Bioorganic & Medicinal Chemistry 19 (2011) 978–984

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Dicationic phenyl-2,2′-bichalcophenes and analogues as antiprotozoal agents

Mohamed A. Ismail [a,e], Serry A. El Bialy [b,e], Reto Brun [c,d], Tanja Wenzler [c,d], Rupesh Nanjunda [e], W. David Wilson [e], David W. Boykin [e,*]

[a] Department of Chemistry, Faculty of Science, Mansoura University, Mansoura 35516, Egypt
[b] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[c] Swiss Tropical and Public Health Institute, Basel CH4002, Switzerland
[d] University of Basel, Basel CH4002, Switzerland
[e] Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA

ARTICLE INFO

Article history:
Received 9 October 2010
Revised 16 November 2010
Accepted 22 November 2010
Available online 5 December 2010

Keywords:
Diamidines
Bichalcophenes
Antiprotozoal agents
Stille coupling
Heck coupling

ABSTRACT

A series of phenyl-2,2'-bichalcophene diamidines **1a–h** were synthesized from the corresponding dinitriles either via a direct reaction with $LiN(TMS)_2$, followed by deprotection with ethanolic HCl or through the bis-*O*-acetoxyamidoxime followed by hydrogenation in acetic acid and EtOH over Pd–C. These diamidines show a wide range of DNA affinities as judged from their ΔT_m values which are remarkably sensitive to replacement of a furan unit with a thiophene one. These differences are explained in terms of the effect of subtle changes in geometry of the diamidines on binding efficacy. Five of the eight compounds were highly active (below 6 nM IC_{50}) in vitro against *Trypanosoma brucei rhodesiense* (*T. b. r.*) and four gave IC_{50}values less than 7 nM against *Plasmodium falciparum* (*P. f.*). Only one of the compounds was as effective as reference compounds in the *T. b. r.* mouse model for the acute phase of African trypanosomiasis.

1. Introduction

Aromatic diamidines exhibit broad-spectrum anti-microbial activity including effectiveness against the protozoan diseases caused by *Trypanosoma* sp. and *Plasmodium* sp.[1] The broad activity of the aromatic diamidines notwithstanding, pentamidine (**I**) is the only compound of this class to be used extensively in the clinic (Fig. 1).[1] Furamidine (**IIa**), a diamidino diphenylfuran, has been shown to be more potent and less toxic than pentamidine in murine models of trypanosomiasis.[2] The oral prodrug of furamidine, 2,5-bis[4-(methoxyamidino)phenyl]furan (pafuramidine), showed promising results in Phase I and II clinical trials against both human African trypanosomiasis (HAT) and malaria.[1] Unfortunately, in an additional safety study of pafuramidine paralleling the Phase III trials, liver and kidney toxicities in some volunteers were found and the development of pafuramidine was terminated.[2] Many of the active aromatic diamidines for various structural classes have been shown to bind to the minor groove of DNA at AT rich sites.[3–9] It has been assumed that the minor groove binding of these type of compounds leads to inhibition of one or more DNA dependant enzymes which gives rise to the anti-microbial effect.[10–12] Recently, we have made compounds in which the phenyl group(s) of furamidine have been replaced with pyridyl group(s) (**IIb**). Several of these aza-analogues show in vivo activity which is superior to that of furamidine.[13] More recently, one of these aza analogs was found to be effective in a mouse model for second stage African sleeping sickness.[14] Furan units are often key structural elements in the aromatic frame work for the more effective diamidines.[15–17] The furan analogue **III** has been found to bind to DNA in a unique stacked dimer array which has potential for development of new gene regulation molecules.[18–21] Compound **III** has shown some activity in an immunosuppressed rat model for *Pneumocystis carinii pneumonia*[18] and in the STIB900 mouse model for acute stage African trypanosomiasis.[22] The bichalcophene diamidine **IV** from our laboratory has been shown to recognize G-quadruplex DNA.[23] More recently, the diverse modes of the interaction of **IV** with multi-stranded DNA structures has been reported.[24]

As part of a research program directed to drug discovery of antiprotozoal agents and due to the unusual DNA binding properties of **III** and **IV** we decided to prepare additional analogues of this series of bichalcophene diamidines in order to investigate structure–activity relationships (SAR) and their DNA binding profiles. We report here the synthesis of novel diamidino phenyl-bifuran derivatives and structural isosteres and their evaluation versus *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*).

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

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.11.047

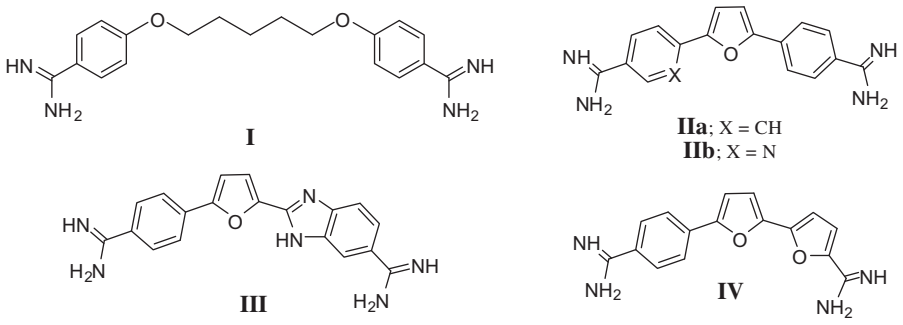


Figure 1.

2. Results and discussion

2.1. Chemistry

Our strategy for the synthesis of diamidines **1** is based upon the conversion of dinitriles either by direct reaction using lithium bis(trimethylsilyl)amide $LiN(TMS)_2$ or through the bis-*O*-acetoxyamidoxime followed by hydrogenation in acetic acid. Retrosynthetic studies (Fig. 2) for this strategy suggested that the triaryl structure **2** could be obtained by the application of palladium catalyzed reactions which permit the formation of C–C bond with full regioselectivity. The bichalcophenes **3** could be converted into dinitriles **2** using the Heck coupling reaction. The compounds **4** could be converted into the dinitriles **2** using a Suzuki coupling reaction. For SAR study, a two-carbon spacer could be inserted into dinitriles **2** using Sonogashira coupling[27] by the reaction of **4a** with trimethyl silyl acetylene derivative. Furthermore, Stille coupling is another Pd catalyzed reaction which is an alternative pathway to furnish certain analogues using compound **5**.

We initiated our study by the synthesis of dinitrile **2a** which was obtained from 2,2′-bifuran-5-carbonitrile (**3a**), prepared according to our published procedure,[25] via a Heck coupling reaction with the commercially available 5-bromopyridine-2-carbonitrile as shown in Scheme 1. The dinitrile **2b** was prepared employing similar procedures used for **2a** using 2,2′-bithiophene-5-carbonitrile (**3b**). The synthesis of dinitriles **2c–f** was accomplished in two steps. The regioselective bromination of **3a–d** using *N*-bromosuccinamide (NBS) in DMF furnished bromo derivatives **4a–d**. Suzuki coupling of **4a–d** with *p*-cyanophenylboronic acid was accomplished in the presence of Pd $(PPh_3)_4$ as a catalyst, Na_2CO_3 as base and toluene as solvent.

Compound **2h** was obtained in three steps starting with a Stille coupling reaction of the readily available 6-(5-bromofuran-2-yl)nicotinonitrile **5** and 2-tributylstannylfuran to form the corresponding 6-(2,2′-bifuran-5-yl)-nicotinonitrile (**6**) (Scheme 2). Bromination of **6** with *N*-bromosuccinimide in DMF, furnished bromo-bifuran derivative **7** in a 58% yield. A subsequent cyanation reaction of compound **7** with Cu(I)CN in DMF gave the dinitrile **2h**.

As outlined in Scheme 3, a series of diamidines **1a–h** was obtained from the dinitriles **2a–h** either by direct reaction using $LiN(TMS)_2$ (cf. **1a–g**) or through the bis-*O*-acetoxyamidoxime followed by hydrogenation in glacial acetic acid as in case of **1c, h**. Thus, 5′-(2-amidinopyridin-5-yl)-2,2′-bifuran-5-carboxamidine (**1a**) was synthesized from the corresponding dinitrile **2a** by treatment with $LiN(TMS)_2$ followed by deprotection with ethanolic HCl(g). 6-(5′-Amidino-2,2′-bifuran-5-yl)-nicotinamidine acetate salt (**1h**) was synthesized from 6-(5′-cyano-2,2′bifuran-5-yl)-nicotinonitrile (**2h**), through the bis-*O*-acetoxyamidoxime followed by hydrogenation. The hydrochloride salts of the diamidines **1a–g** were obtained by passing hydrogen chloride gas into ethanolic solutions of their free bases.

2.2. Biology

Aromatic diamidines are well known cationic molecules that have a strong and reversible interaction with DNA. Recent work

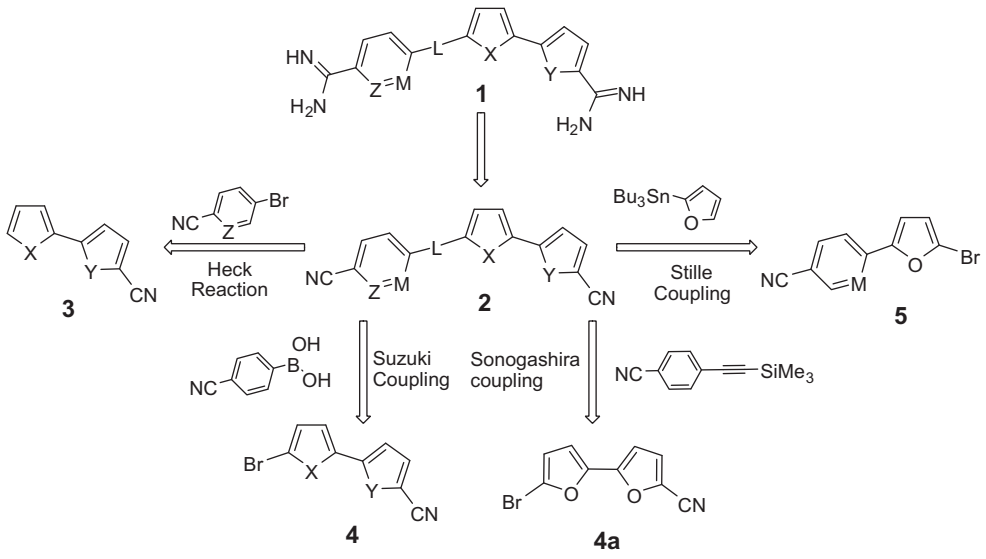
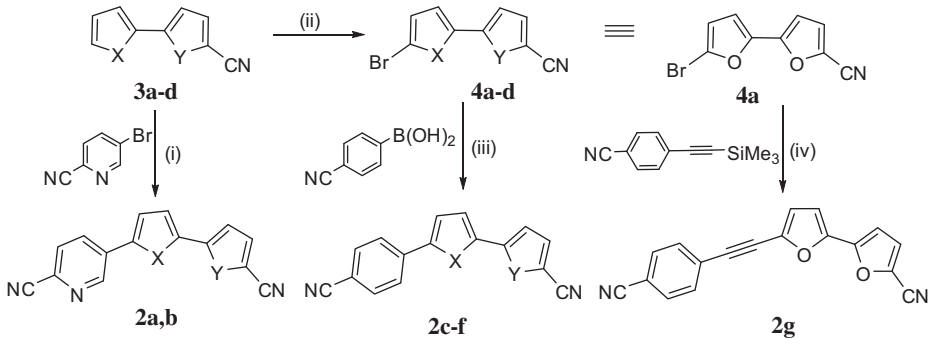
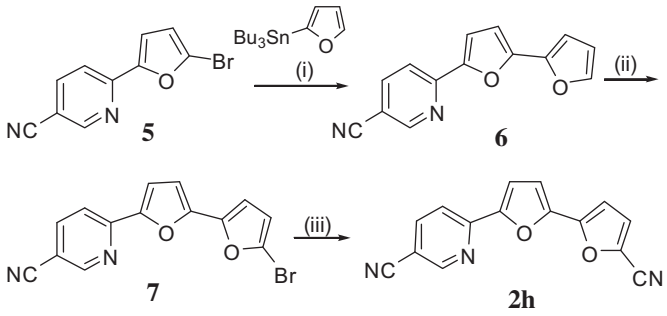


Figure 2. Retrosynthetic study of diamidines.



2	X	Y	Yield (%)
a	O	O	(55)
b	S	S	(60)
c	O	O	(74)
d	O	S	(76)
e	S	S	(70)
f	S	O	(75)
g	O	O	(25)

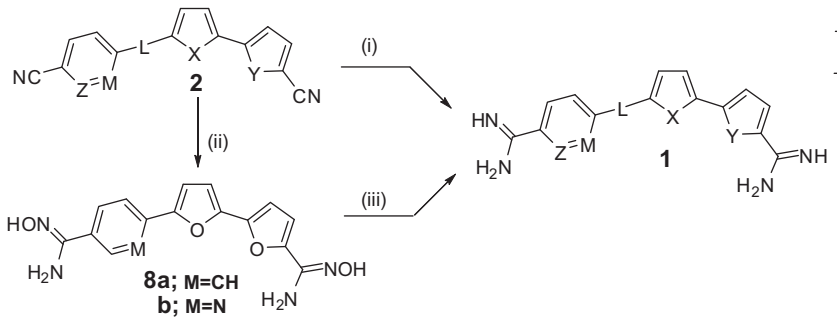
Scheme 1. Reagents and conditions: (i) DMF, KOAc, $Pd(PPh_3)_4$, 125–135 °C; (ii) NBS, DMF; (iii) toluene, $Pd(PPh_3)_4$, Na_2CO_3, 80 °C; (iv) $Pd(OAc)_2$, NaOAc, Bu_4NCl, DMF, 70 °C.



Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, 1,4-dioxan 100–110 °C; (ii) NBS, DMF; (iii) Cu(I)CN, DMF.

has shown that both curved and linear molecules exhibit excellent biological activity against trypanosomes.[22,26] The mechanism of action for anti-trypanosomal activity has been suggested to involve the inhibition of DNA dependent enzymes or direct inhibition of transcription.[1,9,10]

Table 1 contains the DNA binding affinities for the new bichalcophene diamidines as well as the in vitro activities for these compounds against *T. b. r.* and *P. f.* For comparative purposes, similar data for furamidine and pentamidine are included. The use of the thermal melting increase ΔT_m (T_m of complex – T_m of free DNA) is a rapid and reliable method for comparing binding affinities for a large number of diverse classes of diamidines. The complexes between poly (dA–dT) and the bichalcophene diamidines give an unexpected wide range (from 4 to 18 °C) of ΔT_m values. The ΔT_m value (8.5 °C) for the bifuran **1c** is significantly lower than that of both pentamidine (12.6 °C) and furamidine (25 °C). Due to structural similarities this analysis will focus on comparisons between furamidine and the bichalcophene analogues. The large drop in ΔT_m value on replacing one of the furamidine phenyl groups with a furan ring is probably due to a combination of factors presented by **1c** including a more curved structure, a shorter distance between the two amidine units and a smaller stacking surface provided by the three aryl rings. The first two differences seem likely to be the more important contributors to the reduction in ΔT_m value since the more curved structure would be expected to prevent optimal fit in the groove by pushing the central portion of the molecule away from the floor of the groove and the shorter distance between the amidine units could lead to a disadvantageous offset in H-bond indexing with the minor groove base pairs. Replacement of one of the furan rings with a thiophene ring (**1d** or **1f**) results in an increase in the ΔT_m by approximately 5 °C. This result is consistent with the increased affinity of other thiophene based diamidines in comparison to furan counterparts.[28] There is a small increase in bond angle for C–S–C in thiophene compared to that of C–O–C in furan attributable to the differences in van der Waals radii between S and O. This small angle difference when amplified to the terminal amidine units leads to a significant difference in the positions of the amidines in the two different molecules and likely leads to the increase in binding affinities for the thiophene analogues.[28] The bithiophene analog **1e** gives the highest ΔT_m value (18.1 °C) for the bichalcophenes studied, consistent with the previous observations. Introduction of nitrogen into the phenyl ring in this system (**1a**, **1b**, **1h**) results in a 1.4–3.0 °C decrease in ΔT_m values compared to that of their carbocycle analogues. This type decline has been noted previously for other diamidines and has been attributed to differences in hydration of the phenyl and pyridyl ring systems.[13,29–31] The bifuran **1g** in which a carbon–carbon triple bond has been inserted between the furan and the phenyl rings yields the bichalcophene compound with the lowest ΔT_m value of those investigated. A similar reduction in binding affinity was noted in DAPI analogues in which a carbon–carbon triple bond had been inserted.[30] The origin of the decline seems likely to be associated with poor stacking interactions of the triple bond and possible changes in H-bond indexing as a result of further separation of the amidine groups.



1	X	Y	L	M	Z	Yield (%)
a	O	O	-	CH	N	65
b	S	S	-	CH	N	71
c	O	O	-	CH	CH	73
d	O	S	-	CH	CH	70
e	S	S	-	CH	CH	81
f	S	O	-	CH	CH	77
g	O	O	C≡C	CH	CH	78
h	O	O	-	N	CH	59

Scheme 3. Reagents and conditions: (i) (a) $LiN(TMS)_2$, THF, rt, 12 h; (b) HCl(g), dry EtOH, rt, 12 h; (ii) $NH_2OH{\cdot}HCl$/KO-*t*-Bu, DMSO; (iii) (a) $AcOH/Ac_2O$; (b) H_2/Pd–C, AcOH.

Table 1
DNA affinities, in vitro anti-protozoan data and in vivo anti-trypanosomal activity of bichalcophene diamidines

Compound No.	Substitution					DNA affinities and in vitro anti-protozoan data						In vivo anti-trypanosomal activity in the STIB900 mouse model		
	X	Y	L	M	Z	ΔT_m[a] (°C)	Cytotoxicity[b] IC_{50} (μM)	*T. b. rhodesiense*[c] IC_{50} (nM)	SI_T[d]	*P. falciparum*[c] IC_{50} (nM)	SI_p[e]	Dose[f] (mg/kg)	Cures[g]	Survival[h] (days)
Pentamidine						12.6	6	2.2	15,533	6.4	991	5	2/4	>45
Furamidine						25.0	6.4	4.5	2093	15.5	486	5	1/4	>46
1a	O	O	—	CH	N	5.5	38.5	6	6416	1	38,500	5	0/4	41.75
1b	S	S	—	CH	N	16.6	4.0	4	1000	2.3	1739	5	0/4	49
1c	O	O	—	CH	CH	8.5	37.8	12	3150	41.5	910	20	2/4	>45.5
1d	O	S	—	CH	CH	14.1	10.9	6	1816	17.4	626	5	0/4	16.75
1e	S	S	—	CH	CH	18.1	6.0	2	3000	10.4	576	5	0/4	26.25
1f	S	O	—	CH	CH	13.1	29.3	2	14,900	0.9	32,555	5	2/4	>46.5
1g	O	O	C≡C	CH	CH	4.0	63.0	42	1500	14.1	4468	5	0/4	15.25
1h	O	O	—	N	CH	6.0	26.9	97	277	6.4	4203	5	0/4	25.5

[a] Increase in thermal melting of Poly d(A-T)$_n$.[32]
[b] Cytotoxicity was evaluated using cultured L6 rat myoblast cells using an Alamar Blue assay.[33]
[c] 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 determination.[33]
[d] Selectivity index for *T. b. r.* (SI_T) expressed as the ratio: IC_{50} (L6)/IC_{50} (*T. b. r.*).
[e] Selectivity index for *P. f.* (SI_P) expressed as the ratio: IC_{50} (L6)/IC_{50} (*P. f.*).
[f] Intraperitoneal administration.[14]
[g] Number of mice that survive and are parasite free for 60 days.[14]
[h] Average days of survival; untreated controls expired between days 7 and 10 post infection.[14]

The IC_{50} values for the bichalcophene diamidines against *T. b. r.* range from 2 to 97 nM. Five (**1a**, **1b**, **1d**, **1e**, **1f**) of the eight compounds studied give IC_{50} values of 6 nM or less which are all in the range of the values of pentamidine and furamidine. These compounds were quite effective against *P. f.* with IC_{50} values ranging from of 0.9 to 41 nM. Against this organism four compounds (**1a**, **1b**, **1f**, **1h**) gave IC_{50} values less than 7 nM. On comparison of the *T. b. r.* activity of non-pyridyl containing analogues (**1c–g)** the thiophene compounds (**1b**, **1e**) are the most effective. A similar trend is also seen for the pyridyl analogues (**1a**, **1b**, **1h**); however, the difference in IC_{50} values for **1a** and **1b** is within experimental error. There is not an obvious SAR pattern for the *P. f.* activity. As we have noted previously, there is no direct correlation between DNA binding affinity and antiparasitic activity.[1] While some DNA affinity seems essential, transport of diamidines into the parasite plays and important role.[1] There does appear to be a rough correlation between DNA affinity and cytotoxicity.[31] Nevertheless, the selectivity of these compounds for the parasitic organisms is generally quite high as judged from their cytotoxicity to cultured L6 rat myoblast cells shown in Table 1. The selectivity indices range from 277 to 38,500.

Given the in vitro selectivity and activity of the bichalcophenes they were evaluated in the stringent *T. b. r.* mouse model for the acute phase of African trypanosomiasis.[14] The compounds were evaluated by daily intraperitoneal dosage of 5 mg/kg (except for **1c** which was tested in an earlier protocol at 20 mg/kg) for four consecutive days. The results for these studies are included in Table 1. At the low dosage of 5 mg/kg only **1f** provided cures (2/4) which represents activity comparable to that of the reference compounds pentamidine and furamidine. Compound **1f** merits further evaluation in other models for HAT.

2.3. Conclusion

A new series of bichalcophene diamidines has been prepared in good yields. These analogues show a wide range of DNA affinities as judged from their ΔT_m values which are remarkably sensitive to replacement of a furan unit with a thiophene one. These differences are explained in terms of the effect of subtle changes in geometry of the diamidines on binding efficacy. Five of the eight compounds were highly active (below 6 nM IC_{50}) in vitro against *T. b. r.* and four gave IC_{50}values less than 7 nM against *P. f.* Only one of the compounds was as effective as reference compounds in the *T. b. r.* mouse model for the acute phase of African trypanosomiasis.

3. Experimental section

3.1. Biology

3.1.1. 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 added 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.[26,32]

3.1.2. In vitro activity determination

In vitro assays with *T. b. rhodesiense* STIB 900 and *P. falciparum* K1 strain and cell toxicity assays using cultured L6 rat myoblast cells were carried out as previously described.[33]

3.1.3. STIB900 acute mouse model of trypanosomiasis

Experiments were performed as previously reported[14] with minor modifications. Briefly, female NMRI mice were infected intraperitoneally (ip) with 2×10^4 STIB900 bloodstream forms. Groups of four mice were treated ip with the tested dications on 4 consecutive days from day 3 through 6 post infection. A control group was infected but was not treated. The tail blood of all mice was checked for parasitemia until 60 days post infection. Surviving and parasite free mice at day 60 were considered cured and then euthanized. Death of animals (including the aparasitemic mice, >60) was recorded to calculate the mean survival time in days.

3.2. Chemistry

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on Silica Gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^{1}H and ^{13}C NMR spectra were recorded employing a Varian Unity Plus 300 spectrometer and a Bruker Avance 400 MHz spectrometer, and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer. Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ±0.4 of the theoretical values. The compounds reported as salts frequently analyzed correctly for fractional moles by water and/or ethanol of solvation. In each case proton NMR showed the presence of indicated solvent (s). All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific or Frontier.

3.2.1. General procedure of Heck reaction (2a, b)

A mixture of 2,2′-bifuran-5-carbonitrile **3a** or 2,2′-bithiophene-5-carbonitrile **3b** (10 mmol), 5-bromopyridine-2-carbonitrile (1.82 g, 10 mmol), $Pd(PPh_3)_4(0)$ (400 mg), and KOAc (5 g, 50 mmol) in dry DMF (10 mL) was heated under nitrogen at 130–135 °C for overnight. The reaction mixture then poured onto cold-water. The precipitate which formed was collected, recrystallized from DMF.

3.2.1.1. 5′-(2-Cyanopyridin-5-yl)-2,2′-bifuran-5-carbonitrile (2a). Yellow solid, yield 55%, mp 213–214 °C. ^{1}H NMR (DMSO-d_6); δ 7.25 (d, J = 3.6 Hz, 1H), 7.27 (d, J = 3.6 Hz, 1H), 7.59 (d, J = 3.6 Hz, 1H), 7.80 (d, J = 3.6 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.41 (dd, J = 8.0, 2.4 Hz, 1H), 9.22 (d, J = 2.4 Hz, 1H). ^{13}C NMR (DMSO-d_6); δ 149.9, 149.1, 146.5, 145.1, 131.7, 130.7, 129.3, 128.3, 125.6, 124.4, 117.6, 113.1, 112.1, 111.8, 108.5. MS (EI) m/e (rel. int.); 262 (M^++1, 100), 207 (18), 179 (24), 148 (80). Anal. Calcd for $C_{15}H_7N_3O_2$: C, 68.96; H, 2.70. Found: C, 68.72; H, 2.79.

3.2.1.2. 5′-(2-Cyanopyridin-5-yl)-2,2′-bithiophene-5-carbonitrile (2b). Yellow solid, yield 60%, mp 257–258.5 °C. ^{1}H NMR (DMSO-d_6); δ 7.59 (d, J = 4.0 Hz, 1H), 7.72 (d, J = 4.0 Hz, 1H), 7.95 (d, J = 4.0 Hz, 1H), 8.00 (d, J = 4.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.34 (dd, J = 8.0, 2.4 Hz, 1H), 9.14 (d, J = 2.4 Hz, 1H). ^{13}C NMR (DMSO-d_6); δ 147.6, 143.0, 140.4, 138.9, 136.3, 133.6, 132.2, 131.0, 129.3, 129.0, 128.5, 125.4, 117.5, 114.1, 107.0. MS (EI) m/e (rel. int.); 293 (M^+, 10), 152 (12), 133 (15), 69 (100). Anal. Calcd for $C_{15}H_7N_3S_2$: C, 61.41; H, 2.41. Found: C, 61.54; H, 2.47.

3.2.2. General procedure of Suzuki coupling (2c–f)

To a stirred solution of 5′-bromo-2,2′-bifuran-5-carbonitrile[25] **4a** (4.74 g, 20 mmol), and $Pd(PPh_3)_4(0)$ (600 mg) in toluene (40 mL) under a nitrogen atmosphere was added 20 mL of a 1.5 M aqueous solution of Na_2CO_3 followed by 4-cyanophenylboronic acid (3.21 g, 22 mmol) in 5 mL of methanol. The vigorously stirred mixture was warmed to 80 °C for 16 h. The solvent was evaporated, the precipitate was partitioned between CH_2Cl_2 (300 mL) and aqueous solution containing 15 mL of concentrated ammonia. The organic layer was dried (Na_2SO_4), and then concentrated to dryness under reduced pressure.

3.2.2.1. 5′-(4-Cyanophenyl)-2,2′-bifuran-5-carbonitrile (2c). Yellow solid, yield 74%, mp 194–195 °C (DMF).[25] ^{1}H NMR (DMSO-d_6) δ 7.17 (d, J = 3.6 Hz, 1H), 7.21 (d, J = 3.6 Hz, 1H), 7.43 (d, J = 3.6 Hz, 1H), 7.76 (d, J = 3.6 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H).

3.2.2.2. 5-[5-(4-Cyanophenyl)-furan-2-yl]-thiophene-2-carbonitrile (2d). Yellowish green solid, yield 76%, mp 187–188 °C.[25] ^{1}H NMR (DMSO-d_6); δ 7.22 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 3.6 Hz, 1H), 7.62 (d, J = 3.9 Hz, 1H), 7.85–7.94 (m, 5H).

3.2.2.3. 5′-(4-Cyanophenyl)-2,2′-bithiophene-5-carbonitrile (2e). Yellow solid, yield 70%, mp 231–233 °C.[25] ^{1}H NMR (DMSO-d_6) δ 7.50 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.73 (d, J = 3.9 Hz, 1H), 7.84–7.87 (m, 4H), 7.91 (d, J = 3.9 Hz, 1H).

3.2.2.4. 5-[5-(4-Cyanophenyl)-thiophen-2-yl]-furan-2-carbonitrile (2f). Yellow solid, yield 75%, mp 216.5–218 °C.[25] ^{1}H NMR (DMSO-d_6) δ 7.09 (d, J = 3.9 Hz, 1H), 7.64–7.66 (m, 2H), 7.75 (d, J = 3.9 Hz, 1H), 7.84–7.88 (m, 4H).

3.2.3. 5′-[(4-Cyanophenyl)ethynyl]-2,2′-bifuran-5-carbonitrile (2g). A mixture of 4-[(trimethylsilyl)ethynyl]benzonitrile (1.99 g, 10 mmol), 5′-bromo-2,2′-bifuran-5-carbonitrile (**4a**) (1.19 g, 5 mmol), palladium acetate (56 mg, 0.25 mmol), tri(*o*-tolyl)phosphine (152 mg, 0.5 mmol), Bu_4NCl (1.39 g, 5 mmol), and sodium acetate (1.64 g, 20 mmol) in DMF (20 mL) was heated at 70 °C for 3 h. The reaction mixture was poured onto water and extracted with ethyl acetate 200 mL (3×). The extract was evaporated, and the residue was chromatographed on silica gel using hexanes/EtOAc (95:5) as an eluent to furnish compound **2g** as yellow solid in 25% yield, mp 172–173 °C. ^{1}H NMR (DMSO-d_6); δ 7.16 (d, J = 3.6 Hz, 1H), 7.21 (d, J = 3.6 Hz, 1H), 7.23 (d, J = 3.6 Hz, 1H), 7.76–7.79 (m, 3H), 7.93 (d, J = 8.0 Hz, 2H). ^{13}C NMR (DMSO-d_6); δ 148.7, 145.1, 136.1, 132.7, 131.9, 125.6, 125.5, 124.5, 119.8, 118.3, 111.7, 111.2, 108.6, 93.8, 82.5. MS (EI) m/e (rel. int.); 284 (M^+, 10), 228 (35), 201 (40), 164 (60), 64 (100). Anal. Calcd for $C_{18}H_8N_2O_2$: C, 76.05; H, 2.83. Found: C, 75.80; H, 2.96.

3.2.4. 6-(2,2′-Bifuran-5-yl)-nicotinonitrile (6). A mixture of 6-(5-bromofuran-2-yl)nicotinonitrile[13] **5** (2.48 g, 10 mmol), 2-*n*-tributyltin furan (3.58 g, 10 mmol), and tetrakis(triphenylphosphine) palladium (300 mg) in dry dioxane (20 mL) was heated under nitrogen at 100–110 °C for 24 h. The solvent was evaporated under reduced pressure and the resulting residue was dissolved in ethyl acetate. This solution was passed through celite to remove Pd. The solution was evaporated, and the residue was chromatographed on silica gel using hexanes/EtOAc (70:30) as an eluent to furnish compound **6** as a golden yellow solid in 78% yield, mp 169–170 °C. ^{1}H NMR ($CDCl_3$); δ 6.52 (dd, J = 3.6, 1.8 Hz, 1H), 6.72–6.76 (m, 2H), 7.31 (d, J = 3.6 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.95 (dd, J = 8.4, 2.1 Hz, 1H), 8.80 (d, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$); δ 152.5, 151.3, 151.0, 148.7, 145.5, 142.9, 139.6, 117.6, 117.0, 114.3, 111.7, 108.1, 107.1, 106.6. Anal. Calcd for $C_{14}H_8N_2O_2$: C, 71.18; H, 3.41; N, 11.85. Found: C, 70.83; H, 3.61; N, 11.84.

3.2.5. 6-(5′-Bromo-2,2′-bifuran-5-yl)-nicotinonitrile (7). To a solution of **6** (1.41 g, 6 mmol) in DMF (20 mL) was added portionwise *N*-bromosuccinimide (1.07 g, 6 mmol) with stirring. The reaction mixture was stirred overnight, then poured onto cold-water. The precipitate which formed was collected, washed

with water and dried to give **7** as a golden yellow solid in 58% (EtOH), mp 143–145 °C. ^{1}H NMR ($CDCl_3$); δ 6.44 (d, J = 3.6 Hz, 1H), 6.70 (d, J = 3.6 Hz, 1H), 6.75 (d, J = 3.6 Hz, 1H), 7.30 (d, J = 3.6 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.95 (dd, J = 8.4, 2.1 Hz, 1H), 8.80 (d, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$); δ 152.5, 151.2, 151.1, 147.5, 147.3, 139.7, 122.8, 117.7, 117.0, 114.3, 113.5, 109.3, 108.6, 106.9. MS (EI) m/e (rel. int.); 314 (M^+, 60), 285 (10), 235 (20), 207 (100), 179 (10). HRMS calcd for $C_{14}H_7BrN_2O_2$: 313.9690. Observed 313.9661.

3.2.6. 6-(5′-Cyano-2,2′-bifuran-5-yl)-nicotinonitrile (2h). A mixture of **7** (942 mg, 3 mmol) and Cu(1)CN (540 mg, 6 mmol) in dry DMF (25 mL) was refluxed for 48 h. The reaction mixture was poured onto water/ammonia and extracted with methylene chloride. The extract was washed with water and brine, dried over Na_2SO_4, then passed on silica gel to give compound **2h** as yellow solid in 27% yield, mp 209–210.5 °C. ^{1}H NMR (DMSO-d_6); δ 7.23 (d, J = 3.6 Hz, 1H), 7.27 (d, J = 3.6 Hz, 1H), 7.52 (d, J = 3.6 Hz, 1H), 7.79 (d, J = 3.6 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 8.39 (dd, J = 8.4, 2.4 Hz, 1H), 9.03 (d, J = 2.4 Hz, 1H). Anal. Calcd for $C_{15}H_7N_3O_2$: C, 68.96; H, 2.70. Found: C, 68.81; H, 2.64.

3.2.7. General procedure of diamidines synthesis (Method A)

The dinitrile **2a** (0.87 g, 3.34 mmol), suspended in freshly distilled THF (10 mL), was treated with $LiN(TMS)_2$ (1 M solution in THF, 8 mL, 8 mmol) and the reaction was allowed to stir overnight. The reaction mixture was then cooled to 0 °C to which was added HCl saturated ethanol (40 mL) whereupon a precipitate started forming. The mixture was left to run overnight whereafter it was diluted with ether and the resultant solid was collected by filtration. The diamidine was purified by neutralization with 1 N NaOH followed by filtration of the resultant solid and washing with water (3× 10 mL). Finally, the free base was stirred with ethanolic HCl overnight, diluted with ether, and the solid formed was filtered and dried to give the diamidine **1a** hydrochloride salt.

3.2.7.1. 5′-(2-Amidinopyridin-5-yl)-2,2′-bifuran-5-carboxamidine hydrochloride (1a). Yellow solid, yield 65%, mp >320 °C. ^{1}H NMR (DMSO-d_6); δ 7.30 (d, J = 2.4 Hz, 1H), 7.38 (d, J = 2.4 Hz, 1H), 7.67 (d, J = 2.4 Hz, 1H), 8.05 (d, J = 2.4 Hz, 1H), 8.51–8.57 (m, 2H), 9.26 (s, 2H), 9.29 (s, 1H), 9.53 (s, 2H), 9.60 (s, 2H), 9.69 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 161.4, 153.3, 150.3, 148.8, 145.4, 145.0, 142.2, 140.5, 132.1, 129.1, 123.8, 120.9, 113.0, 112.4, 109.5. MS (ESI) m/e (rel. int.); 296 (M^++1, 100), 208 (7), 148 (52). HRMS calcd for $C_{15}H_{14}N_5O_2$: 296.1147. Observed: 296.1144. Anal. Calcd for $C_{15}H_{13}N_5O_2{\cdot}2.0HCl{\cdot}1.25H_2O$: C, 46.10; H, 4.51; N, 17.92. Found: C, 46.01; H, 4.44; N, 17.63.

3.2.7.2. 5′-(2-Amidinopyridin-5-yl)-2,2′-bithiophene-5-carboxamidine hydrochloride (1b). The same procedure described for preparation of **1a** was used starting with the dinitrile **2b**. Orange solid, yield 71%, mp 318–321 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.55 (d, J = 4.0 Hz, 1H), 7.60 (d, J = 4.0 Hz, 1H), 7.82 (d, J = 4.0 Hz, 1H), 7.94 (d, J = 4.0 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.30 (dd, J = 8.4, 2.0 Hz, 1H), 9.06 (d, J = 2.0 Hz, 1H). ^{13}C NMR (D_2O/DMSO-d_6); δ 161.5, 158.4, 146.6, 143.7, 142.4, 139.4, 137.0, 135.8, 134.3, 133.4, 129.2, 128.8, 127.2, 126.3, 123.9. MS (ESI) m/e (rel. int.); 328 (M^+ + 1, 75), 224 (10), 203 (7), 164 (100). HRMS calcd. for $C_{15}H_{14}N_5S_2$: 328.0691. Observed: 328.0696. Anal. Calcd for $C_{15}H_{13}N_5S_2{\cdot}2.0HCl{\cdot}0.5H_2O{\cdot}0.25EtOH$: C, 44.23; H, 4.19; N, 16.64. Found: C, 44.18; H, 4.13; N, 16.61.

3.2.7.3. 5′-(4-Amidinophenyl)-2,2′-bifuran-5-carboxamidine hydrochloride (1c). The same procedure described for preparation of **1a** was used starting with the dinitrile **2c**. Yellow solid, yield 73%, mp >300 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.16 (d, J = 3.6 Hz, 1H), 7.23 (d, J = 3.6 Hz, 1H), 7.36 (d, J = 3.6 Hz, 1H), 7.75 (d, J = 3.6 Hz, 1H), 7.85 (d, J = 7.8 Hz, 2H), 8.01 (d, J = 7.8 Hz, 2H). Anal. Calcd for $C_{16}H_{14}N_4O_2{\cdot}2.0HCl{\cdot}1.0H_2O$: C, 49.88; H, 4.70; N, 14.54. Found: C, 49.92; H, 4.50; N, 14.41.

3.2.7.4. 5-[5-(4-Amidinophenyl)-furan-2-yl]-thiophene-2-carboxamidine hydrochloride (1d). The same procedure described for preparation of **1a** was used starting with the dinitrile **2d**. Brown solid, yield 70%, mp 250–252 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.14 (d, J = 3.6 Hz, 1H), 7.25 (d, J = 3.6 Hz, 1H), 7.58 (d, J = 3.6 Hz, 1H), 7.79 (d, J = 7.8 Hz, 2H), 7.85–7.93 (m, 3H). ^{13}C NMR (D_2O/DMSO-d_6); δ 165.9, 159.2, 153.2, 148.8, 140.2, 136.1, 134.8, 129.8, 127.6, 127.5, 125.5, 125.0, 112.9, 112.7. MS (ESI) m/e (rel. int.); 311 (M^+ +1, 92), 156 (100). HRMS calcd for $C_{16}H_{15}N_4OS$: 311.0967. Observed: 311.0977. Anal. Calcd for $C_{16}H_{14}N_4OS{\cdot}2.0HCl{\cdot}1.5H_2O{\cdot}0.25EtOH$: C, 46.97; H, 4.89; N, 13.28. Found: C, 46.82; H, 4.69; N, 13.13.

3.2.7.5. 5′-(4-Amidinophenyl)-2,2′-bithiophene-5-carboxamidine hydrochloride (1e). The same procedure described for preparation of **1a** was used starting with the dinitrile **2e**. Redish brown solid, yield 81%, mp 271–273 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.49 (d, J = 3.6 Hz, 1H), 7.53 (d, J = 3.6 Hz, 1H), 7.67 (d, J = 3.6 Hz, 1H), 7.84 (s, 4H), 7.94 (d, J = 3.6 Hz, 1H). ^{13}C NMR (D_2O/DMSO-d_6); δ 165.5, 158.7, 144.1, 143.3, 138.4, 136.1, 136.0, 129.8, 129.0, 128.1, 127.5, 127.4, 126.4, 126.2. MS (ESI) m/e (rel. int.); 327 (M^+ +1, 100), 208 (17), 164 (90). HRMS calcd. for $C_{16}H_{15}N_4S_2$: 327.0738. Observed: 327.0739. Anal. Calcd for $C_{16}H_{14}N_4S_2{\cdot}2.0HCl{\cdot}1.5H_2O$: C, 45.06; H, 4.49; N, 13.13. Found: C, 45.00; H, 4.38; N, 13.03.

3.2.7.6. 5-[5-(4-Amidinophenyl)-thiophen-2-yl]-furan-2-carboxamidine hydrochloride (1f). The same procedure described for preparation of **1a** was used starting with the dinitrile **2f**. Yellow solid, yield 77%, mp 248–249.5 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.19 (d, J = 3.6 Hz, 1H), 7.78–7.82 (m, 3H), 7.91–7.96 (m, 4H). ^{13}C NMR (D_2O/DMSO-d_6); δ 165.5, 153.8, 153.4, 143.7, 140.2, 138.5, 131.9, 129.6, 128.9, 127.4, 126.4, 126.0, 123.8, 121.8. MS (ESI) m/e (rel. int.); 311 (M^+ +1, 100), 156 (87). HRMS calcd. for $C_{16}H_{15}N_4OS$: 311.0967. Observed: 311.0966. Anal. Calcd for $C_{16}H_{14}N_4OS{\cdot}2.0HCl{\cdot}1.5H_2O$: C, 46.83; H, 4.67; N, 13.65. Found: C, 46.90; H, 4.66; N, 13.30.

3.2.7.7. 5′-[(4-Amidinophenyl)ethynyl]-2,2′-bifuran-5-amidine hydrochloride (1g). The same procedure described for preparation of **1a** was used starting with the dinitrile **2g**. Yellow solid, yield 78%, mp >320 °C. ^{1}H NMR (DMSO-d_6); δ 7.19 (d, J = 3.6 Hz, 1H), 7.25 (d, J = 3.6 Hz, 1H), 7.32 (d, J = 3.6 Hz, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 3.6 Hz, 1H), 9.26 (s, 2H), 9.40 (s, 2H), 9.58, 9.61 (2s, 4H). ^{13}C NMR (DMSO-d_6); δ 164.9, 153.2, 148.3, 145.4, 140.5, 136.2, 131.4, 128.7, 128.4, 125.9, 120.9, 119.7, 111.5, 109.6, 94.1, 81.95. MS (ESI) m/e (rel. int.); 319 (M^++1, 100), 160 (75). HRMS calcd for $C_{18}H_{15}N_4O_2$: 319.1195. Observed 319.1206. Anal. Calcd for $C_{18}H_{14}N_4O_2{\cdot}2.0HCl{\cdot}1.25H_2O$: C, 52.24; H, 4.51; N, 13.54. Found: C, 52.50; H, 4.54; N, 13.23.

3.2.8. General procedure of diamidines synthesis (Method B)

3.2.8.1. *N*-Hydroxy-5′-[4-(*N*-hydroxyamidino)-phenyl]-2,2′-bifuran-5-carboxamidine (8a). A mixture of hydroxylamine hydrochloride (695 mg, 10 mmol, 10 equiv) in anhydrous DMSO (8 mL) was cooled to 5 °C under nitrogen and potassium *t*-butoxide (1.12 g, 10 mmol, 10 equiv) was added in portions. The mixture was stirred for 30 min. This mixture was added to the dinitrile derivative **2c** (260 mg, 1 mmol, 1 equiv). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then poured slowly onto ice-water. The precipitate was

filtered and washed with water and then ethanol to afford **8a** (free base) in 93%, mp 205–206 °C. ^{1}H NMR (DMSO-d_6); δ 5.83 (br s, 4H), 6.86–6.89 (m, 3H), 7.14 (s, 1H), 7.75 (s, 4H), 9.70 (s, 1H), 9.75 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 152.3, 150.3, 146.7, 145.0, 144.9, 144.1, 132.3, 129.9, 125.8, 123.1, 109.7, 108.5, 107.2. MS (FAB) *m*/*e* (rel. int.); 327 (M^++1, 40), 307 (100), 299 (60), 273 (10), 220 (30). HRMS calcd for $C_{16}H_{15}N_4O_4$ ms 327.1093. Observed 327.1137.

3.2.8.2. *N*-Hydroxy-6-[5′-(*N*-hydroxyamidino)-2,2′-bifuran-5-yl]-nicotinamidine (8b). The same procedure described for **8a** was used starting with **2h**. Yield 89%, mp 248–250 °C. ^{1}H NMR (DMSO-d_6); δ 5.88 (s, 2H), 6.04 (s, 2H), 6.92–6.96 (m, 3H), 7.29 (d, J = 3.6 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 8.11 (dd, J = 8.4, 2.1 Hz, 1H), 8.88 (d, J = 2.1 Hz, 1H), 9.80 (s, 1H), 9.92 (s, 1H). ^{13}C NMR (DMSO-d_6); δ 152.2, 148.7, 147.8, 147.0, 146.6, 146.0, 144.6, 144.0, 133.6, 127.3, 117.7, 111.3, 109.7, 108.5, 107.9. MS (EI) *m*/*e* (rel. int.); 327 (M^+, 15), 311 (5), 295 (10), 278 (85), 261 (100). HRMS calcd for $C_{15}H_{13}N_5O_4$: 327.0967. Observed 327.0974.

3.2.8.3. 5′-(4-Amidinophenyl)-2,2′-bifuran-5-carboxamidine acetate (1c). To a solution of the diamidoxime **8a** (326 mg, 1 mmol) in glacial acetic acid (10 mL) was slowly added acetic anhydride (0.35 mL). After stirring for overnight TLC indicated complete acylation of the starting material, ethanol (10 mL) and 10% palladium on carbon (80 mg) were then added. The mixture was placed on Parr hydrogenation apparatus at 50 psi for 4 h at room temperature. The mixture was filtered through hyflo and the filter pad washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **1c** acetate salt in 67% yield, mp 240–242 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.80 (s, 2 x CH_3), 7.06 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H), 7.36 (d, J = 3.6 Hz, 1H), 7.39 (d, J = 3.6 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H). Anal. Calcd for $C_{16}H_{14}N_4O_2 \cdot 2.0AcOH \cdot 2.4H_2O$: C, 52.48; H, 5.87; N, 12.23. Found: C, 52.28; H, 5.49; N, 11.81.

3.2.8.4. 5′-(4-Amidinophenyl)-2,2′-bifuran-5-carboxamidine (1c). It was prepared by dissolving the acetate salt **1c** (50 mg) in water (5 mL) and by neutralization with 1 N NaOH. The precipitate was filtered, dried to afford free amidine of **1c**. Mp 202–203.5 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 6.93 (d, J = 3.6 Hz, 1H), 7.01 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H), 7.22 (d, J = 3.6 Hz, 1H), 7.83 (s, 4H). ^{13}C NMR (D_2O/DMSO-d_6); δ 162.2, 153.9, 152.4, 147.4, 145.7, 145.0, 134.1, 131.1, 127.3, 123.1, 111.9, 109.4, 109.2, 107.7. MS (EI) *m*/*e* (rel. int.); 294 (M^+, 50), 277 (100), 261 (25). HRMS calcd for $C_{16}H_{14}N_4O_2$: 294.1116. Observed: 294.1101.

3.2.8.5. 6-(5′-Amidino-2,2′-bifuran-5-yl)-nicotinamidine acetate (1h). The same procedure described for preparation of **1c** was used starting with **8b**. Yellow solid, yield 59%, mp 269–271 °C. ^{1}H NMR (D_2O/DMSO-d_6); δ 7.15 (d, J = 3.6 Hz, 1H), 7.23 (d, J = 3.6 Hz, 1H), 7.47 (d, J = 3.6 Hz, 1H), 7.57 (d, J = 3.6 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.99 (s, 1H). MS (EI) *m*/*e* (rel. int.); 296 (M^++1, 100), 273 (12), 239 (40). HRMS calcd for $C_{15}H_{14}N_5O_2$: 296.1147. Observed 296.1189. Anal. Calcd for $C_{15}H_{13}N_5O_2 \cdot 2.0AcOH \cdot 2.65H_2O \cdot 0.5EtOH$: C, 49.41; H, 6.07; N, 14.40. Found: C, 49.72; H, 5.96; N, 14.02.

Acknowledgments

This work was supported by NIH Grant AI64200, and an award from the Bill and Melinda Gates Foundation. S.A.E.B. was supported by a Fulbright Fellowship awarded by the Binational Fulbright Commission of Egypt.

References and notes

1. Tidwell, R. R.; Boykin, D. W. Dicationic DNA Minor Groove Binders as Antimicrobial Agents In *Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes*; Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Wiley-VCH: New York, 2003; Vol. 2, pp 414–460; (b) Wilson, W. D.; Nguyen, B.; Tanious, F. A.; Mathis, A.; Hall, J. E.; Stephens, C. E.; Boykin, D. W. *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 389; (c) Soeiro, M. N. C.; de Souza, E. M.; Stephens, C. E.; Boykin, D. W. *Expert Opin. Invest. Drugs* **2005**, *14*, 957; (d) Dardonville, C. *Expert Opin Ther. Patents* **2005**, *15*, 1241; (e) Werbovetz, K. A. *Curr. Opin. Invest. Drugs* **2006**, *7*, 147; (f) Paine, M. F.; Wang, M. Z.; Generaux, C. N.; Boykin, D. W.; Wilson, W. D.; De Koning, H. P.; Olson, C. A.; Pohling, G.; Burri, C.; Brun, R.; Murilla, G. A.; Thuita, J. K.; Barrett, M. P.; Tidwell, R. R. *Expert Opin. Invest. Drugs* **2010**, *11*, 876.
2. Thuita, J. K.; Karanja, S. M.; Wenzler, T.; Mdachi, R. E.; Ngotho, J. M.; Kagira, J. M.; Tidwell, R. R.; Brun, R. *Acta Trop.* **2008**, *108*, 6.
3. Czarny, A.; Boykin, D. W.; Wood, A.; Nunn, C. M.; Neidle, S.; Zhao, M. R. J.; Wilson, W. D. *J. Am. Chem. Soc.* **1995**, *117*, 4716.
4. Laughton, C. A.; Tanious, F. A.; Nunn, C. M.; Boykin, D. W.; Wilson, W. D.; Neidle, S. *Biochemistry* **1996**, *35*, 5655.
5. Trent, J. O.; Clark, G. R.; Kumar, A.; Wilson, D. W.; Boykin, D. W.; Hall, J. E.; Tidwell, R. R.; Blagburn, B. L.; Neidle, S. *J. Med. Chem.* **1996**, *39*, 4554.
6. Wilson, W. D.; Tanious, F. A.; Ding, D.; Kumar, A.; Boykin, D. W.; Colson, P.; Houssier, C.; Bailly, C. *J. Am. Chem. Soc.* **1998**, *120*, 10310.
7. Boykin, D. W.; Kumar, A.; Spychala, J.; Zhou, M.; Lombardy, R.; Wilson, W. D.; Dykstra, C. C.; Jones, S. K.; Hall, J. E.; Tidwell, R. R.; Laughton, C.; Nunn, C. M.; Neidle, S. *J. Med. Chem.* **1995**, *38*, 912.
8. Boykin, D. W.; Kumar, A.; Xiao, G.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R. *J. Med. Chem.* **1998**, *41*, 124.
9. Francesconi, I.; Wilson, W. D.; Tanious, F. A.; Hall, J. E.; Bender, B. C.; Tidwell, R. R.; McCurdy, D.; Boykin, D. W. *J. Med. Chem.* **1999**, *42*, 2260.
10. Dykstra, C. C.; McClernon, D. R.; Elwell, L. P.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1994**, *38*, 1890.
11. Bailly, C.; Dassonneville, L.; Carrascol, C.; Lucasl, D.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *Anti-Cancer Drug Des.* **1999**, *14*, 47.
12. Fitzgerald, D. J.; Anderson, J. N. *J. Biol. Chem.* **1999**, *274*, 27128.
13. Ismail, M. A.; Brun, R.; Easterbrook, J. D.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2003**, *46*, 4761.
14. Wenzler, T.; Boykin, D. W.; Ismail, M. A.; Hall, E. J.; Tidwell, R. R.; Brun, R. *Antimicrob. Agents Chemother.* **2009**, *53*, 4185.
15. Tidwell, R. R.; Geratz, J. D.; Dann, O.; Volz, G.; Zeh, D.; Loewe, H. *J. Med. Chem.* **1978**, *21*, 613.
16. Del Poeta, M.; Schell, W. A.; Dykstra, C. C.; Jones, S. K.; Tidwell, R. R.; Czarny, A.; Bajic, M.; Bajic, M.; Kumar, A.; Boykin, D.; Perfect, J. R. *Antimicrob. Agents Chemother.* **1998**, *42*, 2495.
17. Del Poeta, M.; Schell, W. A.; Dykstra, C. C.; Jones, S. K.; Tidwell, R. R.; Kumar, A.; Boykin, D. W.; Perfect, J. R. *Antimicrob. Agents Chemother.* **1998**, *42*, 2503.
18. Hopkins, K. T.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R.; Kumar, A.; Bajic, M.; Boykin, D. W. *J. Med. Chem.* **1998**, *41*, 3872.
19. Wang, L.; Bailly, C.; Kumar, A.; Ding, D.; Bajic, M.; Boykin, D. W.; Wilson, W. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12.
20. Wang, L.; Carrasco, C.; Kumar, A.; Stephens, C. E.; Bailly, C.; Boykin, D. W.; Wilson, W. D. *Biochemistry* **2001**, *40*, 2511.
21. Tanious, F. A.; Wilson, W. D.; Wang, L.; Kumar, A.; Boykin, D. W.; Marty, C.; Baldeyrou, B.; Bailly, C. *Biochemistry* **2003**, *42*, 13576.
22. Ismail, M. A.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2004**, *12*, 5405.
23. (a) White, E. W.; Tanious, F. A.; Ismail, M. A.; Reszka, A. P.; Neidle, S.; Boykin, D. W.; Wilson, D. W. *Biophys. Chem.* **2007**, *126*, 140; (b) Tidwell, R. R.; Boykin, D. W.; Ismail, M. A.; Wilson, D. W.; White, E. A. W.; Kumar, A.; Nanjunda, R. Eur. Pat. Appl. 2007, 110; CODEN: EPXXDW EP1792613 A2 20070606.
24. Kaluzhny, D. N.; Borisova, O. F.; Shchyolkina, A. K. *Biopolymers* **2010**, *93*, 8.
25. Ismail, M. A. *J. Chem. Res.* **2006**, 733.
26. Ismail, M. A.; Arafa, R. K.; Brun, R.; Wenzler, T.; Miao, Y.; Wilson, W. D.; Generaux, C.; Bridges, A.; Hall, J. E.; Boykin, D. W. *J. Med. Chem.* **2006**, *49*, 5324.
27. Sørensen, U. S.; Pombo-Villar, E. *Tetrahedron* **2005**, *61*, 2697.
28. Mallena, S.; Lee, M. P. H.; Bailly, C.; Neidle, S.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *J. Am. Chem. Soc.* **2004**, *126*, 13659.
29. Wilson, W. D.; Tanious, F. A.; Mathis, A.; Tevis, D.; Hall, J. E.; Boykin, D. W. *Biochimie* **2008**, *90*, 999.
30. Farahat, A. A.; Kumar, A.; Say, M.; Barghash, A. E. M.; Goda, F. E.; Eisa, H. M.; Wenzler, T.; Brun, R.; Liu, Y.; Mickelson, L.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2010**, *18*, 557.
31. We appreciate a reviewer making this observation.
32. Branowska, D.; Farahat, A. A.; Kumar, A.; Wenzler, T.; Brun, R.; Liu, Y.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2010**, *18*, 3551.
33. Bakunova, S. M.; Bakunov, S. A.; Patrick, D. A.; Suresh Kumar, E. V. K.; Ohemeng, K. A.; Bridges, A. S.; Wenzler, T.; Barszcz, T.; Susan Jones, S. K.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2009**, *52*, 2016.