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ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 2901–2908
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Novel linear triaryl guanidines, *N*-substituted guanidines and potential prodrugs as antiprotozoal agents

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Received 6 August 2007; received in revised form 2 November 2007; accepted 8 February 2008
Available online 29 February 2008

Abstract

A series of triaryl guanidines and *N*-substituted guanidines designed to target the minor groove of DNA were synthesized and evaluated as antiprotozoal agents. Selected carbamate prodrugs of these guanidines were assayed for their oral efficacy. The linear triaryl bis-guanidines **6a**,**b** were prepared from their corresponding diamines **4a**,**b** through the intermediate BOC protected bis-guanidines **5a**,**b** followed by acid catalyzed deprotection. The *N*-substituted guanidino analogues **9c**–**f** were obtained in three steps starting by reacting the diamines **4a**,**b** with ethyl isothiocyanatoformate to give the carbamoyl thioureas **7a**,**b**. Subsequent condensation of **7a**,**b** with various amines in the presence of EDCI provided the carbamoyl *N*-substituted guanidine intermediates **8a**–**f** which can also be regarded as potential prodrugs for the guanidino derivatives. Compounds **9c**–**f** were obtained via the base catalyzed decarbamoylation of **8a**–**f**. The DNA binding affinities for the target dicationic bis-guanidines were assessed by ΔT_m values. In vitro antiprotozoal screening of the new compounds showed that derivatives **6a**, **9c** and **9e** possess high to moderate activity against *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*). While the prodrugs did not yield cures upon oral administration in the antitrypanosomal STIB900 mouse model, compounds **8a** and **8c** prolonged the survival of the treated mice.

Keywords: Antiprotozoan; Bis-guanidines; DNA binding; Triaryl guanidines

1. Introduction

Aromatic dications have been long known to possess significant antimicrobial activity [1]. The aromatic diamidine pentamidine **I**, first introduced in the 1942, belongs to this group of dicationic drugs and is the only one to see significant human clinical use [1,2]. Currently, pentamidine is used in the management of antimony-resistant leishmaniasis, initial stage human African trypanosomiasis (HAT), and as a second line agent for AIDS-related *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) pneumonia [1]. Pafuramidine **III** (Fig. 1), an orally bioavailable prodrug of furamidine **II** (Fig. 1), is currently in phase III trials for pneumocystis pneumonia and HAT [1,3–7]. It has been suggested that DNA minor groove binding at AT rich sites is essential for the antimicrobial activity of dications [1]. The consequent inhibition of transcription and/or DNA dependent enzymes is postulated to be the cause of antimicrobial action [1,8–11]. The belief that complementing geometry to that of the DNA minor groove is a structural necessity for the binding affinity, lead to design and synthesis of many dications with crescent shaped scaffold e.g. pentamidine, furamidine and other analogues [1,12–17].On the other hand, current reports have indicated that linear dicationic diamidines can bind strongly to DNA and possess significant antiprotozoan activity. CGP40215A (**IV**, Fig. 1) is an example of these type molecules with considerable antimicrobial activity [18–20]. Moreover, the high binding affinity to AT rich sites of the DNA minor groove was explained by analysis of the binding data, crystal structure and molecular dynamic simulations which lead to the finding that water-assisted interaction of **IV** simulates the curved structure of DNA minor groove [19,20].

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0223-5234/$ - see front matter © 2008 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2008.02.008

We have most recently reported the marked antiprotozoan activity of the rigid-rod terphenyl diamidine (**V**, Fig. 1) and analogues [21]. Also, the thermal melting data and circular dichroism (CD) studies of these linear derivatives reflect a significant DNA minor groove binding affinity [21]. Structures with rigid frameworks of fused ring systems as linkers for the dicationic groups (e.g. carbazole, benzofuran, benzothiophene, 9*H*-fluorene, fluorenone, acridine and anthraquinone) were also reported as effective antimicrobial agents [22–25] and had high DNA minor groove binding affinity and postulated to involve water mediated interactions [26,27].

Previous studies from our laboratory have demonstrated that guanidine dications display considerable affinity to AT rich sites of the DNA minor groove and possess interesting antiprotozoan activity [25,28–30]. In light of the previous results, we have designed and performed a study aiming at combining the novelty of a rigid rod-like terphenyl framework bearing a bisguanidine. We report here the DNA binding and antiprotozoan activity of series of novel dicationic linear triaryl bis-guanidines.

It is documented that aromatic amidines and guanidines are charged at physiological pH since they have p*K* values of 10 or higher [31,32]. This accounts for the lack of oral bioavailability of these dications [1]. Since oral administration is the preferred route of administration, especially for those diseases requiring a prolonged course of treatment, then a prodrug approach is a plausible solution to circumvent the drawback of limited oral absorption. Carbamates were introduced earlier as potential prodrugs for guanidine derivatives [25,33–35]. In this work a number of carbamate derivatives were prepared and evaluated as potential prodrugs for the bis-guanidine series.

2. Discussion

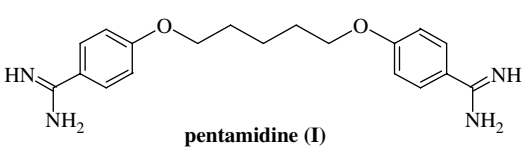
2.1. Chemistry

As depicted in Scheme 1, the linear triaryl bis-guanidines **6a**,**b** were prepared from their corresponding diamines **4a**,**b** through the intermediate BOC protected bis-guanidines **5a**,**b** by the use of bis-Boc-protected *N*-methylpseudothiourea and $HgCl_2$ as a Lewis acid. Treatment of **5a**,**b** with ethanolic HCl at room temperature served the dual purpose of guanidine deprotection, as well as providing the hydrochloride salts of the guanidine free bases **6a**,**b**. While the key intermediate diamine **4a** was commercially available, **4b** was prepared in two steps. The initial step was a suzuki coupling reaction between benzene 1,4-bisboronic acid **1** and 2-chloro-5-nitropyridine **2** to yield the dinitro derivative **3**. Next was the palladium catalyzed hydrogenation of **3** which furnished the desired diamine **4b**.

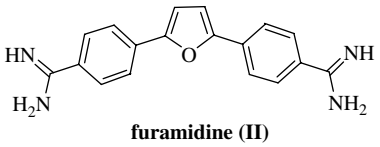
As outlined in Scheme 2, the *N*-substituted alkyl-guanidino analogues **9c–f** were obtained from the diamines **4a**,**b** in three steps. First, the diamines **4a**,**b** were reacted with ethyl isothiocyanatoformate to give the carbamoyl thioureas **7a**,**b**. Subsequently, condensation with variable aliphatic amines in the presence of EDCI provided the carbamoyl *N*-substituted alkyl-guanidines intermediates **8a–f** which can also be regarded as potential prodrugs for the guanidino derivatives. The target compounds **9c–f** were finally obtained via the KOH catalyzed decarbamoylation of **8a–f**. Hydrochloride salts of **9c–f** were prepared by treating an ethanolic solution of the free bases with HCl gas.

2.2. Biological activity

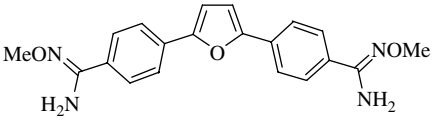
The DNA binding affinity of the dicationic bis-guanidines was assessed by ΔT_m values and the results from in vitro evaluation versus *Trypanosoma brucei rhodesiense* (*T.b.r.*) and *Plasmodium falciparum* (*P.f.*) are shown in Table 1. The DNA affinity of the bis-guanidine **6a** is reduced by about 20% from that of the corresponding diamidine **V**. A similar reduction has also been observed recently in a diphenylthiophene parent system and it seems that amidine dications are generally able to form slightly more favorable complexes with the DNA minor groove than guanidines [36]. *N*-Methyl substitution (**9c**) has little effect on the DNA affinity; however, the larger alkyl group of **9e** results in a reduction of affinity by



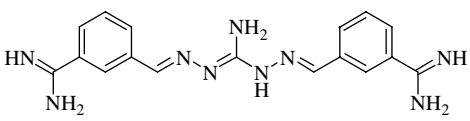
pentamidine (I)



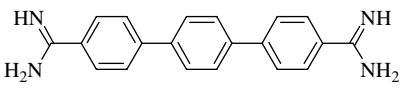
furamidine (II)



Pafuramidine (III)

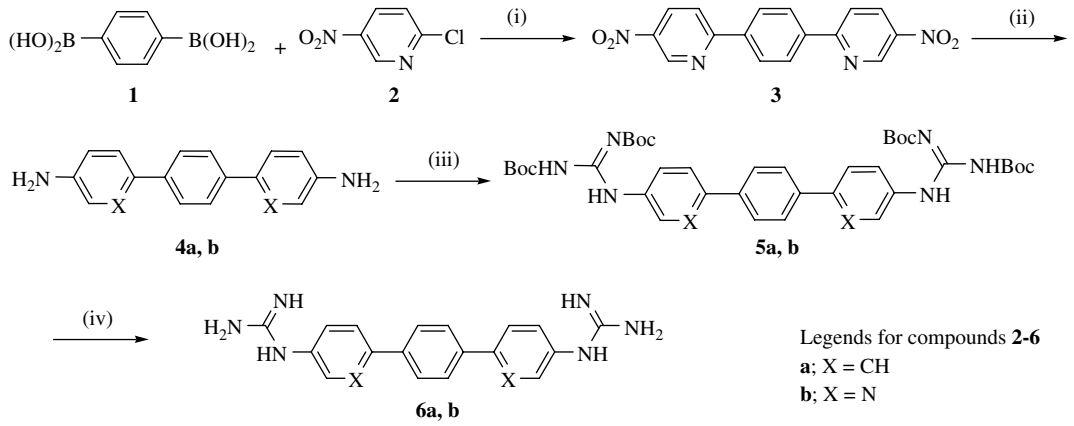


CGP 40215 (IV)



V

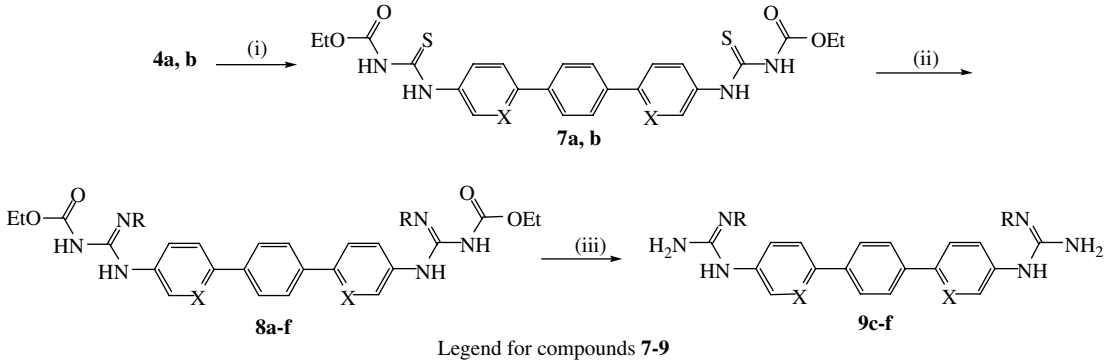
Fig. 1. Structures of key dicationic antiprotozoan agents.



Scheme 1. Reagents and conditions: (i) $Pd(PPh_3)_4$, Na_2CO_3, toluene, 80 °C; (ii) H_2/Pd–C, EtOH, EtOAc; (iii) BocNH(MeS)C=NBoc, $HgCl_2$, TEA, DMF, r.t.; (iv) HCl (g), EtOH, CH_2Cl_2, r.t.

approximately one third. The introduction of nitrogen atoms in the aryl ring (**6b**) also results in approximately a one third reduction in DNA affinity.

The in vitro results against *T.b.r.* and *P.f.* also reflect a reduction in activity compared to that of **V**. Nevertheless, the parent bis-guanidine **6a** and the *N*-methyl analogue **9c** show potent activity against both *T.b.r.* and *P.f.* giving IC_{50} values ranging from 7 to 27 nM. The activity of the *N*-isopropyl analogue **9e** is markedly reduced versus *T.b.r.*; however, its activity versus *P.f.* is essentially the same as **6a** and **9c**. The activity of the compounds with nitrogen atoms in the aryl rings (**6b**, **9d**, and **9f**) in general is markedly reduced from their carbocycle counterparts. In summary, key SAR features noted are that direct substitution on the guanidines with a methyl group is tolerated whereas a moderately large alkyl group (isopropyl) causes significant reduction in *T.b.r.* activity, however, the same pattern is not found against *P.f.* Also, introduction of nitrogen atoms in the linear array results in loss of activity against both parasites. It is unclear whether the origin of these differences are related to the mechanism of action of these molecules or their transport into the parasites. The potential prodrugs (**8b–f**) as expected show essentially no in vitro activity against *T.b.r.*, however, they are active against *P.f.* suggesting the presence of non-specific esterases in the parasite or the culture media. Since the parent molecules **6a**, **6b**, **9c**, **9d** showed promising in vitro activity they were advanced to the STIB900 acute mouse model for African trypanosomiasis and the results are shown in Table 2. The most effective compounds in this model were **6a**, **9c** and **9d**, all extended the average survival time of the animals by greater than 47 days, however, none gave cures at intrapertoneal dose of 20 mg/kg given for 4 days, and **9d** showed acute toxicity. In this experiment the cited three compounds were more effective than the parent diamidine **V**. The two potential prodrugs **8a** and **8c** were tested on oral administration and were found not to be very effective as no cures were achieved and only 20 day survival times were noted. The amidoxime prodrugs of **V** were also only moderately effective which was attributed, at least

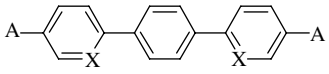


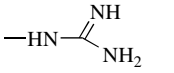
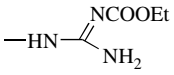
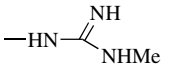
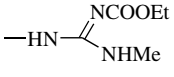
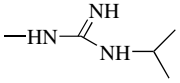
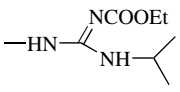
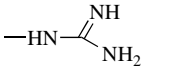
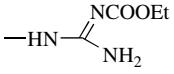
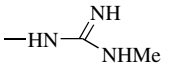
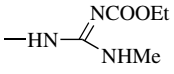
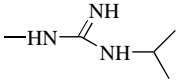
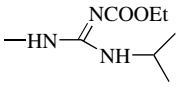
Legend for compounds **7-9**

	X	R
a;	CH	H
b;	N	H
c;	CH	Me
d;	N	Me
e;	CH	*i*-pro
f;	N	*i*-pro

Scheme 2. Reagents and conditions: (i) ethylisothiocyanatoformate, CH_2Cl_2, r.t.; (ii) RNH_2, DIPEA, WSC, r.t.; (iii) KOH, EtOH, 55 °C.

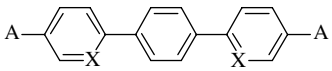
Table 1
DNA binding and in vitro antiprotozoan testing data for linear triaryl dications

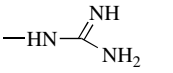
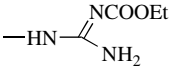
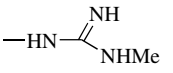
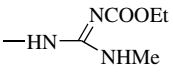
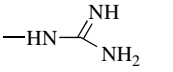
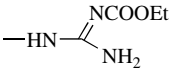
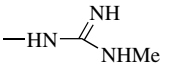
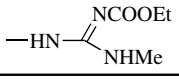


Code	A	X	ΔT_m[a] (°C)	*T.b.r.*[b] IC_{50} (nM)	*P.f.*[b] IC_{50} (nM)	Cytotoxicity[c] IC_{50} (μM)
V		CH	18.2	5	1	22.1
6a		CH	14.2	18	27	25.4
8a		CH	NT[d]	142K	1.7	>181
9c		CH	13.0	18	7	65.1
8c		CH	NT	7.5K	8.9	5.7
9e		CH	9.3	530	14	21.9
8e		CH	NT	14.2K	4.5	5.3
6b		N	9.2	169	151	152
8b		N	NT	61K	1.9	>181
9d		N	10.2	134	54	>161
8d		N	NT	17.8K	2.6	>169
9f		N	8.1	11.9K	393	89.7
8f		N	NT	8.2K	2.9	>135

[a] Poly(d(A-T))$_2$ in MES10 buffer; ratio, compound/DNA is 0.3.
[b] *T.b.r.* strain was STIB900, and the *P.f.* strain was K1. Values are duplicate determinations, see Refs. [36,37].
[c] Cytotoxicity was evaluated using cultered L6 rat myoblast cells using the same assay procedure as for *T.b.r.*
[d] NT = not tested.

Table 2
In vivo antitrypanosomal screening data for linear triaryl dications in STIB900 mouse model[a]



Code	A	X	Dose	Cures[b]	Average survival (days)[c]
V[d]		CH	20 mg/kg ip	0/4	29
6a		CH	20 mg/kg ip	0/4	>54.5
8a		CH	25 mg/kg po	0/4	20.25
9c		CH	20 mg/kg ip	0/4	>56
8c		CH	25 mg/kg po	0/4	20.25
6b		N	20 mg/kg ip	0/4	20.25
8b		N	25 mg/kg po	0/4	8.5
9d[e]		N	20 mg/kg ip	0/2	>47
8d		N	25 mg/kg ip	0/4	7.75

[a] See Ref. [37] for details of the STIB900 mouse model. Dosage was for 4 days and was either intraperitoneal (ip) or oral (po) as noted.
[b] Number of mice that survived 60 days and are parasite-free.
[c] Average days of survival; untreated controls died between day 7 and 9 post infection.
[d] The results for **V** in this experiment were moderately different from that reported in Ref. [21].
[e] Some acute toxicity was apparent as two of the four animals died within one day after the first dose. Due to the modest IC_{50} values for **9e** and **9f**, they were not tested in vivo, nor were their prodrugs **8e** and **8f**.

in part, to poor bioconversion [20]. It is unknown if the poor efficacies of these carbamte prodrugs, which would be converted by a different mechanism, are due to poor absorption/distribution or poor bioconversion.

In conclusion, the linear bis-guanidines were found to bind to DNA at a somewhat reduced level compared to **V**, yet they exhibited very good in vitro activity against *T.b.r.* and *P.f.* although reduced compared with that of **V**. However, the in vivo efficacy of the most active compounds **6a**, **9c** and **9d** was greater than that of **V**. Unfortunately, both the active parent molecules (**6a** and **6b**) and their potential prodrugs (**8a** and **8b**) were only moderately effective in the STIB900 acute mouse model.

3. Experimental section

3.1. Efficacy evaluations

In vitro activity over a 72 h drug exposure time was determined against the erythrocytic stages of the chloroquine and pyrimethamine resistant *P.f.* strain K1 using the 3H-hypoxanthine incorporation assay and against the bloodstream form of the *T.b.r.* strain STIB900 using the Alamar Blue assay. In vivo efficacy was determined in the STIB900 acute mouse model. Four infected mice were treated with the drugs either by the intraperitoneal or for prodrugs by the oral route on days

3–6 post infection and parasitamia checked to day 60. The experiments were carried out as previously reported [37].

3.2. T_m Measurements

Thermal melting experiments were conducted with a Cary 300 spectrophotometer. Cuvettes for the experiment are mounted in a thermal block and the solution temperatures are monitored by a thermistor in 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 curvettes in CAC 10 buffer (cacodylic acid 10 mM, EDTA 1 mM, NaCl 100 mM with NaOH addition 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. The ΔT_m values obtained in this manner are reproducible within ± 0.5 °C.

3.3. Synthetic protocols

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 (Varian, Inc., Palo Alto, California), and chemical shifts (δ) are in ppm relative to TMS as the internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (VG Analytical, Ltd., Manchester, UK). 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 of water and/or ethanol of solvation. In each case, proton NMR showed the presence of indicated solvent(s). All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Lancaster Synthesis, Inc.

3.3.1. 4,4″-Bis(N′,N″-t-butoxycarbonyl)-guanidino-[1,1′;4′,1″]terphenyl (**5a**)

To a solution of 4,4″-diamino-[1,1′;4′,1″]terphenyl (**4a**) (0.30 g, 1.15 mmol) in anhydrous DMF (10 ml) was added 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea (0.71 g, 2.4 mmol), triethylamine (0.74 g, 7.2 mmol) and finally mercury(II) chloride (0.73 g, 2.6 mmol). The suspension was kept stirring at room temperature for 24 h. The reaction, diluted with CH_2Cl_2 and Na_2CO_3 solution, was filtered through a pad of Celite. The organic layer was washed with water (3×) followed by brine and then dried over anhydrous Na_2SO_4. After evaporating the solvent to dryness the obtained residue was recrystallized from CH_2Cl_2/MeOH giving a creamy white solid, yield 79%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.50, 1.59 (2s, 36H), 7.56–7.70 (m, 12H), 10.39 (br s, 2H), 11.65 (br s, 2H). Anal. Calc. for $C_{40}H_{52}N_6O_8$: C, 64.49; H, 7.03. Found C, 64.31; H, 6.85.

3.3.2. 4,4″-Bis-guanidino-[1,1′;4′,1″]terphenyl (**6a**)

The N′,N″-di-BOCguanidine **5a** (0.45 g, 0.60 mmol) was dissolved in CH_2Cl_2 (10 ml), diluted with dry EtOH (15 ml) and the chilled solution was saturated with dry HCl. The reaction was then kept stirring at room temperature for 3 days (drying tube), where upon the product started precipitating. After evaporating the solvent to dryness, the residue was washed with ether multiple times and was dried under vacuum at 50–60 °C overnight to give whitish yellow solid of the bis-guanidine dihydrochloride, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 7.85 (d, $J = 8.4$ Hz, 4H), 7.92 (s, 4H), 8.00 (d, $J = 8.4$ Hz, 4H), 9.09 (br s, 4H), 11.25 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 155.98, 138.35, 137.32, 135.00, 127.79, 127.22, 124.79. MS (ESI) *m/e* (rel. int.): 345 ($M^+ + 1$, 6), 173 (100). Anal. Calc. for $C_{20}H_{20}N_6$–2.0HCl–H_2O–0.2C_2H_5OH: C, 55.11; H, 5.71; N, 18.90. Found C, 55.39; H, 5.50; N, 18.71.

3.3.3. 4,4″-Bis(N′-ethoxycarbonylthiourea)-[1,1′;4′,1″]terphenyl (**7a**)

A solution of **4a** (0.50 g, 1.92 mmol) in CH_2Cl_2 (10 ml), added to which ethyl isothiocyanatoformate (0.55 g, 4.22 mmol), was stirred at room temperature for 24 h. After flash chromatography, the reaction was diluted with hexane and the precipitate formed was collected and dried to yield the bis-carbamoyl thiourea as a white solid, yield 89%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.26 (t, $J = 7.2$ Hz, 6H), 4.22 (q, $J = 7.2$ Hz, 4H), 7.70–7.79 (m, 12H), 11.29 (br s, 2H), 11.61 (br s, 2H). MS (ESI) *m/e* (rel. int.): 522 (M^+, 13), 344 (100), 328 (63). Anal. Calc. for $C_{26}H_{26}N_4O_4S_2$: C, 59.75; H, 5.01. Found C, 59.58; H, 5.23.

3.3.4. 4,4″-Bis(N′-ethoxycarbonyl)-guanidino-[1,1′;4′,1″]terphenyl (**8a**)

A stirred solution of the carbamoyl thiourea **7a** (0.80 g, 1.53 mmol), 0.5 M ammonia solution in dioxane (11.7 ml, 6.12 mmol), and diisopropylethylamine (1.18 g, 9.18 mmol) in anhydrous DMF (10 ml) was cooled to 0 °C. EDCI (1.17 g, 6.12 mmol) was added, and the solution was stirred at room temperature overnight. The reaction mixture poured onto ice/water, the solid collected by vacuum filtration. Finally, the carbamoyl-guanidine was crystallized from EtOH, yield 67%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.17 (t, $J = 6.9$ Hz, 6H), 3.98 (q, $J = 6.9$ Hz, 4H), 7.53–7.71 (m, 16H), 9.05 (br s, 2H). Anal. Calc. for $C_{26}H_{28}N_6O_4$–0.2C_2H_5OH: C, 63.70; H, 5.91; N, 16.88. Found C, 63.66; H, 5.71; N, 16.65.

3.3.5. 4,4″-Bis(N′-ethoxycarbonyl-N″-methyl)-guanidino-[1,1′;4′,1″]terphenyl (**8c**)

A stirred solution of carbamoyl thiourea **7a** (1.00 g, 1.91 mmol), methylamine hydrochloride (0.51 ml, 7.65 mmol), and diisopropylethylamine (1.48 g, 11.48 mmol) in anhydrous CH_2Cl_2 (10 ml) was cooled to 0 °C. EDCI (1.46 g, 7.65 mmol) was added, and the solution was stirred at room temperature overnight. The reaction mixture was

washed with water (3 × 100 mL), followed by brine and dried over anhydrous Na_2SO_4. The residue remaining after removal of the solvent was crystallized from EtOH/water, yield 82%, m.p. 297–299 °C. ^{1}H NMR (DMSO-d_6): δ 1.15 (t, $J = 7.2$ Hz, 6H), 3.32 (s, 6H), 3.95 (q, $J = 7.2$ Hz, 4H), 7.45 (br s, 4H), 7.68–7.75 (m, 12H). ^{13}C NMR (DMSO-d_6): δ 163.01, 158.53, 138.25, 137.50, 135.41, 126.76, 126.66, 124.14, 59.60, 28.17, 14.54. MS (ESI) *m/e* (rel. int.): 517 (M^+, 30), 259 (100). Anal. Calc. for $C_{28}H_{32}N_6O_4$–$0.25C_2H_5OH$: C, 64.81; H, 6.39; N, 15.91. Found C, 64.84; H, 6.20; N, 15.82.

3.3.6. 4,4″-Bis(N′-methyl)-guanidino-[1,1′;4′,1″]terphenyl (**9c**)

The bis substituted carbamoyl-guanidine **8c** (0.5 g, 0.96 mmol) was suspended in EtOH (10 ml). KOH (1 N, 9.6 ml, 9.6 mmol) was then added and the reaction mixture was kept stirring overnight maintaining the temperature at 55–60 °C. The reaction mixture was diluted with water and the solid formed was collected by filtration, washed multiple times with water and recrystallized from aqueous EtOH to give a tan white solid, yield 75%, m.p. 243–244 °C. ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 6H), 5.35 (br s, 2H), 6.87 (d, $J = 8.4$ Hz, 4H), 7.53 (d, $J = 8.4$ Hz, 4H), 7.64 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 152.2, 149.8, 138.2, 131.3, 126.5, 126.0, 123.2, 27.5. MS (ESI) *m/e* (rel. int.): 373 (M^+, 6), 187 (100).

Hydrochloride salt of **9c**. The free base was dissolved in dry EtOH (20 ml) and the solution was chilled in an ice-bath. After passing HCl gas for 10 min, the reaction was concentrated under reduced pressure and then diluted with ether. The precipitate formed was collected by filtration, m.p. 211–214 °C. ^{1}H NMR (DMSO-d_6): δ 2.83, 2.85 (2 s, 6H), 7.33 (d, $J = 8.4$ Hz, 4H), 7.77–7.80 (m, 8H), 7.96 (br s, 4H), 10.02 (br s, 4H). Anal. Calc. for $C_{22}H_{24}N_6$–2.0HCl–$1.25H_2O$–$0.3C_2H_5OH$: C, 56.34; H, 6.33; N, 17.42. Found C, 56.69; H, 6.07; N, 17.05.

3.3.7. 4,4″-Bis(N′-ethoxycarbonyl-N″-isopropyl)-guanidino-[1,1′;4′,1″]terphenyl (**8e**)

The procedure described for **8a** was adopted. Yield 89%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.11–1.18 (m, 18H), 3.94 (q, $J = 7.2$ Hz, 4H), 4.01–4.14 (m, 2H), 7.45 (d, $J = 7.8$ Hz, 4H), 7.68–7.75 (m, 8H), 9.24 (br s, 4H). ^{13}C NMR (DMSO-d_6): δ 163.40, 157.15, 138.22, 137.49, 135.21, 126.72, 126.57, 123.95, 59.58, 42.40, 22.54, 14.53. MS (ESI) *m/e* (rel. int.): 573 (M^+, 17), 287 (100). Anal. Calc. for $C_{32}H_{40}N_6O_4$: C, 67.11; H, 7.03; N, 14.67. Found C, 66.95; H, 7.16; N, 14.37.

3.3.8. 4,4″-Bis(N′-isopropyl)-guanidino-[1,1′;4′,1″]terphenyl (**9e**)

The procedure described for **9c** was adopted. Yield 73%, m.p. 246–247 °C. ^{1}H NMR (DMSO-d_6): δ 1.11 (d, $J = 6.3$ Hz, 12H), 3.81–3.89 (m, 2H), 5.38 (br s, 6H), 6.87 (d, $J = 8.4$ Hz, 4H), 7.53 (d, $J = 8.4$ Hz, 4H), 7.74 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 150.95, 149.36, 138.23, 131.47, 126.69, 126.14, 123.36, 41.56, 22.84. MS (ESI) *m/e* (rel. int.): 429 (M^+, 8), 215 (100).

Hydrochloride salt of **9e**. M.p. 275–277 °C. ^{1}H NMR (DMSO-d_6): δ 1.19 (d, $J = 6.6$ Hz, 12H), 3.85–3.96 (m, 2H), 5.38 (br s, 6H), 7.31 (d, $J = 8.4$ Hz, 4H), 7.77–7.80 (m, 8H), 8.13 (br s, 2H), 9.91 (br s, 2H). Anal. Calc. for $C_{26}H_{32}N_6$–2.0HCl–$1.5H_2O$–$0.25C_2H_5OH$: C, 58.93; H, 7.18; N, 15.56. Found C, 59.00; H, 6.82; N, 15.33.

3.3.9. 1,4-Bis-[5′-nitropyridin-2′-yl]phenylene (**3**)

To a stirred solution of 2-chloro-5-nitropyridine (3.16 g, 20 mmol), and tetrakis(triphenylphosphine) palladium (800 mg) in toluene (40 mL) under a nitrogen atmosphere was added 20 mL of a 2 M aqueous solution of Na_2CO_3 followed by 1,4-phenylenebisboronic acid (1.64 g, 10 mmol) in 10 mL of methanol. The vigorously stirred mixture was warmed to 80 °C for 12 h. The solvent was evaporated, the precipitate was filtered off, washed with ethanol, recrystallized from DMF to afford **3** as a yellow solid in 89% yield, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 8.35 (d, $J = 8.7$ Hz, 2H), 8.39 (s, 4H), 8.67 (dd, $J = 2.1$, 8.7 Hz, 2H), 9.47 (d, $J = 2.1$ Hz, 2H). MS (ESI) *m/e* (rel. int.): 323 ($M^+ + 1$, 100), 307 (80), 287 (12).

3.3.10. 1,4-Bis-[5′-aminopyridin-2′-yl]phenylene (**4b**)

A suspension of the bis-nitro compound **3** (2.58 g, 8.0 mmol) in EtOAc (150 ml) and EtOH (50 ml) was hydrogenated with 10% Pd/C (1.0 g, Lancaster) at 60 psi until uptake subsided (8 h). The mixture was then filtered over Celite and concentrated in vacuo to afford a buff solid in 77% yield, m.p. 250–251 °C. ^{1}H NMR (DMSO-d_6): δ 5.46 (s, 4H, $2NH_2$), 6.98 (dd, $J = 2.4$, 8.4 Hz, 2H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.93 (s, 4H), 8.02 (d, $J = 2.4$ Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 143.4, 137.9, 136.0, 125.0, 120.5, 120.0. MS (ESI) *m/e* (rel. int.): 263 ($M^+ + 1$, 100).

3.3.11. 1,4-Bis-{5′-[(N′,N″-t-butoxycarbonyl)-guanidino]pyridin-2′-yl}phenylene (**5b**)

The same procedure described for **5a** was used starting with **4b**. Yield 82%, m.p. >300 °C. ^{1}H NMR ($CDCl_3$); δ 1.51, 1.56 (2s, 36H), 7.79 (d, $J = 8.4$ Hz, 2H), 8.07 (s, 4H), 8.28 (dd, $J = 2.4$, 8.4 Hz, 2H), 8.80 (d, $J = 2.4$ Hz, 2H), 10.46 (s, 2H), 11.63 (s, 2H). Anal. Calc. for $C_{38}H_{50}N_8O_8$: C, 61.11; H, 6.75. Found C, 61.43; H, 6.69.

3.3.12. 1,4-Bis-[5′-guanidinopyridin-2′-yl]phenylene (**6b**)

The same procedure described for **6a** was used starting with **5b**. Yield 55%, m.p. 271–273 °C. ^{1}H NMR (D_2O/DMSO-d_6): δ 7.82 (s, 4H), 8.15–8.25 (m, 4H), 8.62 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 156.4, 152.3, 144.7, 137.8, 133.7, 131.7, 126.9, 121.2. MS (ESI) *m/e* (rel. int.): 347 ($M^+ + 1$, 25), 305 (40), 288 (5), 263 (7), 174 (100). Anal. Calc. for $C_{18}H_{18}N_8$–4.0HCl–$1.1H_2O$–$1.0C_2H_5OH$: C, 43.04; H, 5.42; N, 20.07. Found C, 43.03; H, 5.10; N, 19.78.

*3.3.13. 1,4-Bis-[5′-(N′-ethoxycarbonylthiourea)pyridin-2′-yl]-phenylene (**7b**)*

The same procedure described for **7a** was used starting with **4a**. Yield 89%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6); δ 1.29 (t, $J = 7.2$ Hz, 6H), 4.25 (q, $J = 7.2$ Hz, 4H), 8.02–8.20 (m, 8H), 8.80 (s, 2H), 11.12 (s, 2H), 11.51 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 179.3, 153.0, 152.4, 145.4, 138.1, 133.9, 132.6, 126.4, 119.3, 61.7, 13.6. MS (ESI) *m/e* (rel. int.): 525 (M^+, 10), 409 (25), 241 (40), 163 (100). Anal. Calc. for $C_{24}H_{24}N_6O_4S_2$: C, 54.95; H, 4.61. Found C, 54.76; H, 4.89.

*3.3.14. 1,4-Bis-{5′-(N′-ethoxycarbonylguanidino)pyridin-2′-yl}phenylene (**8b**)*

The procedure described for **8a** was used. Yield 70%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.19 (t, $J = 7.2$ Hz, 6H), 4.03 (q, $J = 7.2$ Hz, 4H), 7.53 (br s, 4H), 7.92–8.03 (m, 4H), 8.13 (s, 4H), 8.66 (s, 2H), 9.08 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 163.0, 155.4, 149.6, 142.3, 138.2, 135.4, 129.0, 126.2, 119.9, 59.8, 14.5. MS (ESI) *m/e* (rel. int.): 491 ($M^+ + 1$, 40), 436 (5), 402 (10), 284 (10), 246 (100). Anal. Calc. for $C_{24}H_{26}N_8O_4$–$0.35H_2O$: C, 58.02; H, 5.41; N, 22.55. Found C, 58.05; H, 5.41; N, 22.20.

*3.3.15. 1,4-Bis-{5′-[(N′-ethoxycarbonyl-N″-methyl) guanidino]pyridin-2′-yl}phenylene (**8d**)*

The procedure used for **8a** was adopted. Yield 65%, m.p. >300 °C; ^{1}H NMR (DMSO-d_6): δ 1.14 (t, J = 7.2 Hz, 6H), 2.92 (s, 6H), 3.95 (q, $J = 7.2$ Hz, 4H), 7.93–8.18 (m, 12H), 8.71 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 163.2, 159.0, 144.7, 138.3, 134.2, 132.1, 126.4, 119.7, 59.6, 28.3, 14.6. MS (ESI) *m/e* (rel. int.): 519 (M^+, 40), 488 (5), 464 (10), 391 (145), 298 (20), 260 (100). Anal. Calc. for $C_{26}H_{30}N_8O_4$–$0.25C_2H_5OH$: C, 60.04; H, 5.98; N, 21.13. Found C, 59.80; H, 5.90; N, 20.91.

*3.3.16. 1,4-Bis-[5′-(N′-methylguanidino)-pyridin-2′-yl]-phenylene (**9d**)*

The procedure described for **9c** was used. Yield 58%, m.p. 262–264 °C. ^{1}H NMR (DMSO-d_6): δ 2.75 (s, 6H), 5.40 (br s, 6H), 7.21 (dd, $J = 8.4$, 2.4 Hz, 2H), 7.80 (d, $J = 8.4$ Hz, 2H), 8.06 (s, 4H), 8.12 (d, $J = 2.4$ Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 153.4, 146.7, 146.4, 144.8, 138.2, 130.2, 125.6, 119.9, 27.7. MS (ESI) *m/e* (rel. int.): 375 ($M^+ + 1$, 10), 344 (10), 226 (25), 188 (100).

Hydrochloride salt of **9d**. M.p. 292–294 °C. Anal. Calc. for $C_{20}H_{22}N_8$–4.0HCl–$1.5H_2O$–$0.25C_2H_5OH$: C, 44.06; H, 5.48; N, 20.05. Found C, 44.19; H, 5.18; N, 19.81.

*3.3.17. 1,4-Bis-{5′-[(N′-ethoxycarbonyl-N″-iso-propyl) guanidino]pyridin-2′-yl}-phenylene (**8f**)*

Yield 64%, m.p. >300 °C. ^{1}H NMR (DMSO-d_6): δ 1.11–1.21 (m, 18H), 3.95 (q, $J = 7.2$ Hz, 4H), 4.05–4.12 (m, 2H), 7.86 (d, $J = 8.7$ Hz, 2H), 8.01 (d, $J = 8.7$ Hz, 2H), 8.17 (s, 4H), 8.63 (s, 2H), 9.25 (br s, 4H). ^{13}C NMR (DMSO-d_6): δ 162.7, 157.0, 150.2, 144.3, 138.1, 135.3, 131.2, 126.1, 119.4, 59.6, 42.4, 22.3, 14.2. MS (ESI) *m/e* (rel. int.): 575 (M^+, 100), 529 (20), 503 (10). Anal. Calc. for $C_{30}H_{38}N_8O_4$–$0.5H_2O$: C, 61.73; H, 6.73; N, 19.19. Found C, 61.75; H, 6.55; N, 18.87.

*3.3.18. 1,4-Bis-[5′-(N′-isopropylguanidino)-pyridin-2′-yl]-phenylene (**9f**)*

The procedure described for **9c** was adopted. Yield 59%, m.p. 259–260.5 °C. ^{1}H NMR (DMSO-d_6): δ 1.16 (d, $J = 6.6$ Hz, 12H), 3.88–3.96 (m, 2H), 6.30 (br s, 6H), 7.38 (dd, $J = 8.4$, 2.1 Hz, 2H), 7.85 (d, $J = 8.4$ Hz, 2H), 8.08 (s, 4H), 8.26 (d, $J = 2.1$ Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 152.3, 148.3, 144.7, 142.0, 138.1, 130.5, 125.7, 119.7, 42.0, 22.4. MS (ESI) *m/e* (rel. int.): 431 (M^+, 100), 372 (5), 216 (60).

Hydrochloride salt of **9f**. M.p. 278–280 °C. Anal. Calc. for $C_{24}H_{30}N_8$–4.0HCl–$0.75H_2O$: C, 48.90; H, 6.06; N, 18.99. Found C, 49.15; H, 6.09; N, 18.62.

Acknowledgment

This work was supported by an award from the Bill and Melinda Gates Foundation and NIH grants GM61587 and AI46365.

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