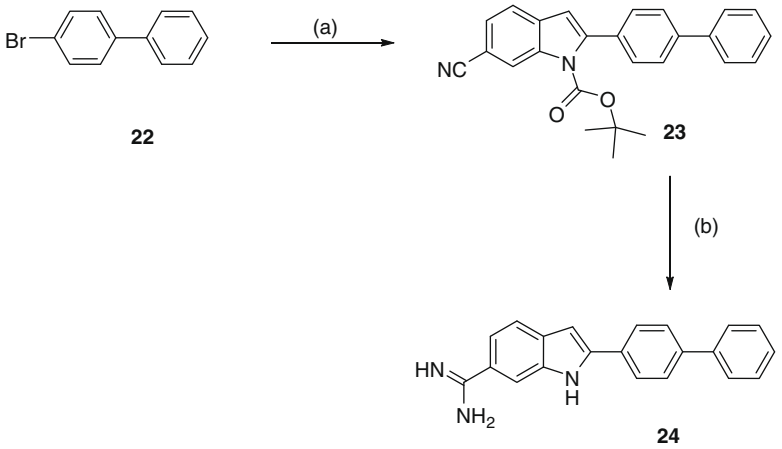
2.2. Biology

The results for assessment of DNA binding affinity and the activity against *P. f.* K1 and *T. b. r.* STIB900 for seven new indole analogues are presented in Table 1. For comparative purposes related data for pentamidine, furamidine, DAPI and DB921 are also included in Table 1. The DNA binding affinity as deduced from ΔT_m values varies with structure and ranges from quite strong to moderate. For a relatively small molecule the affinity of DAPI is quite strong as previously reported[32] and in the current work a ΔT_m value of greater than 27 °C is found. Extension of the length of DAPI by insertion of a carbon–carbon triple bond at position-2 (**16a**) results in a reduction in ΔT_m value of about 30% of that of DAPI. In contrast, an insertion of a phenyl group at position-2 (**21b**) results in a molecule with comparable DNA affinity to that of DAPI.

Since the overall dimensions of **16a** and **21b** are roughly similar, it seems likely that the lower affinity of **16a** is attributable, in large part, to weaker stacking interactions of **16a** with the groove. The two isomers **21b** and **21a** show significantly different affinities. Compound **21b** shows a larger ΔT_m value than **21a**. Molecular modeling suggests that the 20% difference in affinity is likely due to the fact that in the complex **21b** can form a strong hydrogen bond involving the indole N–H to a thymine carbonyl. Whereas in the analogous complex for **21a** such is not possible as only the 3-postion C–H is pointed into the groove. Removal of the amidine on the terminal phenyl ring to form **24** results in about 40% reduction of affinity consistent with the loss of one key cationic binding



Scheme 4. Reagents: (a) $Pd(PPh_3)_4$, Na_2CO_3, toluene; (b) 1-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)-1*H*-indole-5 or 6-carbonitrile, $Pd(PPh_3)_4$, dioxane; (c) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.



Scheme 5. Reagents: (a) 1-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)-1*H*-indole-6-carbonitrile, $Pd(PPh_3)_4$, dioxane; (b) (i) $LiN(TMS)_2$/THF; (ii) HCl gas/EtOH.

Table 1
DNA affinities and antiprotozoal activity for DAPI analogues

Compd	ΔT_m[a] (°C)	Cyctoxicity[c] (μM)	*T. b. r.*[b] (nM)	SI_T[d]	P.f.[b] (nM)	SI_P[e]
Pentamidine	12.6	46	2.2	15,533	46.4	991
Furamidine	25	6.7	4.5	2093	15.5	486
DB921	>27	17	7.7	2207	0.5	34,000
DAPI	>27	0.86	18	51	3	287
16a	19.0	8.7	6	1450	3.8	2289
16b	19.1	1.6	3	533	3.1	516
16c	12.2	13.0	215	60	14.1	933
16d	16.0	4.5	9	500	5.9	763
21a	20.9	38.9	4	9725	0.7	55,571
21b	>27	10.5	2	5250	3.3	3182
24	16.0	8.4	8723	0.96	858	9.8

[a] Increase in thermal melting of poly(dA-dT)$_n$.[38]
[b] The *T. b. r.* (*Trypanosoma brucei rhodesiense*) strain was STIB900 and the *P. f.* (*Plasmodium falciparum*) strain was K1. The values are the average of duplicate determinations.[37]
[c] Cyctotoxicity was evaluated using cultered L6 rat myoblast cells.[37]
[d] Selectivity index for *T. b. r.* expressed as the ratio: IC_{50}(L6)/IC_{50}(*T.b.r*).
[e] Selectivity index for *P. f.* expressed as the ratio: IC_{50}(L6)/IC_{50}(*P.f.*)

center. A limited study of the effect of substitution at the benzamidine portion of **16a** shows that DNA affinity is sensitive to such variations. Introduction of nitrogen *ortho* to the amidine **16b** was well tolerated, however when nitrogen is placed *meta* to the amidine **16c** the ΔT_m value is reduced by approximately one-third. Similar trends have been noted for other aza-analogues of minor groove binders and are believed to arise from differences in hydration of the small molecules.[33] Introduction of a fluorine atom *ortho* to the benzamidine **16d** results in about 15% reduction in affinity, perhaps due to twisting of the amidine group relative to the phenyl ring. In order to determine if the new analogues **16a** and **21b** are binding in the DNA minor groove and to compare them to DAPI, we acquired CD spectra of these compounds on binding to DNA (see Fig. 2). Minor groove binding agents give a large positive induced CD signal on binding to AT DNA sequences and cause only small changes in the shape of the DNA CD spectrum.[34] Such a result is observed on adding DAPI to poly(dA)·poly (dT) (Fig. 2) with a strong, positive induced signal between 350 and 400 nm and little change in the DNA signal near 260 nm. As can be seen in Figure 2, both **16a** and **21b** have similar CD spectra with poly(dA)·poly (dT). The biphenyl indole, **21b** has a particularly strong induced CD signal at around 390 nm indicating a strong interaction with the AT base pairs. These results clearly support a minor groove binding mode for **16a** and **21b** which is the case for DAPI.[34] A detailed analysis of the effect of structure induced variation in DAPI analogues DNA affinities awaits the results of the quantitative biophysical studies which are in progress.

The IC_{50} values of the new diamidino indoles against *T. b. r.* range from 2 to 9 nM (with the exception of **16c**; IC_{50} = 215 nM), which are in the range of those for furamidine and pentamidine, but 2–9-folds more active than DAPI. Consistent with other monoamidines of active diamidines, **24** is essentially inactive (IC_{50} = 8.7 μM).[3a] The activity of the diamidines against *P. f.* followed a similar trend, **16c** was the least active and the other diamidines gave IC_{50} values ranging from 0.7 to 5.9 nM. The activity of the monoamidine **24** is greatly reduced (IC_{50} = 0.86 μM) in comparison to **21a** and **21b**. The antiplasmodial activity of the indoles is superior to that of furamidine and pentamidine and comparable to that of DAPI. The potency of **21a** (IC_{50} = 0.7 nM) places it in a small group of highly active diamidines in the STI screen.[35] The selectivity indices for both *T. b. r.*(SI_T) and *P. f.* (SI_P) are listed in Table 1. The SI_T values for the five most active compounds (**16a**, **16b**, **16d**, **21a**, **21b**) range from 500 to 9725, a clear improvement over the value of 51 for DAPI. The SI_P values for the same five compounds ranges from 516 to 55571which are all superior to the DAPI value of 287. Clearly these compounds merit in vivo evaluation against both organisms.

Given the general use of DAPI in molecular biology, in large part due to its fluorescence properties, we have recorded fluorescence data for the new indoles which is found in Table 2. For comparative evaluation, also included in Table 2 are results for DAPI. The absorption maximum for the new indoles ranges from 340 to 368 nm (with the exception of the fluoro analogue **16d** which are comparable to that of DAPI (341 nm). The emission maximum ranges from 425 to 514 nm in comparison of a value of 458 nm for DAPI. With two exceptions, a qualitative comparison of quantum yield to that of DAPI shows a loss of efficacy by 13–43-fold. One exception, **16a** shows only a twofold decline and interestingly the monoamidine **24** shows a moderate increase compared to that of DAPI. An important feature for the molecular biology use of DAPI fluorescence is that on binding to DNA, its fluorescence is enhanced by about 20-folds.[36] Therefore, we compared the fluorescence of the new analogues to DAPI on binding to DNA. Figure 3 shows the expected enhancement of DAPI fluorescence on interaction with DNA, however none of the new analogues cause a similar enhancement; illustrated in Figure 3 with compound **21b**. We conclude that the fluorescence properties of these new molecules do not offer any advantage over those of DAPI.

2.3. Conclusion

The new indoles show significant DNA binding affinities which vary with structure consistent with that noted for other diamidines. The diamidines are highly active in vitro against both *T. b. r.* and *P. f.* and are scheduled for in vivo evaluation. The fluorescent

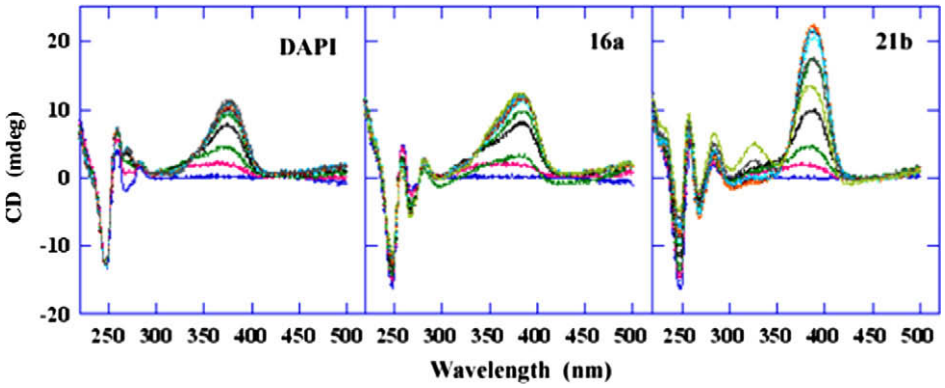


Figure 2. Comparison of CD spectra of poly(dA)·poly(dT) in complex with different compounds at various mixing ratios. (A) CD titration spectra of poly(dA)·poly(dT) binding with DAPI. (B) CD titration spectra of poly(dA)·poly(dT) binding with **16a**. (C) CD titration spectra of poly(dA)·poly(dT) binding with **21b**.

Table 2
Fluorescence data for DAPI analogues

Compd	λ_{ex}[a,b] (nm)	λ_{em}[a,b] (nm) Sw/ExSw 5/20	
DAPI	341	458	821
16a	343	465	378
16b	348	461	27
16c	368	514	42
16d	290	425	19
21a	341	511	32
21b	348	461	63
24	340	454[c]	721

[a] Wavelenths(λ) indicated are for excitation and emission.
[b] Measurements were made in 0.001 M solution in distilled water.
[c] λ_{em} obtained at Ex. sw/Em. sw 2.5/20.

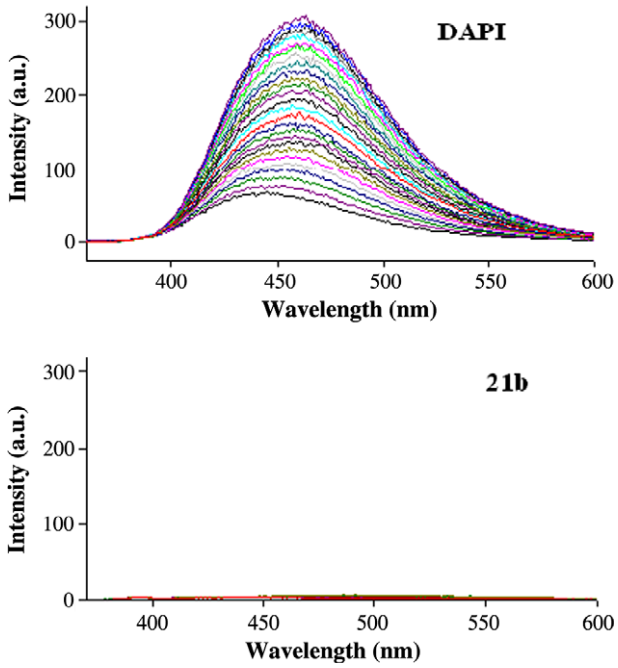


Figure 3. Excitation spectra for DAPI and **21b** are plotted as intensity (arbitary units) versus wavelength. The free compound concentrations were 0.5 μM, poly(dA)·poly(dT) DNA was titrated into the cell at 0.1 μM increments. Both excitation and emission slit widths were 5 nm. The buffer was 10 mM cacodylic acid, 1 mM EDTA and 0.1 M NaCl with the pH adjusted to 6.2 with NaOH prior to dilution to the final volume. Excitation wavelength is the same as listed in Table 1. DAPI gave the expected fluorescence enhancement but none of the DAPI analogues gave any significant enhancement of fluorescence when bound to the AT DNA polymer. All were similar to **21b** in the Figure.

properties of the new molecules do not vary greatly from that of DAPI.

3. Experimental

3.1. Biology

3.1.1. In vitro activity determination

In vitro assays with *T. b. r.* STIB900 and *P. f.* K1 strain and cell toxicity assays using cultured L6 rat myoblast cells were carried out as previously reported.[37]

3.1.2. ΔT_m Measurements

Thermal melting experiments were conducted with a Cary 300 spectrophotometer. Cuvettes for the experiment are mounted in a thermal block and the solution temperatures are monitored by a thermistor in the reference cuvette. Temperatures were maintained under computer control and are increased at 0.5 °C/min. The experiments were conducted in 1 cm path length quartz cuvettes in CAC 10 buffer (cacodylic acid 10 mM, EDTA 1 mM, NaCl 100 mM with NaOH add to give pH 7.0). The concentrations of DNA were determined by measuring the absorbance at 260 nm. A ratio of 0.3 mol compound per mole of DNA was used for the complex and DNA with no compound was used as a control.[38]

3.1.3. Circular dichroism (CD)

CD spectra were collected with a Jasco J-810 spectrometer at different ratios of compound to DNA at 25 °C in MES 10 buffer. A DNA solution in a 1-cm quartz cuvette was first scanned over a desired wavelength range. Compounds **16a** and **21b** at increasing ratios were then titrated into the same cuvette and the complexes rescanned under same conditions.[39]

3.2. 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. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using the indicated solvents. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Elemental analysis were performed by Atlantic Microlab Inc., Norcross, GA.

3.2.1. General procedure for the synthesis of 4-[(trimethylsilyl)-ethynyl] arylnitrile (3a–d)

A dry flask was charged with 4-bromoarylnitrile (**1a–d**) (55 mmol), $PdCl_2(PPh_3)_2$ (2 g, 2.75 mmol), and CuI (1.3 g, 5.5 mmol). After three successive nitrogen/vacuum cycles, degassed anhydrous tetrahydrofuran (200 mL), trimethylsilylacetylene (**2**) (16.05 mL, 110 mmol), and degassed anhydrous triethylamine (250 mL) were introduced via a syringe. Once triethylamine was added, the reaction mixture turned black. The reaction mixture was stirred under a nitrogen atmosphere at room temperature for 24 h, passed through celite to remove the catalyst, concentrated under vacuum, extracted with ethyl acetate (3 × 100 mL), washed with ammonium chloride (10% solution) to remove copper salts and then with water, dried over magnesium sulfate and concentrated. Purification by column chromatography on silica gel using hexanes/ethyl acetate (95/5, v/v).

3.2.1.1. 4-[(Trimethylsilyl)ethynyl]benzonitrile (3a). Yellowish brown solid (8.4 g, 83%), mp 109–111 °C, literature[14] mp 109–111 °C; spectroscopic data are consistent with those reported previously for this compound.

3.2.1.2. 5-[(Trimethylsilyl)ethynyl]pyridine-2-carbonitrile (3b). Yellow solid (9.47 g, 86%), mp 91 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.39 (br s, 1H), 7.85 (d, 1H, J = 8 Hz), 7.64 (d, 1H, J = 8 Hz), 0.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 153.6, 139.4, 132.1, 127.6, 123.9, 116.8, 103.7, 99.7, −0.4; ESI-MS: m/z calcd for $C_{11}H_{12}N_2Si$: 200.31, found: 201.1 (M^++1). Anal. Calcd for $C_{11}H_{12}N_2Si$: C, 65.96; H, 6.04; N, 13.98. Found: C, 66.22; H, 6.14; N, 13.82.

3.2.1.3. 6-[(Trimethylsilyl)ethynyl] pyridine-3-carbonitrile (3c). White solid (10 g, 91%), mp 127 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82 (br s, 1H), 7.91 (dd, 1H, J = 2 Hz, J = 8 Hz), 7.53 (d, 1H, J = 8 Hz), 0.23 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 152.4, 146.2, 139.3, 126.9, 116.2, 108.6, 102, 100.7, −0.5; ESI-MS: m/z calcd for $C_{11}H_{12}N_2Si$: 200.31, found: 201.1 (M^++1). Anal. Calcd for $C_{11}H_{12}N_2Si$: C, 65.96; H, 6.04; N, 13.98. Found: C, 66.12; H, 5.96; N, 13.91.

3.2.1.4. 2-Fluoro-4-[(trimethylsilyl)ethynyl]benzonitrile (3d).

Brown solid (9.32 g, 78%), mp 112 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57–7.54 (m, 1H,), 7.33 (m, 2H), 0.27 (s, 9H); ESI-MS: *m*/*z* calcd for $C_{12}H_{12}FNSi$: 217.31, found: 218.1 (M^++1). Anal. Calcd for $C_{12}H_{12}FNSi$: C, 66.32; H, 5.57; N, 6.45. Found: C, 66.51; H, 5.72; N, 6.12.

3.2.2. General procedure for the synthesis of 4-ethynylarylnitriles (4a–d)

A solution of tetrabutyl ammonium fluoride trihydrate (12.1 g, 35.2 mmol) in tetrahydrofuran (50 mL) was added to a solution of the silylated compounds (**3a–d**) (32 mmol) in dichloromethane (100 mL). The reaction mixture was stirred at 0 °C for 2 h, washed twice with water, dried over magnesium sulfate and concentrated. Purification by column chromatography on silica gel using hexanes/ethyl acetate (97/3, v/v).

3.2.2.1. 4-Ethynylbenzonitrile (4a). Yellow solid (3.19 g, 78%), mp 156–158 °C, literature[14] mp 156–158 °C; spectroscopic data are consistent with those reported previously for this compound.

3.2.2.2. 5-Ethynyl pyridine-2-carbonitrile (4b). Yellow solid (3.36 g, 82%), mp 126–126.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.78 (dd, 1H, *J* = 0.8 Hz, *J* = 2 Hz), 7.92 (dd, 1H, *J* = 2 Hz, *J* = 8 Hz), 7.69 (dd, 1H, *J* = 0.8 Hz, *J* = 8 Hz), 3.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 153.7, 139.9, 132.6, 127.7, 122.9, 116.8, 84.9, 78.9; ESI-MS: *m*/*z* calcd for $C_8H_4N_2$: 128.13, found: 129.1 (M^++1). Anal. Calcd for $C_8H_4N_2$: C, 74.99; H, 3.15; N, 21.86. Found: C, 75.12; H, 3.19; N, 21.92.

3.2.2.3. 6-Ethynyl pyridine-3-carbonitrile (4c). Greenish white solid (3.03 g, 74%), mp 158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.56 (d, 1H, *J* = 2 Hz), 7.97 (dd, 1H, *J* = 2 Hz, *J* = 8 Hz), 7.59 (d, 1H, *J* = 8 Hz), 3.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 153.7, 139.9, 132.6, 127.7, 122.9, 116.7, 84.9, 78.9; ESI-MS: *m*/*z* calcd for $C_8H_4N_2$: 128.13, found: 129.0 (M^++1). Anal. Calcd for $C_8H_4N_2$: C, 74.99; H, 3.15; N, 21.86. Found: C, 74.77; H, 3.11; N, 21.89.

3.2.2.4. 4-Ethynyl-2-fluorobenzonitrile (4d). Yellow solid (3.24 g, 70%), mp 120 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70–7.15 (br m, 3H), 3.4 (s, 1H); ESI-MS: *m*/*z* calcd for C_9H_4FN: 145.13, found: 146.2 (M^++1). Anal. Calcd for C_9H_4FN: C, 74.48; H, 2.78; N, 9.65. Found: C, 74.61; H, 2.89; N, 9.42.

3.2.3. Synthesis of 4-(2,2-dibromovinyl)-3-nitrobenzonitrile (8)

Triphenylphosphine (7.9 g, 30 mmol) was added portionwise to an ice cold solution of 4-cyano-2-nitro-benzaldehyde[15,16] (**7**) (1.76 g, 10 mmol), carbon tetrabromide (5.0 g, 15 mmol) and zinc powder (0.65 g, 10 mmol) in anhydrous methylene chloride (100 mL), stirred overnight, filtered to remove triphenylphosphine oxide and zinc bromide, concentrated, purification by column chromatography on silica gel using hexanes/dichloromethane (70/30, v/v) to give yellow crystals (2.95 g, 89%), mp 101–102 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.43 (d, 1H, *J* = 1.6 Hz), 7.96 (dd, 1H, *J* = 1.6 Hz, *J* = 8 Hz), 7.82 (d, 1H, *J* = 8 Hz), 7.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 136.1, 135.5, 132.9, 132.1, 128.6, 116.2, 113.7, 100, 95.9. Anal. Calcd for $C_9H_4Br_2N_2O_2$: C, 32.56; H, 1.21; N, 8.44. Found: C, 32.63; H, 1.32; N, 8.22.

3.2.4. Synthesis of 3-amino-4-(2,2-dibromovinyl)benzonitrile (9)

Pd/C (10%) (0.3 g) and ammonium metavanadate (0.017 g, 1 mol %) was added to a solution of the nitro compound (**8**) (5 g, 15 mmol) in toluene: ethyl acetate (30 mL: 10 mL) mixture. Shaking in a Parr hydrogenator under 50 psi was continued until the uptake of hydrogen ceased; the solution was passed through celite to remove the catalyst, and then concentrated under reduced pressure. Purification by column chromatography on silica gel using hexanes/ethyl acetate (60/40, v/v) gave yellow crystals (4.18 g, 92%), mp 142–142.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (d, 1H, *J* = 8 Hz), 7.07 (dd, 1H, *J* = 1.6 Hz, *J* = 8 Hz), 6.97 (d,1H, *J* = 8 Hz), 3.95 (br s, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 137.1, 135, 131.2, 130.3, 126.5, 119.3, 112.5, 99, 95.1; ESI-MS: *m*/*z* calcd for $C_9H_6Br_2N_2$: 301.97, found: 303 (M^++1). Anal. Calcd for $C_9H_6Br_2N_2$: C, 35.80; H, 2.00; N, 9.28. Found: C, 35.99; H, 2.27; N, 9.32.

3.2.5. Synthesis of 2-[(4-(cyanophenyl)ethynyl]-1*H*-indole-6-carbonitrile (10)

A dry flask was charged with 4-ethynylbenzonitrile (**4a**) (0.63 g, 4.95 mmol), dibromoolefin (**9**) (1 g, 3.3 mmol), Pd/C (0.176 g, 0.165 mmol), CuI (0.063 g, 0.33 mmol) and 4-trimethoxytriphenylphosphine (0.11 g, 0.33 mmol). After three successive nitrogen/vacuum cycles, degassed anhydrous toluene (20 mL) and degassed anhydrous isopropyl amine (0.72 mL, 8.25 mmol), were introduced via a syringe. The reaction mixture was stirred at 100 °C under a nitrogen atmosphere for 24 h, passed through celite to remove the catalyst, concentrated under vacuum, extracted with ethyl acetate (3 × 50 mL), washed with ammonium chloride (10% solution) to remove copper salts and then with water, dried over magnesium sulfate and concentrated. Purification by column chromatography on silica gel using hexanes/dichloromethane (50/50, v/v) gave brownish white crystals (0.13 g, 15%), mp 205–206 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.5 (s, NH); 7.94 (d, 2H, *J* = 8 Hz), 7.89 (br s, 1H), 7.79 (d, 2H, *J* = 8 Hz), 7.76 (d, 1H, *J* = 8 Hz), 7.40 (dd, 1H, *J* = 1.2 Hz, *J* = 8 Hz), 7.06 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 135.8, 133.2, 132.2, 130.7, 126.6, 122.9, 122.2, 121.9, 120.6, 118.8, 116.7, 111.9, 109.6, 105.1, 92.4, 85.9; ESI-MS: *m*/*z* calcd for $C_{18}H_9N_3$: 267.28, found: 268.1 (M^++1). Anal. Calcd for $C_{18}H_9N_3$: C, 80.88; H, 3.39; N, 15.72. Found: C, 80.64; H, 3.48; N, 15.49.

3.2.6. Synthesis of 1-(*tert*-butoxycarbonyl)-2-iodo-1*H*-indole-6-carbonitrile (14)

A molecular iodine (25 g, 98.76 mmol) solution in dry tetrahydrofuran (100 mL) was added slowly to a solution of the indole stannane[22] (**13**) (20 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, then with water, passed through sodium sulfate and evaporated. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (97/3, v/v) gave white solid (16 g, 88%), mp 72 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (d, 1H, *J* = 1.2 Hz), 7.54 (dd, 1H, *J* = 0.8 Hz, *J* = 8.4 Hz), 7.46 (dd, 1H, *J* = 1.2 Hz, *J* = 8.4 Hz), 7.07 (d, 1H, 0.8 Hz), 1.79 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 148.4, 136.4, 134, 126, 121.6, 120, 119.9, 119.7, 107.1, 86.8, 80, 28.2; ESI-MS: *m*/*z* calcd for $C_{14}H_{13}IN_2O_2$: 368.17, found: 369.1 (M^++1). Anal. Calcd for $C_{14}H_{13}IN_2O_2$: C, 45.67; H, 3.55; N, 7.60. Found: C, 45.91; H, 3.71; N, 7.35.

3.2.7. General procedure for the synthesis of 1-(*tert*-butoxycarbonyl)-2-[4-(ethynylarylnitrile)]-1*H*-indole-6-carbonitrile (15a–d)

A dry flask was charged with 4-ethynylarylnitrile (**4a–d**) (10.1 mmol), iodoindole derivative (**14**) (2.5 g, 6.79 mmol), $PdCl_2(PPh_3)_2$ (0.246 g, 0.33 mmol), CuI (0.12 g, 0.67 mmol), after three successive nitrogen/vacuum cycles, degassed anhydrous tetrahydrofuran (30 mL) and degassed anhydrous triethylamine (10 mL), were introduced via a syringe. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 24 h. The reaction mixture was passed through celite to remove the catalyst, concentrated under vacuum, extracted with ethyl acetate (3 × 100 mL), washed with ammonium chloride (10%

solution) to remove copper salts and then with water, dried over magnesium sulfate and concentrated. Purification by column chromatography on silica gel using hexanes/ethyl acetate (75/25, v/v) and then crystallization by hexanes/ethyl acetate mixture.

3.2.7.1. 1-(*tert*-Butoxycarbonyl)-2-[(4-cyanophenyl)ethynyl]-1-*H*-indole-6-carbonitrile (15a). White solid (1.74 g, 70%), mp 175–176 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54 (d, 1H, J = 1.6 Hz), 7.71 (d, 2H, J = 6.8 Hz), 7.67 (d, 2H, J = 6.8 Hz), 7.64 (d, 1H, J = 8 Hz), 7.52 (dd, 1H, J = 1.6 Hz, J = 8 Hz), 7.07 (s, 1H), 1.74 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 148.6, 135.2, 132.2, 132, 131.6, 127.2, 126.3, 123.1, 121.6, 120.3, 119.7, 118.2, 116.5, 112.3, 108.6, 94.9, 85.9, 85, 28.2; ESI-MS: m/z calcd for $C_{23}H_{17}N_3O_2$: 367.40, found: 368.2 (M^++1). Anal. Calcd for $C_{23}H_{17}N_3O_2$: C, 75.18; H, 4.66; N, 11.43. Found: C, 74.98; H, 4.79; N, 11.12.

3.2.7.2. 1-(*tert*-Butoxycarbonyl)-2-[(6-cyano-3-pyridyl)ethynyl]-1*H*-indole-6-carbonitrile (15b). Yellow solid (1.8 g, 72%), mp 175 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.89 (br s, 1H), 8.53 (br s, 1H), 7.99 (brd, 1H, J = 8 Hz), 7.74 (d, 1H, J = 8 Hz), 7.67 (d, 1H, J = 8 Hz), 7.53 (d, 1H, J = 8 Hz), 7.13 (s, 1H), 1.74 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 153, 148.4, 139, 135.2, 132.4, 131.5, 127.8, 126.4, 123.3, 122.4, 121.8, 120.4, 119.6, 117.2, 116.8, 109, 91.8, 88.4, 86.2, 28.2; ESI-MS: m/z calcd for $C_{22}H_{16}N_4O_2$: 368.38, found: 369.2 (M^++1). Anal. Calcd for $C_{22}H_{16}N_4O_2$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.65; H, 4.77; N, 14.86.

3.2.7.3. 1-(*tert*-Butoxycarbonyl)-2-[(5-cyano-2-pyridyl)ethynyl]-1*H*-indole-6-carbonitrile (15c). Yellow solid (1.72 g, 69%), mp 186 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.92 (d, 1H, J = 2.4 Hz), 8.55 (s, 1H), 8.01 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz), 7.69 (d, 1H, J = 8.4 Hz), 7.67 (d, 1H, J = 8.8 Hz), 7.53 (dd, 1H, J = 1.2 Hz, J = 8.8 Hz), 7.18 (s, 1H), 1.74 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 152.6, 147.5, 139, 137.1, 132.4, 132, 127.8, 126.9, 123.3, 123.1, 121.2, 120.4, 118.9, 117, 116, 108.8, 91.8, 89.1, 86.7, 28.1; ESI-MS: m/z calcd for $C_{22}H_{16}N_4O_2$: 368.38, found: 369.2 (M^++1). Anal. Calcd for $C_{22}H_{16}N_4O_2$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.65; H, 4.29; N, 15.17.

3.2.7.4. 1-(*tert*-Butoxycarbonyl)-2-[(4-cyano-3-fluorophenyl)ethynyl]-1*H*-indole-6-carbonitrile (15d). Brown solid (2 g, 80%), mp 185 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (s, 1H), 7.72–7.63 (m, 2H), 7.52 (dd, 1H, J = 1.2 Hz, J = 8 Hz), 7.46 (dd, 1H, J = 1.2 Hz, J = 8 Hz), 7.41 (dd, 1H, J = 1.2 Hz, J = 8 Hz), 7.10 (s, 1H), 1.73 (s, 9H); ESI-MS: m/z calcd for $C_{23}H_{16}FN_3O_2$: 385.39, found: 385.00 (M^+). Anal. Calcd for $C_{23}H_{16}FN_3O_2$: C, 71.68; H, 4.18; N, 10.90. Found: C, 71.39; H, 4.15; N, 10.82.

3.2.8. General procedure for the synthesis of 2-((4-amidinoaryl)ethynyl)-6-amidino-1*H*-indole dihydrochlorides (16a–d)

The dinitriles (**15a–d**) (0.66 mmol) were suspended in freshly distilled THF (5 mL), and treated with lithium trimethylsilylamide 1 M solution in tetrahydrofuran (4 mL, 3.98 mmol), the mixture was stirred for three days at room temperature. The reaction mixture was then cooled to 0 °C and HCl saturated ethanol (2 mL) was added. 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 solution followed by filtration of the resultant solid and washing with water and drying. Finally, the free base was stirred with ethanolic HCl for one week to make sure that the $(Boc)_2O$ group was completely removed, diluted with ether, and the solid formed was filtered and dried to give the diamidine salt.

3.2.8.1. 2-((4-Amidinophenyl)ethynyl)-6-amidino-1*H*-indole dihydrochloride (16a). Yellow solid (0.113 g, 40%), mp 260–262 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.65 (s, 1H), 9.58 (s, 2H), 9.34 (s, 2H), 9.26 (s, 2H), 9.17 (s, 2H), 7.93 (br s, 1H), 7.92 (d, 2H, J = 7.2 Hz), 7.78 (d, 2H, J = 7.2 Hz), 7.73 (d, 1H, J = 8 Hz), 7.5 (d, 1H, J = 8 Hz), 6.96 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.8, 165.4, 136, 132.5, 131.4, 129.2, 128.6, 127.1, 122.4, 122.3, 121.4, 119.5, 112.7, 109.2, 92.4, 85.5; ESI-MS: m/z calcd for $C_{18}H_{15}N_5$: 301.35, found: 302.20 (amidine base M^++1). Anal. Calcd for $C_{18}H_{15}N_5{\cdot}2HCl{\cdot}3.1H_2O$: C, 50.35; H, 5.45; N, 16.32. Found: C, 50.00; H, 5.22; N, 16.69.

3.2.8.2. 2-[(6-Amidino-3-pyridyl)ethynyl]-6-amidino-1*H*-indole dihydrochloride (16b). Yellow solid (0.115 g, 40%), mp 273–276 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.75 (s, 1H), 9.78 (br, 2H), 9.62 (br, 2H), 9.40 (br, 2H), 9.20 (br, 2H), 9.03 (br s, 1H), 8.48 (d, 1H, J = 8.4 Hz), 8.39 (d, 1H, J = 8.4 Hz),, 8.03 (br s, 1H), 7.81 (d, 1H, J = 8.4 Hz),, 7.51 (d, 1H, J = 8.4 Hz),, 6.99 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.7, 161.8, 151.8, 143.5, 140.7, 136.2, 131.3, 123.7, 123.5, 122.8, 121.6, 121.4, 119.6, 112.9, 109.8, 89.7, 88.7; ESI-MS: m/z calcd for $C_{17}H_{14}N_6$: 302.33, found: 303.20 (amidine base M^++1). Anal. Calcd for $C_{17}H_{14}N_6{\cdot}$-$2HCl{\cdot}1.65H_2O$: C, 50.41; H, 4.80; N, 20.75. Found: C, 50.55; H, 4.75; N, 20.37.

3.2.8.3. 2-[(5-Amidino-2-pyridyl)ethynyl]-6-amidino-1*H*-indole (16c) dihydrochloride. Yellow solid (0.111 g, 39%), mp 249–252 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.7 (br s, 1H), 9.64 (br s, 2H), 9.37 (br s,4H), 9.09 (d, 1H, 2.4 Hz), 9.06 (br, 2H), 8.34 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz), 8.19 (d, 1H, J = 8.4 Hz), 8.0 (s, 1H), 7.96 (br s, 1H), 7.83 (d, 1H, J = 8.4), 7.48 (dd, 1H, J = 1.6, J = 8.4 Hz), 7.19 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.7, 160.3, 151.3, 143.5, 140, 135.2, 131.3, 124.2, 123.5, 122.8, 122.2, 120.3, 119, 111.9, 109.8, 90.2, 88.1; ESI-MS: m/z calcd for $C_{17}H_{14}N_6$: 302.33, found: 303.22 (amidine base M^++1). Anal. Calcd for $C_{17}H_{14}N_6{\cdot}$-$3HCl{\cdot}1.75H_2O$: C, 46.19; H, 4.67; N, 19.02. Found: C, 46.29; H, 4.71; N, 18.76.

3.2.8.4. 2-[(4-Amidino-3-fluorophenyl)ethynyl]-6-amidino-1*H*-indole dihydrochloride (16d). Brown solid (0.144 g, 51%), mp 258–260 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.55 (s, 1H), 9.55 (br s, 2H), 9.37 (br s, 2H), 9.29 (br s, 2H), 8.96 (br s, 2H), 7.9 (br s, 1H), 7.79–7.73 (m, 2H), 7.72 (d, 1H, J = 1.2 Hz), 7.61 (dd, 1H, J = 1.6 Hz, J = 8 Hz), 7.45 (dd, 1H, J = 1.6 Hz, J = 8 Hz), 6.03 (d, 1H, J = 1.2 Hz); ESI-MS: m/z calcd for $C_{18}H_{14}FN_5$: 319.34, found: 320.20 (amidine base M^++1). Anal. Calcd for $C_{18}H_{14}FN_5{\cdot}2HCl{\cdot}2.5H_2O$: C, 49.43; H, 4.84; N, 16.01. Found: C, 49.51; H, 4.93; N, 15.77.

3.2.9. Synthesis of 4′-bromobiphenyl-4-carbonitrile (19)

5 mL deaireated 2 M aqueous solution of Na_2CO_3 and 4-bromophenyl boronic acid (**17**) (1 g, 5 mmol) in 5 mL deaireated methanol were added to a stirred solution of 4-iodobenzonitrile (**18**) (1.14 g, 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. Evaporation of the solvent under reduced pressure, the solid was partitioned between ethyl acetate (200 mL) and 2 M aqueous Na_2CO_3 solution (25 mL) containing 5 mL of concentrated ammonia, to destroy the palladium complex, then washed with water, passed through celite to remove the catalyst, and finally passed through sodium sulfate, and evaporated. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (85/15, v/v) gave white solid (1 g, 79%), mp 155–155.5 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.9 (m, 4H), 7.7 (br s, 4H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 143.3, 137.4, 132.9, 132, 129.1, 127.5, 122.4, 118.7, 110.4. Anal. Calcd for $C_{13}H_8BrN$: C, 60.49; H, 3.12; N, 5.43. Found: C, 60.33; H, 2.99; N, 5.15.

3.2.10. General procedure for the synthesis of 1-(*tert*-butoxycarbonyl)-2-(4′-cyanobiphenyl-4-yl)-1*H*-indole-5 or-6-carbonitrile (20a,b)

Tetrakistriphenylphosphine palladium (0.288 g, 0.25 mmol) were added to a stirred mixture of the *N*-BOC-5 or 6-cyanoindole stannane (2.02 g, 5 mmol) and the 4′-bromobiphenyl-4-carbonitrile (**19**) (1.29 g, 5 mmol) in deaireated dioxane (20 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 90–100 °C for 24 h. Evaporation of the solvent under reduced pressure, the solid was partitioned between ethyl acetate (200 mL) containing 5 mL of concentrated ammonia to destroy the palladium complex, then washed with water, passed through celite to remove the catalyst, and finally passed through sodium sulfate, and evaporated. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (80/20, v/v).

3.2.10.1. 1-(*tert*-Butoxycarbonyl)-2-(4′-cyanobiphenyl-4-yl)-1*H*-indole-5-carbonitrile (20a). White solid (0.91 g, 45%), mp 191–192 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.09 (br s, 1H), 8.06–7.87 (m, 8H), 7.58 (d, 1H, J = 8.1 Hz), 7.47 (d, 1H, J = 8.1 Hz), 7.16 (s, 1H), 1.51 (s, 9H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 148.7, 144.2, 139.9, 139.4, 138.1, 133.3, 131.9, 128.8, 128.1, 127.8, 126.4, 126.1, 125, 121.1, 119.3, 113, 110.6, 102.1, 100.5, 84.3, 27.2; ESI-MS: m/z calcd for $C_{27}H_{21}N_3O_2$: 419.47, found: 420.2 (M^++1). Anal. Calcd for $C_{27}H_{21}N_3O_2$: C, 77.31; H, 5.05; N, 10.02. Found: C, 77.35; H, 5.31; N, 9.72.

3.2.10.2. 1-(*tert*-Butoxycarbonyl)-2-(4′-cyanobiphenyl-4-yl)-1*H*-indole-6-carbonitrile (20b). White solid (1 g, 49%), mp 202–202.5 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.59 (s, 1H), 7.82 (m, 4H), 7.70 (d, 2H, J = 8.0 Hz), 7.66 (d, 1H, J = 8.4 Hz), 7.58 (d, 2H, J = 8.0 Hz), 7.54 (d, 1H, J = 8.0 Hz), 6.70 (s, 1H), 1.40 (s, 9H).; ^{13}C NMR (DMSO-d_6, 400 MHz) δ 149.3, 144.8, 143.1, 139.1, 136.4, 134.1, 132.7, 132.4, 129.5, 127.6, 126.8, 126.2, 121.3, 120, 119.9, 118.8, 111.3, 110.1, 107.2, 85, 27.6; ESI-MS: m/z calcd for $C_{27}H_{21}N_3O_2$: 419.47, found: 420.2 (M^++1). Anal. Calcd for $C_{27}H_{21}N_3O_2$: C, 77.31; H, 5.05; N, 10.02. Found: C, 77.29; H, 5.15; N, 9.89.

3.2.11. General procedure for the synthesis of 2-(4′-amidinobiphenyl-4-yl)-5 or 6-amidino-1*H*-indole dihydrochlorides (21a,b)

The dinitriles (**20a**,**b**) (0.276 g, 0.66 mmol) were suspended in freshly distilled THF (5 mL), and treated with lithium trimethylsilylamide 1 M solution in tetrahydrofuran (4 mL, 3.98 mmol), the mixture was stirred for three days at room temperature. The reaction mixture was then cooled to 0 °C and HCl saturated ethanol (2 mL) was added. 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 solution followed by filtration of the resultant solid and washing with water and drying. Finally, the free base was stirred with ethanolic HCl for one week to make sure that the $(Boc)_2O$ group was completely removed, diluted with ether, and the solid formed was filtered and dried to give the diamidine salt.

3.2.11.1. 2-(4′-Amidinobiphenyl-4-yl)-5-amidino-1*H*-indole dihydrochloride (21a). Yellow solid (0.126 g, 42%), mp >300 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.55 (s, 1H),), 9.55 (s, 2H), 9.30 (br s, 4H), 9.06 (s, 2H), 8.22–7.81 (m, 8H), 7.63 (br s, 2H), 7.23 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.9, 165.6, 144.8, 140.6, 138, 132, 129.3, 128.7, 128, 127.3, 126.7, 126.2, 121.8, 121.6, 119.1, 112.3, 100.8, 100.5; ESI-MS: m/z calcd for $C_{22}H_{19}N_5$: 353.4, found: 354.2 (amidine base M^++1). Anal. Calcd for $C_{22}H_{19}N_5{\cdot}2HCl{\cdot}2.45H_2O$: C, 56.16; H, 5.54; N, 14.88; Found: C, 56.33; H, 5.67; N, 14.62.

3.2.11.2. 2-(4′-Amidinobiphenyl-4-yl)-6-amidino-1*H*-indole dihydrochloride (21b). Yellow solid (0.154 g, 51%), mp >300 °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.65 (s, 1H), 9.56 (s,2H), 9.36 (s,4H), 9.13 (s, 2H), 8.17 (d, 2H, J = 7.2 Hz), 8.07–7.96 (m, 5H), 7.88 (d, 2H, J = 7.2 Hz), 7.76 (d, 1H, 8.4 Hz), 7.48 (d, 1H, J = 8.4 Hz), 7.19 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 165.7, 165.4, 147, 138.6, 137.8, 137.3, 133.3, 129.4, 129.3, 127.3, 126.6, 126.3, 125.6, 121.7, 119.8, 110.5, 101.7; ESI-MS: m/z calcd for $C_{22}H_{19}N_5$: 353.4, found: 354.2 (amidine base M^++1). Anal. Calcd for $C_{22}H_{19}N_5{\cdot}2HCl{\cdot}1.85H_2O$: C, 57.48; H, 5.41; N, 15.23. Found: C, 57.12; H, 5.63; N, 14.96.

3.2.12. 1-(*tert*-Butoxycarbonyl)-2-(biphenyl-4-yl))-1*H*-indole-6-carbonitrile (23)

Tetrakistriphenylphosphine palladium (0.288 g, 0.25 mmol) were added to a stirred mixture of the *N*-BOC-6-cyanoindole stannane (**13**) (2.02 g, 5 mmol) and the 4-bromobiphenyl (**22**) (1.16 g, 5 mmol) in deaireated dioxane (20 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 90–100 °C for 24 h. Evaporation of the solvent under reduced pressure, the solid was partitioned between ethyl acetate (200 mL) containing 5 mL of concentrated ammonia to destroy the palladium complex, then washing with water, passed through celite to remove the catalyst, and finally passed through sodium sulfate, and evaporated. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (90/10, v/v). Afforded white solid (1.13 g, 58%), mp 192–193 °C ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1H), 7.17–7.64 (m, 5H), 7.54–7.49 (m, 5H), 7.27 (dd, 1H, J = 7.2 Hz), 6.68 (s, 1H), 1.38 (s, 9H).; ^{13}C NMR ($CDCl_3$, 400 MHz) δ 149.5, 143.8, 141.3, 140.4, 136.5, 132.7, 132.5, 129.2, 128.9, 127.7127.1, 126.1, 121.2, 120.2, 119.9, 109.7, 106.9, 84.9, 27.5; ESI-MS: m/z calcd for $C_{26}H_{22}N_2O_2$: 394.47, found: 395.3 (M^++1). Anal. Calcd for $C_{26}H_{22}N_2O_2$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.1; H, 5.65; N, 7.18.

3.2.13. 2-(Biphenyl-4-yl)-6-amidino-1*H*-indole hydrochloride (24)

The mononitrile (**23**) (0.26 g, 0.66 mmol) was suspended in freshly distilled THF (5 mL), and treated with lithium trimethylsilylamide 1 M solution in tetrahydrofuran (2 mL, 1.99 mmol), the mixture was stirred for three days at room temperature. The reaction mixture was then cooled to 0 °C and HCl saturated ethanol (1 mL) was added. 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 solution followed by filtration of the resultant solid and washing with water and drying. Finally, the free base was stirred with ethanolic HCl for one week to make sure that the $(Boc)_2O$ group was completely removed, diluted with ether, and the solid formed was filtered and dried to give the diamidine salt as yellow solid (0.133 g, 55%), mp >280 dec °C ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.40 (s, 1H), 9.30 (s, 2H), 8.98 (s, 2H), 8.08 (d, 2H, J = 8.4 Hz), 7.96 (s, 1H), 7.84 (d, 2H, J = 8.4 Hz), 7.79–740 (m, 3H), 7.55–7.38 (m, 4H), 7.15 (s, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166, 144.8, 140.5, 137.1, 136.9, 132.8, 129.1, 128.7, 127.6, 126.6, 125.8, 125.4, 121.3, 120.1, 109.4, 100.1; ESI-MS: m/z calcd for $C_{21}H_{17}N_3$: 311.38, found: 312.2 (amidine base M^++1). Anal. Calcd for $C_{21}H_{17}N_3{\cdot}1HCl{\cdot}1.15H_2O$: C, 68.43; H, 5.55; N, 11.4. Found: C, 68.11; H, 5.4; N, 11.13.

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