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Short communication

Synthesis and antiparasitic evaluation of bis-2,5-[4-guanidinophenyl]thiophenes

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Abstract

A series of bis-2,5-[4-guanidinophenyl]thiophenes were prepared in a five step process starting from 2,5-bis[trimethylstannyl]thiophene. The compounds were evaluated in vitro against *Trypanosoma brucei rhodesiense* (*T. b. r.*), *Plasmodium falciparum* (*P. f.*), *Leshmania donovani* (*L. d.*) and *Trypanasoma cruzi* (*T. c.*), and in vivo against *T. b. r.* Certain compounds show promising in vitro activity against *T. b. r.* and *P. f.* and have superior in vivo activity against *T. b. r.* to that of pentamidine and furamidine.

Keywords: Diguanidino thiophenes; Antiparasitic agents

1. Introduction

Over three billion people are estimated to be at risk for the parasitic diseases such as malaria, African (sleeping sickness) and American (Chagas disease) trypanosomiasis and leishmaniasis [1]. Furthermore, the combined number of cases of these diseases is estimated to be approximately 300 million [1,2]. A relatively new development further increases the public health burden, through transmission of Chagas disease by travelers with blood transfusions in countries not normally affected by the diseases [3]. Malaria is widely distributed throughout the tropics and it is estimated that between 350 and 500 million clinical episodes occur annually in areas where some 3.2 billion people are at risk [4]. *Plasmodium falciparum* (*P. f.*) and *Plasmodium vivax* cause the majority of human cases. Sleeping sickness caused by the parasites *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Trypanosoma brucei gambiense*, is fatal if not treated and impacts the socioeconomic well being of millions of people in central Africa [1]. Chagas disease, caused by *Trypanosoma cruzi* (*T. c.*), is found in much of South America, all of Central America and in Mexico. The *Leishmania* parasite is broadly distributed in humans and animals. It is found in the Far East, southern Europe and now even in the United States [1]. Many of the drugs currently in use for the treatment of these parasitic infections were developed over 50 years ago and have major limitations including significant toxicity, variable efficacy, lack of oral bioavailability, extensive courses of parenteral administration, and problems of cost and supply. Furthermore, there is considerable evidence that extended use of these drugs is leading to the development of resistance [5]. The need for the discovery of new drugs to treat these diseases is clear.

Dicationic molecules were first reported to have significant antiprotozoal activity in the 1930's [5]. Despite numerous studies of various classes of dications, the diamidine pentamidine (**I**) is the only compound from this class which has seen significant human use. Pentamidine is used to treat early stage

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T. b. g. human African trypanosomiasis (HAT), antimony-resistant leishmaniasis and AIDS-related *P. jiroveci* pneumonia [9]. The diamidine furamidine and its analogues have shown significant activity against various parasites, and pafuramidine, an orally effective prodrug of furamidine, was in Phase II clinical trials against malaria, and is in Phase III trials against HAT and pneumocystis pneumonia [5–9]. The mode of action of this class of compounds is still being elucidated. However, transport mediated uptake appears to be a key step. It has been suggested that these dicationic molecules act by binding in the minor groove of DNA at AT rich sites [5]. Binding to DNA does not directly kill the parasite, but there is considerable evidence that minor groove binding leads to inhibition of DNA dependant enzymes or possibly direct inhibition of transcription [5,10–13].

The structurally related dicationic diguanidines have received relatively little attention compared to the extensive studies of diamidines. However, a few reports have recently appeared which show that these types of molecules exhibit significant antiprotozoal activity. For example, 4,4′-bis[guanidino]diphenylamine has shown promising in vitro and in vivo activity against *T. b. r.* [14,15]. In a study of fused ring diguanidino compounds, several bisguanidino indenes have been found to exhibit potent in vitro activity against both *P. f.* and *T. b. r.* and significant in vivo activity was found in a mouse model for African trypanosomiasis [16]. Bis-2,5-[4-guanidinophenyl]furans [17] and bis-2,5-[3- or 4-guanidinophenyl]-*N*-methylpyrroles [18] have shown promising in vitro antifungal activity. Also, the bis-2,5-[4-guanidinophenyl]furans [19] were active in vitro against *L. d.* and *T. c.* Collectively, these results for various aryl diguanidino compounds led us to synthesize and evaluate the antiprotozoal activity of bis-2,5-[4-guanidinophenyl]thiophenes.

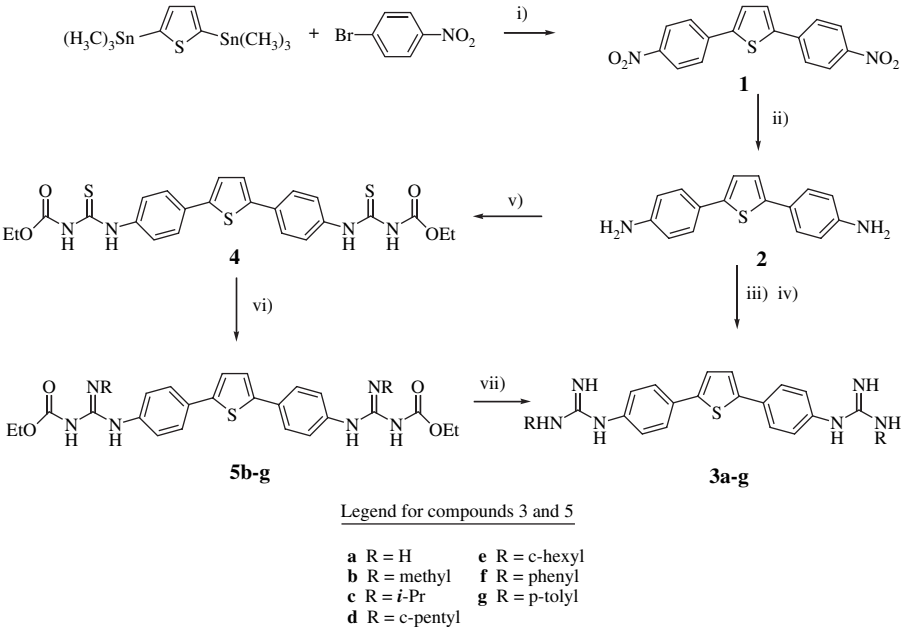
2. Chemistry

The synthesis of the various bis-2,5-[4-guanidinophenyl]thiophenes is outlined in Scheme 1 and is patterned after the approach we developed to make the corresponding furan analogs [17]. Stille coupling of bis-2,5-[trimethylstannyl]thiophene and 4-bromonitrobenzene gave bis-2,5-[4-nitrophenyl]thiophene (**1**) in high yield. Stannous chloride reduction of **1** gave bis-2,5-[4-aminophenyl]thiophene (**2**) in very good yield.

The diamine **2** was used to prepare bis-2,5-[4-guanidinophenyl]thiophene (**3a**) and the various bis-2,5-[4-(*N*-alkyl and *N*-aryl)guanidinophenyl]thiophenes (**3b–3g**). The parent molecule **3a** was made in a two-step process in which **2** was allowed to react with Boc-protected *S*-methylthiourea in the presence of mercuric chloride. The Boc-protected analogue of **3a** was obtained in 70% yield and it was subsequently deprotected using anhydrous HCl in ethanol/dichloromethane solution. The *N*-alkyl and *N*-aryl diguanidines **3b–3g** were obtained first by the reaction of **2** with ethyl isothiocyanatoformate to yield the carbethoxy thiourea **4** [20]. The reaction of **4** with the appropriate amine in the presence of carbodiimide EDCI yielded the corresponding carbethoxy diguanidines **5b–5g** [21]. The action of KOH/EtOH provide the diguandine free bases **3b–3g** which were readily converted to dihydrochloride salts.

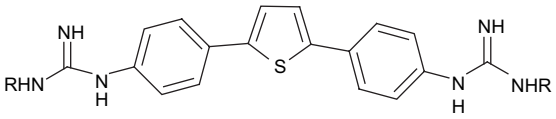
3. Biology

The DNA affinity as measured by ΔTm for their complexes with polydA.polydT and the in vitro efficacy of **3a–3g** against four different parasites *T. b. r.*, *P. f.*, *Leishmania donovani* (*L. d.*) and *T. c.* are presented in Table 1. The DNA affinity of the parent thiophene **3a** is slightly less than that of its furan counterpart [17]. The smaller *N*-alkyl substituted compounds **3b**



Scheme 1. (i) $Pd(PPh_3)_4$, 1,4-dioxane; (ii) $SnCl_2$ dihydrate, EtOH; (iii) *S*-methyl-di-Bocthiourea, $HgCl_2$, TEA, DMF; (iv) HCl, CH_2Cl_2, EtOH; (v) ethyl isothiocyanatoformate, CH_2Cl_2; (vi) NH_2R, DIPEA, EDCI, CH_2Cl_2; (vii) KOH, EtOH.

Table 1
DNA affinity and in vitro antiparasitic activities of diguanidino dications



Code	R	ΔTm	IC_{50} (nM)		IC_{50} (μM)		
		poly dA.dT [a]	*T. b. r.*[b]	*P. f.* [b,c]	*L. d.*[b,d]	T. c.[b,e]	L-6[b,f]
Chloroquine	NA	–	–	201	–	–	188.5
Pentamidine	NA	12. 6	2.8	58.4	1.9	5.35	46.6
Furamidine	NA	25.0	4.3	15.5	2.8	23.3	6.4
3a	H	19.9	28	3.6	6.7	154.0	100.3
3b	Me	16.2	6.2	23	6.2	30.5	54.3
3c	*i*-Pr	15.4	116	107	11	28.2	69.4
3d	*c*-pent	19.4	306	134	1.5	8.4	15.9
3e	*c*-hex	17.8	354	160	2.5	1.7	4.3
3f	Phenyl	19.5	1900	154	35	9.9	2.5
3g	4-Mephenyl	16.8	573	42.7	16	3.7	1.1

[a] DNA: polydA.polydT; buffer MES 10; compound/DNA ratio = 0.3 see Ref. [24].
[b] Activities represent the mean of at least two independent experiments; IC_{50} values used to calculate the average for a given compound are within a factor of two.
[c] The *T. b. r.* strain used was STIB900 and the *P. f.* strain was K1; see Refs. [15,22–24].
[d] See Refs. [26,27] for details of this amastigote screen.
[e] Benznidazole in this screen IC_{50} = 1.5 μM; see Ref. [25] for details of this screen.
[f] Cytotoxicity was evaluated using cultured L-6 rat myoblast cells using the alamar blue assay, see Ref. [23].

and **3c** exhibited ΔTm values which are reduced by about 20–30% compared to the parent **3a**, whereas the larger *N*-alkyl and *N*-aryl compounds (**3d**–**3g**) only show a modest drop in affinity. Generally, the ΔTm values of guanidines **3a**–**3g** are 20–40% smaller than that of furamidine, the prototype diamidine. Nevertheless, these diguanidino analogues show strong DNA affinity, superior to that of pentamidine [22].

The IC_{50} values of this series of diguanidines against *T. b. r.* range from 6.2 to 1900 nM. The most active compounds are the parent **3a** and its *N*-methyl analog **3b**; progressively larger substituents than methyl lead to significant reduction in activity. The activity of these compounds against *P. f.* shows a similar structure–activity relationship but with a less dramatic drop in activity with large substituents. The IC_{50} values range from 3.6 to 160 nM against *P. f.* with the most active antimalarials being **3a** and **3b**. The IC_{50} values of these compounds against *L. d.* and *T. c.* are modest and range from 2 to 35 μM versus *L. d.* and from 1 to 154 μM against *T. c.* With the exception of **3e**–**3g**, the compounds do not show cytotoxicity and thus are reasonably selective for *T. b. r.* and *P. f.*

Given the good in vitro activity against *T. b. r.* of several of these diguanidines and the availability of the well-established *T. b. r* STIB900 mouse model, we decided to evaluate the in vivo activity of the 4 most active compounds (Table 2). The mouse model used is very stringent and the extended survival indicates improved in vivo activity for all the four tested diguanidines over the current drug pentamidine. The three *N*-alkyl analogues tested (**3b**–**3d**) gave 1/4 or 2/4 cures in this model, activity which is superior to that of both pentamidine and furamidine. Interestingly, both **3c** and **3d**, whose intrinsic antitrypanosomal activity in vitro is significantly less than that of **3a** and **3b** is more active in vivo, suggesting superior pharmacodynamic properties.

4. Conclusions

In summary, we report an efficient synthetic approach to bis-2,5-[4-guanidinophenyl]thiophenes and their promising antiparasitic activity, especially against *T. b. r.* and *P. f.* The in vivo studies in the STIB900 mouse model show that the diguandino compounds are well tolerated and efficacious against African trypanosomes. These and other diguanidino compounds clearly merit further study as antiparasitic agents.

5. Experimental section

5.1. Biology

In vitro assays with *T. b. r.* STIB900, *P. f.* K1 and *T. cruzi* Tulahuen Lac Z C4 strain as well as the efficacy study in an

Table 2
In vivo antitrypanosomal activity of dicationic compounds in the STIB900 mouse model[a]

Code	Dosage[b] mg/kg	Cures[c]	Survival[d] (days)
Pentamidine	4 × 20	0/4	40.8
Furamidine	4 × 20	0/4	>52.5
3a	4 × 20	0/4	43
3b	4 × 20	2/4	>40.25
3c	4 × 5	1/4	>34
3d	4 × 20	2/4	>39.5

[a] See Refs. [15,22–24] for details of the *T. b. r.* mouse model STIB900 strain.
[b] Dosage = intraperitoneal for four days.
[c] Number of mice that survive and are parasite free for 60 days post infection.
[d] Average days of survival; untreated control animals expire between day 7 and 8 post infection. The > symbol reflects the fact that animals are alive at the end of the experiment (60 days) and so an absolute value for the survival time cannot be given.

acute mouse model for *T. b. r.* STIB900 were carried out as previously reported [15,22–25]. Assays against *L. donovani* axenic amastigotes were performed as outlined earlier [26,27].

5.2. Tm measurements

Thermal melting experiments were conducted with a Cary 300 spectrophotometer. For these measurements cuvettes are mounted in a thermal block and the solution temperatures are monitored by a thermistor in a reference cuvette. Temperatures are maintained under computer control and are increased at 0.5 °C/min. The experiments were MES 10 buffer (MES 10 mM, EDTA 1 mM, NaCl 100 mM) are conducted in 1 cm path length quartz cuvettes. The concentrations of DNA were determined by measuring the absorbance at 260 nm. A ratio of 0.3 compound per base was used for the complex and DNA with no compound was used as a control.

5.3. Chemistry

Melting points were determined in open capillary tubes with a Mel-Temp 3.0 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity 300 and Varian VRX 400 instruments and chemical shifts are reported in parts per million relative to TMS. Mass spectra (MS) were performed by the Georgia Tech Mass Spectra Laboratory at the Georgia Institute of Technology in Atlanta, GA. Elemental analyses were performed by Atlantic Microlab in Norcross, GA. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or ethanol of solvation; in all such cases proton NMR confirmed the presence of solvent (s). All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific.

5.3.1. 2,5-Bis(4-nitrophenyl)thiophene (**1**)

To a solution of 4-bromonitrobenzene (1.39 g, 6.90 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.15 g) in anhydrous 1,4-dioxane (20 mL) was added 1.00 g (3.40 mmol) of 2,5-bis[trimethylstannyl]thiophene [28] and the mixture was heated at reflux overnight under nitrogen. The resulting orange suspension was diluted with hexanes, cooled to rt, and filtered. The obtained solid was crystallized from acetone to yield 1.09 g (99%, mp 261.9–263.1 °C, lit. [29] mp 257–258 °C) of 2,5-bis(4-nitrophenyl)thiophene as a yellow crystalline solid. ^{1}H NMR (300 MHz, DMSO-d_6) 8.30 (d, 4H, $J = 8.1$ Hz), 8.02 (d, 4H, $J = 8.1$ Hz), 7.94 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) 146.5, 142.5, 139.1, 128.5, 126.1, 124.4.

5.3.2. 2,5-Bis(4-aminophenyl)thiophene (**2**)

A mixture of 0.85 g (2.60 mmol) of **1**, 5.86 g (10.0 mmol) of stannous chloride dihydrate and 40 mL of ethanol was heated at 70 °C under nitrogen for 5 h. The mixture was allowed to cool, treated with an aqueous sodium hydroxide solution until a pH of 8–9 was reached and then extracted with ethyl acetate. The organic extract was washed with water, brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the solid obtained was washed with hexanes and crystallized from acetone to yield 0.63 g (91%, mp 169.2–170.8 °C, lit. [26] mp 168–169 °C) of 2,5-bis(4-aminophenyl)thiophene. ^{1}H NMR (300 MHz, DMSO-d_6) 7.29 (d, 4H, $J = 8.4$ Hz), 7.10 (s, 2H), 6.58 (d, 4H, $J = 8.4$ Hz), 5.39 (br s, 4H); ^{13}C NMR (300 MHz, DMSO-d_6) 148.4, 141.7. 126.3, 122.3, 121.8, 114.6.

5.3.3. 2,5-Bis(4-guanidinophenyl)thiophene (**3a**)

2,5-Bis(4-guanidinophenyl)thiophene was prepared according to the general two-step literature procedure used for the synthesis of the analogous furan [17]. Bis-Boc derivative: yellow solid. Yield: 70%; ^{1}H NMR ($CDCl_3$) 10.41 (br s, 2H), 7.67 (d, 4H), 7.59 (d, 4H), 7.28 (s, 2H), 1.54–1.57 (2s, 36H). Dihydrochloride: yellow/pale green hygroscopic solid. ^{1}H NMR (DMSO-d_6) 10.14 (br s, 2H), 7.74 (d, 4H), 7.59 (br s, 8H), 7.56 (s, 2H), 7.28 (d, 4H). MS (FAB, thioglycerol): *m/z* 351 (MH^+). Anal. Calcd. for $C_{18}H_{18}N_6S \cdot 2.0HCl \cdot 0.5H_2O \cdot 0.2EtOH$: C, 50.04; H, 4.84; N, 19.03. Found: C, 50.28; H, 4.94; N, 18.9.

5.3.4. 2,5-Bis[4-(*N*-ethoxycarbonylthiourea)-phenyl]thiophene (**4**)

A solution of ethyl isothiocyanatoformate (0.17 g, 1.30 mmol) in 20 mL of dichloromethane was cooled to 0 °C (ice bath) before adding **2** (0.17 g, 0.65 mmol). The ice bath was removed and the solution was stirred at rt overnight under nitrogen. The mixture was poured into 50 mL of pentane and the precipitate was collected by filtration and dried under reduced pressure to yield 0.26 g (75%, mp above 350 °C) of 2,5-bis[4-(*N*-ethoxycarbonylthiourea)phenyl]thiophene. ^{1}H NMR (300 MHz, DMSO-d_6) 11.61 (s, 2H), 11.30 (s, 2H), 7.69 (m, 8H), 7.54 (s, 2H), 4.21 (q, 4H, $J = 6.9$ Hz), 1.25 (t, 6H, $J = 6.9$ Hz). Anal. Calcd. for $C_{24}H_{24}N_4O_4S_3$: C, 54.52; H, 4.58; N, 10.60. Found: C, 54.80; H, 4.60; N, 10.55.

5.3.5. 2,5-Bis[4-(*N*′-ethoxycarbonyl-*N*″-methylguanidino)-phenyl]thiophene (**5b**)

To a 2.0 M dioxane solution of *N*-methylamine (1.89 mL, 3.78 mmol) in 20 mL of anhydrous CH_2Cl_2, 0.73 g (5.65 mmol) of DIPEA and 0.05 g (0.95 mmol) of **4** were added. The suspension was cooled to 0 °C using an ice bath and 0.73 g (3.78 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride was added and the mixture was stirred for 1 h at 0 °C. The mixture was allowed to warm to rt and stirred overnight. The solvent was removed under reduced pressure to yield a yellow solid that was washed several times with water. The solid was suspended in EtOH and heated at reflux for 1 h, allowed to cool to rt, filtered and dried under reduced pressure to yield 0.34 g (69%, mp 181.2–183.4 °C) of 2,5-bis[4-(*N*′-ethoxycarbonyl-*N*″-methylguanidino)phenyl]thiophene. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.64 (d, 4H, $J = 8.6$ Hz), 7.48 (s, 2H), 7.42 (br d, 4H, $J = 8.6$ Hz), 3.95 (q, 4H, $J = 7.2$ Hz), 2.85 (s, 6H), 1.15 (t, 6H, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 163.6, 159.0, 142.3, 130.0, 125.8, 124.9, 124.6, 60.2, 28.4,

14.9. Anal. Calcd. for $C_{26}H_{30}N_6O_4S$: C, 59.75; H, 5.79; N, 16.08. Found: C, 59.72; H, 5.77; N, 16.09.

5.3.6. 2,5-Bis[4-(N-methylguanidino)phenyl]-thiophene dihydrochloride (3b)

The substituted carbethoxy guanidine **5b** (0.11 g, 0.21 mmol) was suspended in 1 mL of EtOH. To this stirred suspension, 1.7 mL (1.7 mmol) of 1 M aqueous potassium hydroxide solution was added. The temperature was maintained at approximately 60 °C for 20 h. The solvent was evaporated, the resultant solid was washed with water several times and then suspended in EtOH. This EtOH suspension was heated at reflux, cooled in an ice bath and collected by filtration to yield 0.07 g (89%, mp 138.5–141.0 °C) of 2,5-bis[4-(*N*-methylguanidino)phenyl]thiophene. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.50 (d, 4H, $J = 7.8$ Hz), 7.32 (s, 2H), 6.86 (br d, 4H, $J = 7.8$ Hz), 2.69 (s, 6H). The above free base (0.31 g, 0.82 mmol) was suspended in 10 mL of ethanol and saturated with HCl gas and then stirred at rt for 1 h. The solvent was removed under reduced pressure and the solid obtained was collected by filtration, washed with ether and dried in a vacuum oven to yield 0.18 g (44%, mp 187.6–189.3 °C) of 2,5-bis[4-(*N*-methylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.73 (d, 4H, $J = 8.0$ Hz), 7.55 (s, 2H), 7.28 (d, 4H, $J = 8.0$ Hz), 2.84 (s, 6H); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 155.4, 141.9, 135.2, 131.1, 126.3, 125.1, 124.7, 28.4. Anal. Calcd. for $C_{20}H_{22}N_6S \cdot 2HCl \cdot H_2O \cdot 0.43C_2H_4OH$: C, 51.21; H, 5.89; N, 17.18; O, 4.68; S, 6.55; Cl, 14.49. Found: C, 50.86; H, 5.54; N, 16.82; S, 6.93; Cl, 14.74.

5.3.7. 2,5-Bis[4-(N′-ethoxycarbonyl-N″-isopropylguanidino)-phenyl]thiophene (5c)

The *N*-carbethoxy thiourea **4** was allowed to react with isopropyl amine as described above for **5b** and yielded after crystallization from EtOH 0.37 g (67%, mp 156.8–159.2 °C) of 2,5-bis[4-(*N*′-ethoxycarbonyl-*N*″-isopropylguanidino)phenyl] thiophene as a yellow powder solid. ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) 7.64 (d, 4H, $J = 7.8$ Hz), 7.48 (s, 2H), 7.42 (br d, 4H, $J = 7.8$ Hz), 4.10 (m, 2H), 3.94 (q, 4H, $J = 7.2$ Hz), 1.16 (d, 12H, $J = 6.4$ Hz), 1.13 (t, 6H, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 163.4, 157.1, 129.4, 125.3, 124.4, 124.0, 59.6, 42.4, 22 6, 14.5. Anal. Calcd. for $C_{30}H_{38}N_6O_4S$: C, 62.26; H, 6.62; N, 14.52; O, 11.06; S, 5.54. Found C, 62.18; H, 6.48; N, 14.50.

5.3.8. 2,5-Bis[4-(N-isopropylguanidino)phenyl]-thiophene dihydrochloride (3c)

The carbethoxy guanidine **5c** was treated with KOH as described above for **5b** to yield 0.11 g (100%, mp 215.9–217.3 °C) of 2,5-bis[4-(*N*-isopropylguanidino)phenyl]-thiophene. ^{1}H NMR (400 MHz, DMSO-d_6) 7.44 (d, 4H, $J = 8.4$ Hz), 7.25 (s, 2H), 6.78 (d, 4H, $J = 8.4$ Hz), 5.34 (br s, 2H), 4.94 (br s, 4H), 3.84 (m, 2H, $J = 6.4$ Hz), 1.09 (d, 12H, $J = 6.4$ Hz).

The free base was converted into the salt as described above for **3b** to yield 0.09 g (82%, mp 261.9–263.8 °C) of 2,5-bis [4-(*N*-isopropylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) 7.73 (d, 4H, $J = 8.6$ Hz), 7.55 (s, 2H), 7.26 (d, 4H, $J = 8.6$ Hz), 3.88 (m, 2H, $J = 6.0$ Hz), 1.18 (d, 12H, $J = 6.0$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 153.6, 142.1, 135.6, 131.2, 126.6, 125.4, 124.7, 43.9, 22.4. Anal. Calcd. for $C_{24}H_{30}N_6S \cdot 2HCl \cdot 1.0H_2O$: C, 54.85; H, 6.52; N, 16.00; O, 3.04; S, 6.10; Cl, 13.49. Found: C, 55.15; H, 6.31; N, 15.90; Cl, 13.46.

5.3.9. 2,5-Bis[4-(N-cyclopentylguanidino)phenyl]-thiophene dihydrochloride (3d)

The carbethoxy guanidine **5d** obtained was an oil and was treated directly with KOH as described above for **5b** to yield 0.20 g (93%, mp 212.9 –214.9 °C) of 2,5-bis[4-(*N*-cyclopentylguanidino)phenyl]thiophene. ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) ~7.44 (d, 4H, $J = 7.60$ Hz), 7.25 (s, 2H), 6.79 (br d, 4H, $J = 7.60$), 4.01 (m, 2H), 1.85 (br m, 4H), 1.05 (br m, 12H); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 152.5, 150.7, 142.6, 126.7, 126.4, 124.4, 123.5, 52.4, 33.4, 23.9. The free base was converted into the salt as described above for **3b** to yield 0.17 g (73%, mp 231.8–234.7 °C) of 2,5-bis[4-(*N*-cyclopentylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.73 (d, 4H, $J = 7.8$ Hz), 7.55 (s, 2H), 7.27 (d, 4H, $J = 7.8$ Hz), 4.03 (br m, 2H), 1.95 (br m, 4H), 1.61 (br m, 12H); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 154.4, 142.6, 135.7, 131.9, 127.1, 125.9, 53.6, 32.7, 23.9. Anal. Calcd. for $C_{28}H_{34}N_6S \cdot 2HCl \cdot 1.05H_2O$: C, 58.13; H, 6.64; N, 14.53; O, 2.90; S, 5.54; Cl, 12.26. Found: C, 58.49; H, 6.69; N, 14.13; Cl, 11.73.

5.3.10. 2,5-Bis[4-(N′-ethoxycarbonyl-N″-cyclohexylguanidino)-phenyl]thiophene (5e)

The *N*-ethoxycarbonylthiourea **4** was allowed to react with cyclohexylamine as described above for **5b** and yielded an oily residue which was passed through a short column of silica gel using a mixture of ethyl acetate/hexane (1:1) as the mobile phase. After the removal of the solvent, 0.22 g (63%, mp 186.6–188.9 °C) of 2,5-bis[4-(*N*′-ethoxycarbonyl-*N*″-cyclohexylguanidino)phenyl]thiophene was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) 7.63 (d, 4H, $J = 7.6$), 7.47 (s, 2H), 7.42 (br d, 4H), 3.92 (q, 4H, $J = 6.53$), 3.78 (br d, 2H), 1.88 (br m, 4H), 1.67 (br d, 4H), 1.55 (br d, 2H), 1.28 (m, 10H), 1.13 (t, 6H). Anal. Calcd. for $C_{36}H_{46}N_6O_4S$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.37; H, 7.10; N, 12.73.

5.3.11. 2,5-Bis[4-(N-cyclohexylguanidino)phenyl]-thiophene dihydrochloride (3e)

The carbethoxy guanidine **5e** was treated with KOH as described above for **5b** to yield 0.15 g (77%, mp 252.4–254.8 °C) of 2,5-bis[4-(*N*-cyclohexylguanidino)phenyl] thiophene. ^{1}H NMR (400 MHz, DMSO-d_6) 7.45 (d, 4H, $J = 8.2$ Hz), 7.27 (s, 2H), 6.78 (d, 4H, $J = 8.2$ Hz), 5.40 (br s, 2H), 4.95 (br s, 4H), 3.56 (br s, 2H), 1.91 (br d, 4H), 1.63 (m, 6H), 1.21 (m, 8H); ^{13}C NMR (300 MHz, DMSO-d_6) 151.1, 150.5, 142.1, 126.3, 125.8, 123.6, 122.8, 48.51, 33.0, 25.51, 34.5.

The free base was converted into the salt as described above for **3b** to yield 0.11 g (66%, mp 209.5–210.8 °C) of 2,5-bis[4-(*N*-cyclohexylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.76 (d, 4H, $J = 8.4$ Hz), 7.55 (s, 2H), 7.29 (d, 4H, $J = 8.4$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6) 154.0, 142.6, 135.6, 127.1, 126.0, 51.1, 32.6, 31.3, 25.3, 24.8. Anal. Calcd. for $C_{30}H_{38}N_6S \cdot 2HCl \cdot 1.75H_2O$: C, 58.19; H, 7.08; N, 13.57. Found: C, 58.38; H, 6.72; N, 13.26.

*5.3.12. 2,5-Bis[4-(N-phenylguanidino)phenyl]-thiophene dihydrochloride (**3f**)*

The carbethoxy guanidine **5f** obtained using aniline was an oil and was treated directly with KOH as described above for **5b** and the solid free base obtained was washed with water, air dried and converted to the salt without further characterization to yield 0.28 g (79%, mp 225.4–227.3 °C, dec) of 2,5-bis[4-(*N*-phenylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.72 (s, 2H), 7.2–7.65 (br m, 18H); ^{13}C NMR (300 MHz, DMSO-d_6/D_2O) 154.3, 142.6, 135.8, 135.5, 132.1, 130.5, 127.4, 127.0, 126.0, 125.4, 124.8. Anal. Calcd. for $C_{30}H_{26}N_6S \cdot 2.1HCl \cdot 2.0H_2O \cdot 0.4C_2H_5OH$: C, 58.38; H, 5.49; N, 13.26; Cl, 11.75. Found: C, 58.40; H, 5.12; N, 12.94; Cl, 12.01.

*5.3.13. 2,5-Bis[4-(N-p-tolylguanidino)phenyl]-thiophene dihydrochloride (**3g**)*

The carbethoxy guanidine **5g** obtained was an oil and was treated directly with KOH as described above for **5b** and the solid free base obtained was washed with water, air dried and converted to the salt without further characterization to yield 0.21 g (100%, mp 237.5–238.6 °C, dec) of 2,5-bis[4-(*N*-*p*-tolylguanidino)phenyl]thiophene dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) 7.71 (d, 4H, $J = 8.4$ Hz), 7.50 (s, 2H), 7.33 (d, 4H, $J = 8.4$ Hz), 7.22 (br m, 8H), 2.33 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) 154.0, 141.8, 135.7, 135.1, 132.7, 131.1, 129.9, 126.1, 125.0, 124.4, 124.2, 20.4. Anal. Calcd. for $C_{32}H_{30}N_6S \cdot 2.0HCl \cdot 1.6\ H_2O$: C, 60.79; H, 5.61; N, 13.29. Found: C, 61.18; H, 5.39; N, 12.89.

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