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Synthesis and Antiprotozoal Activity of 2,5-Bis[amidinoaryl]thiazoles

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Abstract

Seven novel diamidino 2,5-bis(aryl)thiazoles (**5a–5g**) were synthesized and evaluated against *Trypanosoma brucei rhodensiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*). The diamidines were obtained directly from the corresponding bis-nitriles (**4a–g**) by the action of lithium bis(trimethylsilyl)amide. The bis-nitriles **4a–4f** were synthesized in four steps starting with the Stille coupling of 2-tributyltinthiazole with the appropriate cyanoaryl halide. The bis-nitrile **5g** was obtained by the palladium facilitated coupling of the mixed tinsilyl reagent 2-trimethylsilyl-5-trimethyltinthiazole with 2-bromo-5-cyanopyridine. The amidoxime potential prodrugs **6a–e**, **6g** were obtained by the reaction of hydroxylamine with the bis-nitriles. *O*-Methylation of the amidoximes gave the corresponding *N*-methoxyamidines **7a–c**, **7e**, **7g**. The diamidines showed strong DNA binding affinity as reflected by ΔT_m measurements. Four of the diamidines **5a**, **5b**, **5d** and **5e** were highly active in vitro against *P. f.* giving IC_{50} values between 1.1 and 2.5 nM. The same four diamidines showed IC_{50} values between 4 and 6 nM against *T. b. r.* The selectivity indices ranged from 233 to 9175. One diamidine **5a** produced one of four cures at an ip dose of 4×5 mg/kg in the STIB900 mouse model for acute African trypanosomiasis. The amidoxime and *N*-methoxyamidine of **5a** were the only prodrugs to provide cures (1/4 cures) in the same mouse model on oral dosage at 4×25 mg/kg.

1. Introduction

It has been estimated that approximately 2.2 billion people are at risk for the related protozoan diseases malaria and trypanosomiasis.¹ Available drugs for treatment of both of these diseases exhibit significant shortcomings. The growth of multi-drug resistance strains of *Plasmodium falciparum*, the species which causes the most severe cases of malaria, has created an urgent need for new antimalarials with different modes of action.^{2,3} Presently, only three such compounds are noted as undergoing advanced evaluation.³ Currently four compounds are registered for use against human African trypanosomiasis (HAT); two are used for 1st stage disease [suramin and pentamidine(**I**)] and two are used against 2nd stage disease [melarsoprol

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and eflornithine].^{4,5} All of these drugs are administered by injection and have undesirable side effects. Thus, the need for new drugs to treat diseases caused by these two parasites is evident.

Aromatic diamidines, pentamidine(**I**) being the preeminent member of the class, have been studied extensively as antiprotozoal agents for nearly eight decades.⁶ Despite broad spectrum antimicrobial activity of this class, pentamidine remains the only member of this class of compounds to see significant human use. Pafuramidine (**IIb**), a prodrug of furamidine (**IIa**) (Figure 1), was subjected to extensive clinical evaluation advancing to Phase III trials against both HAT and AIDS-related *Pneumocystis jiroveci* pneumonia and to Phase II trials against malaria.^{7, 8} Unfortunately, in an additional safety study which paralleled the phase III studies some volunteers exhibited liver and renal toxicities and trials with pafuramidine were discontinued.⁷ Thus it is important to continue to search for effective and safe members of this highly active class of compounds. We have shown that replacement of the phenyl rings of furamidine with pyridyls leads to compounds which are active in a model for CNS stage HAT.⁹ Both the azafuramidine **IIc** and its prodrug **IId** were effective in mouse models for both stages of the disease.^{9,10} A number of studies have focused upon replacement of the central furan ring of **IIa** with other 5-ring heterocyclic systems including pyrrole^{11,12}, thiophene(**IIe**)^{11,12}, imidazole¹¹, oxazole^{11,13}, thiazole¹³, oxadiazole¹³, as well as several with several 6-ring heterocyclic units.^{14,15} Despite these extensive studies it appears that use of a thiazole central linker has not been investigated. This report describes the synthesis and evaluation of diamidino 2,5-diarylthiazoles including several analogues in which the aryl units are pyridyls. Such compounds are expected to have different absorption and distribution properties than **IIa** and **IIe** and thus may exhibit different toxicity profiles. Given the locations where these diseases occur it is highly desirable to develop orally effective antiprotozoal drugs.^{4,16} Due to the dicationic nature of diamidines they typically exhibit quite poor oral bioavailability. This problem has been overcome in several cases by use of amidoxime prodrugs.^{17,18} Consequently, we also report the synthesis and evaluation of several amidoxime prodrugs in the thiazole series.

2. Results and discussion

2.1. Chemistry

The key step(s) in the synthesis of the various diarylthiazoles employs palladium catalyzed coupling reaction which we have previously extensively used.^{10,19–21} The 2,5-bis-(amidinophenyl)thiazoles **5a–c** were synthesized from 2,5-bis(4-cyanophenyl)thiazoles **4a–c** which were obtained in four steps (Scheme 1). The first step involves a Stille coupling reaction between 2-tributylstanyl thiazole and 4-bromocyanophenyl (**1a**), 5-bromo-2-cyanopyridine (**1b**) and 6-chloronicotinonitrile (**1c**), respectively, to form the mono-aryl thiazoles **2a–c**. Bromination of **2a–c** with *N*-bromosuccinimide in DMF solution, furnished **3a–c** in good yield in the second step. Subsequent Suzuki coupling of **3a–c** with 4-cyanophenyl boronic acid gave **4a–c** in good yield (Scheme 1). In a similar way, the bis-nitriles **4d–4f** were prepared from **2a–c** however in this case 5-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-2-cyanopyridine was used to couple with the bromothiazoles **3a–3c**. The target bis-amidines **5a–f** were obtained from the bis-nitriles **4a–4f** by the action of lithium bis(trimethylsilyl)amide in THF.²²

As a part of this study, we also prepared a bis-amidine with both pyridyl nitrogen atoms adjacent to thiazole core (Scheme 2). The synthesis of the bis-amidine **5g** required the corresponding bis-nitrile **4g** (Scheme 2). The preparation of **4g** was achieved by using the mixed tin and silyl reagent 2-trimethylsilyl-5-trimethylthiazole²³ in a palladium catalyzed coupling reaction with 2-bromo-5-cyanopyridine to give **4g** in a modest yield. The bis-amidine **5g** was obtained in good yield employing lithium bis(trimethylsilyl)amide as previously described.

The potential prodrugs of the bis-amidines were prepared as outlined in Scheme 3. The bis-amidoximes **6a–e,g** were readily obtained by the reaction of the corresponding bis-nitriles **4**

with hydroxylamine. Methylation of the diamidoximes **6** with dimethylsulfate in the presence of aqueous lithium hydroxide gave the *N*-methoxyamidines **7a–c,e,g** in reasonable yield.

2.2 Biology

Table 1 summarizes the DNA binding affinities for the thiazole diamidines and the in vitro evaluation of the dications against *Plasmodium falciparum* (*P. f.*) and *Trypanosoma brucei rhodensense* (*T. b. r.*). For comparative purposes similar data for furamide (**IIa**), azafuramide (**IIc**) and the thiophene analogue (**IIe**) are also provided in Table 1. The former two compounds are included since promising antiprotzoan data has been reported for them and the latter is included due to the close structural analogy with the thiazoles. We have found that the determination of melting temperature increases ΔT_m (T_m of complex – T_m of free DNA) provides a rapid and accurate method for ranking the binding affinities for a variety of aryl diamidines.^{6a,24,25} Typical results are illustrated in Figure 2. The ΔT_m values of the thiazole diamidines for their complexes with poly (dA–dT) range from 10 to 22.9°C. The ΔT_m value for the parent thiazole diamidine **5a** is 21.1°C. This value is approximately 20% less than that for both **IIa** and **IIe**. The differences in ΔT_m values for the furamide and **IIe** and **5a** reflects the introduction of the nitrogen atom into the five membered ring heterocycle and is consistent with the “*N*-effect” we have noted for a number of cases of replacement of phenyl rings with pyridyl rings in other triaryl diamidine systems.²⁶ On binding to DNA, transfer of the aza-analogues from aqueous solution into the less polar environment of the minor groove is less favorable than that for furamide and **IIe**.²⁶ The thiazole analogues which contain pyridyl rings replacing one or both of the phenyl rings of **5a** generally show a decline in ΔT_m values. However, there is not an obvious systematic change in ΔT_m with structure which suggests that small effects from subtle differences in van der Waals, H-bonding interactions and molecular geometry are likely contributors to the variation in values. Consistent with other series of aryl diamidines²⁶ generally the compounds with higher DNA affinities exhibit greater antiprotzoan activity.

In order to determine if these thiazole analogues are binding in the DNA minor groove, we acquired CD spectra for **5a** along with **IIa** and **IIe**, for comparison, on binding to DNA (see Figure 3). Minor groove binding compounds give a large positive induced CD signal on binding to AT DNA sequences and cause only small changes in the pattern of the DNA CD spectrum.²⁷ Such a pattern is observed on adding **IIa** and **IIe** to polydA.polydT (Figure 3) with a strong, positive induced signal between 350 to 430 nm smaller changes in the DNA spectral region below 300 nm. As can be seen in Figure 2, **5a** exhibits a similar CD spectra with that of **IIa** and **IIe**. These results clearly support a minor groove binding mode for the thiazole **5a**.²⁷ A detailed analysis of the effect of structure induced variation by thiazoles analogues awaits the results of the biophysical studies which are in progress.

The four thiazoles **5a, 5b, 5d, 5e** were highly active against *P. f.* exhibiting IC_{50} values between 1.1 and 2.5 nM which are about nearly a factor of ten more active than **IIa** and **IIe**. These low nanomolar values meet our group’s criteria of IC_{50} values of less than 100 nM to enter animal studies and the properties of these compounds are in general agreement with criteria suggested by others.¹⁶ The thiazoles **5a–5f** are highly active in vitro versus *T. b. r.* giving IC_{50} values ranging between 4 and 38 nM. Four (**5a, 5b, 5d, 5e**) of the seven new diamidines gave IC_{50} values (4–6 nM) comparable to furamide (3.2 nM) and **IIe** (3 nM) and pentamidine (2.8 nM). The selectivity indices for the four most active compounds against *T. b. r.* are reasonable ranging from 233 to 9175, however they are not as large as that of pentamidine (16,643). Based upon activity and selectivity the compounds were advanced to the rigorous *T. b. r.* STIB900 mouse model of infection.

Data for the parent diamidines **5a–5f** as well as data for the prodrugs **6a–6e, 6g** and **7a–c, 7e, 7g** obtained from the STIB900 mouse model for the acute phase of African trypanosomiasis

are presented in Table 2. The parent diamidines were administered by intraperitoneal injection at the screening dose of 4×5 mg/kg whereas the potential prodrugs were given orally at the screening dose of 4×25 mg/kg. At this low dose level only the parent diamidine **5a** cured 1 of four infected test animals. This result is comparable to that for **IIa** and inferior to that for **IIc** and **IIf** which gave 3/4 and 2/4 cures, respectively, at this dose level. Only two thiazole prodrugs **6e** and **7e**, prodrugs of **5e**, cured; 1/4 infected mice. The results for **6e** and **7e** are inferior to that for pafuramidine and significantly less effective than those for **IIId** which gave 4/4 cures at both 25mg/kg and the quite low dose of 5 mg/kg. This relatively modest in vivo activity may be due to poor PK. Currently, we are using **IIId** as the lead compound that new compounds must match or surpass in efficacy to be taken into preclinical evaluations for 2nd stage HAT, none of the thiazoles meet this standard and consequently they will not be pursued further.

3. Experimental

3.1 Biology

3.1.1 Efficacy evaluations—In vitro assays²⁸ with *T. b. r.* STIB 900 and *P. f.* K1 strain as well as the efficacy study in an acute mouse model for *T. b. r.* STIB 900²⁹ were carried out as previously reported.

3.1.2 Tm 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 10mM, EDTA 1mM, NaCl 100mM with NaOH added to give pH = 7.0). The concentrations of DNA were determined by measuring the absorbance at 260nm. A ratio of 0.3 moles compound per mole of DNA was used for the complex and DNA with no compound was used as a control.³⁰ ΔT_m values are determined by the peak in first derivative curves (dA/dT).

3.1.3 Circular Dichroism (CD)—CD spectra were collected with a Jasco J-810 spectrometer at different ratios of compound to DNA at 25 °C in MES 10 buffer. A DNA solution in a 1-cm quartz cuvette was first scanned over a desired wavelength range. Compounds **IIa**, **IIc** and **5a** at increasing ratios were then titrated into the same cuvette and the complexes rescanned under same conditions.³¹

3.2 Chemistry Experimental Section

Melting points were recorded using a Mel-Temp capillary melting apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F254 precoated aluminum sheets and detected under UV light. ¹H and ¹³C NMR spectra were recorded employing a Bruker 400 Ultrashield™ or Varian Unity Plus™ 300 spectrometer and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on an Applied Biosystems MALDI-TOF-TOF MS (4800) 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 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., VWR, Fischer Scientific, or Frontier and were used as received. The synthesis of 2-trimethylsilyl-5-trimethyltin-thiazole has been previously reported.²³

5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)-2-cyanopyridine—To a round-bottom flask were added 2.35 g (12.8 mmol) 5-bromo-2-cyanopyridine in 45 mL DMSO, 3.76 g (38.4

mmol) potassium acetate, 3.5 g (15.4 mmol) bis(neopentylglycolato)diboron and 0.282 g (3 mol%) PdCl₂(dppf). The mixture was heated at 80°C for 2 days. The reaction solution was cooled to room temperature and poured into ice-water. The mixture was extracted with ethyl acetate, washed with saturated brine, dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by crystallization from a mixture of EtOAc/hexane to give 0.98 g (36%), white solid, mp 126°C. ¹H NMR (300 MHz, CDCl₃) δ: 9.05 (s, 1H), 8.20 (d, 1H, J = 7.5 Hz), 7.68 (d, 1H, J = 7.5 Hz), 3.83 (s, 4H), 1.07 (s, 6H). ¹³C NMR (75 MHz, CDCl₃) δ: 22.1, 31.2, 32.3, 72.7, 117.7, 127.8, 135.4, 142.6, 156.1. Anal. Calcd for C₁₁H₁₃N₂BO₂ C, 61.15; H, 6.07; N, 12.97. Found: C, 61.39; H, 6.07, N, 12.93.

General procedure for Stille coupling reaction—To a round-bottom flask were added 5.49 g (30 mmol) 4-bromo-2-cyanopyridine in 70 mL 1,4-dioxane, 0.69 g (2 mol%) Pd(PPh₃)₄ and 11.22 g (30 mmol) 2-tributylstannanylthiazole. The mixture was allowed to reflux overnight. The solvent was removed under reduced pressure. Hexane was added to the residue. The precipitate was filtered. The solid was dissolved in EtOAc and washed with 5% NaF. The organic layer was dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography using hexane/EtOAc (20:1).

2-(Benzonitrile-4-yl)thiazole (2a)—The physical properties observed are consistent with those previously reported.³²

2-(2-Cyanopyridin-5-yl)thiazole (2b)—Yield (82%), white solid, mp 167°C. ¹H NMR (400 MHz, CDCl₃) δ: 7.51(d, 1H, J = 3.2 Hz), 7.8(dd, 1H, J₁ = 0.8, J₂ = 8 Hz), 8.01(d, 1H, J = 3.2 Hz), 8.40(dd, 1H, J₁ = 2.4, J₂ = 5.6 Hz), 9.29(d, 1H, J = 2.4 Hz). ¹³C NMR (100 MHz, CDCl₃) δ: 117.3, 121.8, 128.9, 132.5, 134.2, 134.4, 145.2, 149.0, 162.8. Anal. Calcd for C₉H₅N₃S: C, 57.74; H, 2.69; N, 22.44. Found: C, 57.64; H, 2.73; N, 22.31.

2-(3-Cyanopyridin-6-yl)thiazole (2c)—Yield (74%), white solid, mp 167°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.60(d, 1H, J = 3 Hz), 8.03(d, 1H, J = 3 Hz), 8.09(dd, 1H, J₁ = 2.1, J₂ = 8.4 Hz), 8.35(d, 1H, J = 8.1 Hz), 8.89(d, 1H, J = 2.1 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 110.2, 116.8, 119.7, 123.8, 140.6, 145.2, 152.5, 154.3, 167.2. Anal. Calcd for C₉H₅N₃S: C, 57.74; H, 2.69; N, 22.44. Found: C, 57.92; H, 2.70; N, 22.55.

General procedure for bromination of (2a–c)—To a round-bottom flask were added **2a** (3.55 g, 19 mmol, 1 eq) in anhydrous DMF (45 mL) and (4.1 g, 23 mmol, 1.2 eq) NBS. The mixture was stirred overnight at room temperature and was poured into ice-water. The precipitate was filtered and washed with water. The crude product was crystallized from a mixture of EtOAc: hexane.

5-Bromo-2-(4-cyanophenyl)thiazole (3a)—Yield (89%), white solid, mp 149°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.77(d, 2H, J = 8.1 Hz), 7.85 (s, 1H), 8.00 (d, 2H, J = 8.1 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 110.7, 113.6, 118.2, 126.6, 132.9, 136.7, 145.6, 166.8. Anal. Calcd for C₁₀H₅BrN₂S·0.1H₂O: C, 44.99; H, 1.96; N, 10.49. Found: C, 44.89; H, 1.79; N, 10.26.

5-Bromo-2-(2-cyanopyridin-5-yl)thiazole (3b)—Yield (78%), white solid, mp 159–160°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.77(d, 1H, J = 8.1 Hz), 7.85 (s, 1H), 8.00 (d, 2H, J = 8.1 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 112.1, 117.1, 128.9, 131.9, 134.1, 134.6, 146.4, 148.6, 163.8. Anal. Calcd for C₉H₄BrN₃S·0.1H₂O: C, 40.35; H, 1.58; N, 15.68. Found: C, 40.49; H, 1.52; N, 15.53.

5-Bromo-2-(3-cyanopyridin-6-yl)thiazole (3c)—Yield (78%), white solid, mp 208–209°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.90 (s, 1H), 8.09 (dd, 1H, J₁ = 2.1 Hz, J₂ = 8.4 Hz), 8.26 (d, 1H, J = 8.1 Hz), 8.86 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ: 110.4, 114.8, 116.7, 119.1, 140.7, 146.4, 152.6, 153.6, 168.3. Anal. Calcd for C₉H₄BrN₃S: C, 40.62; H, 1.52; N, 15.79. Found: C, 40.67; H, 1.61; N, 15.63.

General procedure for Suzuki coupling of 3a–c—A mixture of **3a** (2.65 g, 10 mmol), 4-cyanophenyl boronic acid or 5-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-2-cyanopyridine (1.65 g, 12 mmol), Pd(PPh₃)₄ (0.23 g, 2 mol%) and K₂CO₃ (4.14 g, 30 mmol) in 50 mL anhydrous 1,4-dioxane was allowed to reflux overnight. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO₄, and concentrated. The crude product **4a** was purified by crystallization from a mixture of EtOAc:hexane for **4a** and from DMF for **4b–g**.

2,5-Bis(4-cyanophenyl)thiazole (4a)—Yield (77%), yellow solid, mp 276–277°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.76 (s, 4H), 7.80 (d, 2H, J = 8.4 Hz), 8.11 (d, 2H, J = 8.4 Hz), 8.22 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ: 118.6, 127.2, 127.4, 133.2, 133.3, 137.2, 139.2, 141.8. Anal. Calcd for C₁₇H₉N₃S·0.1H₂O: C, 70.62; H, 3.21; N, 14.53. Found: C, 70.63; H, 3.03; N, 14.25.

5-(4-Cyanophenyl)-2-(2-cyanopyridin-5-yl)thiazole (4b)—Yield (87%), yellow solid, mp 308°C. ¹H NMR (300 MHz, DMSO) δ: 8.0 (s, 4H), 8.22 (d, 1H, J = 8.1 Hz), 8.60 (d, 1H, J = 8.1 Hz), 8.74 (s, 1H), 9.36 (s, 1H). Anal. Calcd for C₁₆H₈N₄S·0.3H₂O: C, 65.42; H, 2.95; N, 19.07. Found: C, 65.57; H, 2.73; N, 19.09.

5-(4-Cyanophenyl)-2-(3-cyanopyridin-6-yl)thiazole (4c)—Yield (87%), yellow solid, mp 357°C. ¹H NMR (300 MHz, DMSO) δ: 9.14 (s, 1H), 8.72 (d, 1H, J = 6.8 Hz), 8.33 (d, 1H, J = 9.6 Hz), 8.31 (d, 1H, J = 9.6 Hz), 8.00 (dd, 4H, J₁ = 4.4 Hz, J₂ = 2.0 Hz). Anal. Calcd for C₁₆H₈N₄S·0.3H₂O: C, 65.42; H, 2.95; N, 19.07. Found: C, 65.65; H, 2.79; N, 19.13.

2-(4-Cyanophenyl)-5-(2-cyanopyridin-5-yl)thiazole (4d)—Yield (27%), yellow solid, mp 292–293°C. ¹H NMR (400 MHz, DMSO) δ: 8.02 (d, 2H, J = 8.4 Hz), 8.16 (d, 1H, J = 8 Hz), 8.18 (d, 2H, J = 8.4 Hz), 8.43 (dd, 1H, J₁ = 2.4 Hz, J₂ = 7.6 Hz), 8.75 (s, 1H), 9.19 (d, 1H, J = 2 Hz). ¹³C NMR (100 MHz, DMSO) δ: 113.6, 118.8, 119.2, 127.4, 130.0, 130.8, 132.1, 133.9, 135.4, 135.8, 136.7, 144.4, 149.0, 165.5. Anal. Calcd for C₁₆H₈N₄S·0.2H₂O: C, 65.83; H, 2.90; N, 19.19. Found: C, 65.70; H, 2.85; N, 19.03.

2,5-Bis(2-cyanopyridin-5-yl)thiazole (4e)—Yield (63%), yellow solid, mp 333°C. ¹H NMR (400 MHz, DMSO) δ: 7.96 (d, 1H, J = 6.8 Hz), 8.16 (d, 1H, J = 8.0 Hz), 8.40 (dd, 1H, J₁ = 2.4 Hz, J₂ = 8.4 Hz), 8.57 (dd, 1H, J₁ = 2.4 Hz, J₂ = 8.4 Hz), 8.72 (s, 1H), 9.17 (d, 1H, J = 2.4 Hz), 9.33 (d, 1H, J = 2.0 Hz). ESI-MS *m/z*: (M - H) 288. Anal. Calcd for C¹⁵H⁷N⁵S·0.6H₂O: C, 60.03; H, 2.75; N, 23.33. Found: C, 60.33; H, 2.49; N, 22.98.

5-(2-Cyanopyridin-5-yl)-2-(3-cyanopyridin-6-yl)thiazole (4f)—Yield (61%), yellow solid, mp 368°C. ¹H NMR (400 MHz, DMSO) δ: 9.26 (d, 1H, J = 2.4 Hz), 9.15 (d, 1H, J = 2.0 Hz), 8.82 (s, 1H), 8.52 (dd, 1H, J₁ = 2.0 Hz, J₂ = 8.0 Hz), 8.48 (dd, 1H, J₁ = 2.4 Hz, J₂ = 8.4 Hz), 8.33 (dd, 1H, J₁ = 0.8 Hz, J₂ = 8.4 Hz), 8.18 (dd, 1H, J₁ = 0.8 Hz, J₂ = 8.0 Hz). ESI-MS *m/z*: (M - H) 288. Anal. Calcd for C₁₅H₇N₅S·0.2H₂O: C, 61.51; H, 2.55; N, 23.91. Found: C, 61.42; H, 2.43; N, 23.71.

Synthesis of 2,5-bis(3-cyanopyridin-6-yl)thiazole (4g)—To a round-bottom flask were added 2-trimethylsilyl-5-trimethylthiazole¹ (1.5 g, 4.73 mmol) in dioxane (50 mL), 2-

bromo-5-cyanopyridine (1.7 g, 9.45 mmol) and Pd(PPh₃)₄ (2 mol%). The reaction mixture was allowed to reflux overnight. The solvent was evaporated and the crude product was crystallized from DMF to give 0.38 g (28%), yellow solid. mp 366°C. ¹H NMR (400 MHz, DMSO) δ: 8.33 (s, 1H), 8.35 (s, 1H), 8.46(dd, 1H, J₁ = 1.2, J₂ = 8.4 Hz), 8.50 (dd, 1H, J₁ = 2.0, J₂ = 8.4 Hz), 8.95 (s, 1H), 9.08 (d, 1H, J = 2 Hz), 9.15(d, 1H, J = 1.6 Hz). Anal. Calcd for C₁₅H₇N₅S-0.3H₂O: C, 61.13; H, 2.60; N, 23.76. Found: C, 61.10; H, 2.54, N, 23.65.

General procedure for conversion of dinitriles 4a–g to diamidines 5a–g

2,5-Bis(4-amidinophenyl)thiazole dihydrochloride (5a): The bis-nitrile **4a** (0.28 g, 1 mmol) was suspended in anhydrous THF (8 mL) and LiN[(CH₃)₃Si]₂ solution 1.0 M THF (5 mL) was added to the flask and the mixture was stirred overnight at room temperature, saturated ethanolic HCl (10 mL) was carefully added, and the mixture was stirred overnight. The precipitate was filtered, washed with ether, and dried. The salt of **5a** was put into water, basified with aqueous NaOH 10%, and stirred vigorously for 24 h. The precipitate was then filtered, washed with water, ether, and dried to afford **5a** (free base) as a yellow solid, which was placed in a 250 mL flask, EtOH was added to the flask, and the solution was chilled in an ice-bath. After passing dry HCl gas for 15 minutes the mixture was stirred overnight. The precipitate was filtered, washed with ether to give a pale yellow solid in 84% yield. mp. >330°C. ¹H NMR (300 MHz, CDCl₃) δ: 7.97(d, 2H, J₁ = 8.4 Hz), 8.02(d, 2H, J = 8.4 Hz), 8.03(d, 2H, J = 8.4 Hz), 8.23(d, 2H, J = 8.4 Hz), 8.67(s, 1H), 9.26(brs, 2H), 9.30(brs, 2H), 9.50(brs, 2H), 9.55(brs, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 126.5, 126.8, 126.9, 127.1, 127.2, 129.1, 129.8, 135.9, 137.5, 142, 143.1, 165.5, 166. ESI-MS *m/z*: (M⁺ +H) 322. Anal. Calcd for C₁₇H₁₅N₅S-2HCl-1.0H₂O-0.2EtOH: C, 49.57; H, 4.83; N, 16.61. Found: C, 49.58; H, 4.52; N, 16.36.

5-(4-Amidinophenyl)-2-(2-amidinopyridin-5-yl)thiazole dihydrochloride (5b): Yield 73 % yellow solid, mp >320°C. ¹H NMR (300 MHz, DMSO) δ: 7.98(d, 2H, J = 8.4 Hz), 8.05(d, 2H, J = 8.4 Hz), 8.48(d, 1H, J = 8.5 Hz), 8.72(dd, 1H, J₁ = 2.2, J₂ = 8.0 Hz), 8.75(s, 1H), 9.21 (brs, 2H), 9.38(d, 1H, J = 2.4 Hz), 9.48(brs, 4H), 9.71(brs, 2H). ¹³C NMR (100 MHz, DMSO) δ: 124.4, 127.4, 128.4, 129.7, 133, 135.8, 135.9, 140.2, 143.1, 145.4, 147.2 162.3, 163.2, 165.7. ESI-MS *m/z*: (M⁺ +H) 323. Anal. Calcd for C₁₆H₁₄N₆S-2HCl-0.2EtOH-1.3H₂O: C, 46.02; H, 4.66; N, 19.63. Found: C, 45.97; H, 4.27; N, 19.31.

5-(4-Amidinophenyl)-2-(3-amidinopyridin-6-yl)thiazole dihydrochloride (5c): Yield 74 % pale yellow solid, mp >350°C. ¹H NMR (400 MHz, DMSO) δ: 7.95(d, 2H, J = 6.3 Hz), 8.08 (d, 2H, J = 8.8 Hz), 8.35(d, 1H, J = 8.4 Hz), 8.41(dd, 1H, J₁ = 2.4, J₂ = 8.4 Hz), 8.73(s, 1H), 9.06(d, 1H, J = 2.0 Hz), 9.14(brs, 2H), 9.32(brs, 2H), 9.45 (s, 2H), 9.65(s, 2H). ¹³C NMR (100 MHz, DMSO) δ: 119.1, 126.0, 127.3 128.3, 129.7, 136.0, 138.6, 141.1, 143.6, 149.9, 154, 163.9, 165.2, 167.4. HRMS: *m/z* calcd. C₁₆H₁₅N₆S: 323.1079; found 323.1082. Anal. Calcd for C₁₆H₁₄N₆S-3.5HCl-0.4H₂O-0.2EtOH: C, 42.23; H, 4.21; N, 18.02. Found: C, 42.53; H, 4.06; N, 17.63.

2-(4-Amidinophenyl)-5-(2-amidinopyridin-5-yl)thiazole dihydrochloride (5d): Yield 67% pale yellow solid, mp >350°C. ¹H NMR (400 MHz, DMSO) δ: 8.02 (d, 2H, J = 8.4 Hz), 8.24 (d, 2H, J = 8.4 Hz), 8.46(d, 1H, J = 8.4 Hz), 8.56 (d, 1H, J = 8.4 Hz), 8.81 (s, 1H), 9.23 (d, 1H, J = 2 Hz), 9.28(brs, 2H), 9.46 (brs, 2H), 9.55(brs, 2H), 9.67 (brs, 2H). ¹³C NMR (100 MHz, DMSO) δ: 124.3, 127, 129.9, 130.3, 131.5, 135.6, 135.8, 137.3, 143.2, 144.4, 147.5, 161.7, 165.4, 167.1. Anal. Calcd for C₁₆H₁₄N₆S-3HCl-0.5EtOH-1.1H₂O: C, 43.02, H, 4.71, N, 17.70. Found: C, 43.24, H, 4.33, N, 17.92.

2,5-Bis(2-amidinopyridin-5-yl)thiazole dihydrochloride (5e): Yield 71% pale yellow, mp >300°C. ¹H NMR (400 MHz, DMSO) δ: 8.51(d, 1H, J = 8.4 Hz), 8.55(d, 1H, J = 8.0 Hz), 8.59

(dd, 1H, $J_1 = 2\text{ Hz}$, $J_2 = 7.6\text{ Hz}$), 8.72(dd, 1H, $J_1 = 2.0\text{ Hz}$, $J_2 = 8.0\text{ Hz}$), 8.88(s, 1H), 9.23(d, 1H, $J = 2\text{ Hz}$), 9.39(d, 1H, $J = 2\text{ Hz}$), 9.50(bris, 2H), 9.60(bris, 2H), 9.73(bris, 2H), 9.78(bris, 2H). ^{13}C NMR (100 MHz, DMSO) δ : 124.3, 124.5, 131.2, 132.5, 135.8, 136, 136.1, 136.6, 143.5, 143.8, 144.6, 145.5, 147.3, 161.8, 164.2. Anal. Calcd for $\text{C}_{15}\text{H}_{13}\text{N}_7\text{S}\cdot 2\text{HCl}\cdot 0.5\text{EtOH}\cdot 1.2\text{H}_2\text{O}$: C, 43.58, H, 4.66, N, 22.23. Found: C, 43.94, H, 4.26, N, 21.85.

2-(3-Amidinopyridin-6-yl)-5-(2-amidinopyridin-5-yl)thiazole dihydrochloride (5f): Yield 70%, pale yellow solid, mp >350°C. ^1H NMR (400 MHz, DMSO) δ : 8.35 (d, 1H, $J = 8.4\text{ Hz}$), 8.43 (dd, 1H, $J_1 = 2\text{ Hz}$, $J_2 = 7.6\text{ Hz}$), 8.49(d, 1H, $J = 8.4\text{ Hz}$), 8.61 (dd, 1H, $J_1 = 2.4\text{ Hz}$, $J_2 = 8.4\text{ Hz}$), 8.86 (s, 1H), 9.00 (d, 1H, $J = 2\text{ Hz}$), 9.27(d, 1H, $J = 2\text{ Hz}$), 9.47 (bris, 2H), 9.51(bris, 2H), 9.73 (bris, 2H), 9.76 (bris, 2H). ^{13}C NMR (100 MHz, DMSO) δ : 124.5, 126.9, 127.3, 129.6, 131.3, 135, 135.7, 139.3, 140.3, 141.9, 142.8, 147.3, 154.4, 165.8, 167.7. HRMS: m/z calcd. $\text{C}_{15}\text{H}_{14}\text{N}_7\text{S}$: 324.1031: found 324.1044. Anal. Calcd for $\text{C}_{15}\text{H}_{13}\text{N}_7\text{S}\cdot 2\text{HCl}\cdot 0.2\text{EtOH}\cdot 1.6\text{H}_2\text{O}$: C, 42.59, H, 4.50, N, 22.59. Found: C, 42.64, H, 4.05, N, 22.20.

2,5-Bis-(3-amidinopyridin-6-yl)thiazole dihydrochloride (5g): Yield 82%, pale yellow solid, mp >350°C. ^1H NMR (400 MHz, DMSO) δ : 8.34 (d, 1H, $J = 8.4\text{ Hz}$), 8.37 (d, 1H, $J = 8.8\text{ Hz}$), 8.42(dd, 1H, $J_1 = 2.0$, $J_2 = 8.8\text{ Hz}$), 8.44 (dd, 1H, $J_1 = 2.4$, $J_2 = 8.4\text{ Hz}$), 9.07 (d, 1H, $J = 1.6\text{ Hz}$), 9.11(d, 1H, $J = 2\text{ Hz}$), 8.95(s, 1H), 9.49(bris, 2H), 9.54(bris, 2H), 9.77(bris, 2H), 9.82(bris, 2H). ^{13}C NMR (100 MHz, DMSO) δ : 119.3, 120, 123.7, 125.8, 138.0, 138.6, 142.5, 145.1, 145.2, 149.9, 154, 154.1, 163.8, 163.9, 169.4. Anal. Calcd for $\text{C}_{15}\text{H}_{13}\text{N}_7\text{S}\cdot 3\text{HCl}\cdot 0.8\text{H}_2\text{O}\cdot 0.3\text{C}_2\text{H}_5\text{OH}$: C, 40.64; H, 4.24; N, 21.26. Found: C, 40.94; H, 4.15, N, 20.88.

General procedure for the synthesis of *N*-hydroxyamidines—To a solution of hydroxylamine hydrochloride (0.695 g, 10 mmol) in anhydrous DMSO (15 mL) was added *t*-BuOK (1.12 g, 10 mmol) in a few portions at 5°C and stirred for 30 minutes. To this solution was added the bis-nitrile (1 mmol) and the reaction mixture was stirred overnight. The reaction mixture was poured into ice water (100 mL) and the light yellow precipitate was filtered, washed with water and dried under reduced pressure. The free base was converted to the hydrochloride salt by treatment with ethanol saturated with HCl gas.

General procedure for the synthesis of *N*-methoxyamidines—A mixture of *N*-dihydroxyamidine (1 mmol) in DMF (10 mL) was stirred at room temp. A solution of lithium hydroxide monohydrate [$\text{LiOH}\cdot\text{H}_2\text{O}$] (6 mmol) was dissolved in H_2O (3 mL) and added dropwise to the reaction mixture and stirring was continued for 30 min. Dimethylsulfate (5 mmol) was added dropwise and the mixture was allowed to stir overnight. The reaction mixture was poured slowly into ice water (100 mL). The precipitate formed was filtered washed with water and dried under reduced pressure. The crude product was purified by column chromatography using a mixture EtOAc/hexane (1:1), (3:1) and (4:1) to give a pale yellow compound. The free base was converted to the hydrochloride salt by treatment with ethanol saturated with HCl gas.

2,5-Bis-(4-*N*-hydroxyamidinophenyl)thiazole dihydrochloride (6a)—Free base: yield (97%), mp 191–192°C. ^1H NMR (400 MHz, DMSO) δ : 5.89(s, 2H), 5.92(s, 2H), 7.74(d, 2H, $J = 8.4\text{ Hz}$), 7.78(d, 2H, $J = 8.4\text{ Hz}$), 7.83(d, 2H, $J = 8.4\text{ Hz}$), 7.97(d, 2H, $J = 8.4\text{ Hz}$), 8.39 (s, 1H), 9.76(s, 1H), 9.83(s, 1H). ^{13}C NMR (100MHz, DMSO) δ : 126.3, 126.6, 126.6, 131.5, 133.6, 133.7, 135.5, 139.0, 140.9, 150.7, 166.0.

Salt: Yield (89%), mp >260 °C. ^1H NMR (DMSO, 400 MHz) δ : 7.85(m, 6H), 8.16(d, 2H, $J=8.4\text{ Hz}$), 8.63(s, 1H), 9.25(bs, 2H). ^{13}C NMR (DMSO, 100MHz) δ : 125.8, 126.8, 126.9, 127.6, 129.5, 129.7, 137.2, 139.1, 141.8, 142.8, 159, 166.1, 167.7. Anal. Calcd for

$C_{17}H_{15}N_5O_2S \cdot 2HCl \cdot 1.4H_2O \cdot 0.2C_2H_5OH$: C, 45.36; H, 4.59; N, 15.19. Found: C, 45.49; H, 4.40, N, 15.01.

2,5-Bis-(4-*N*-methoxyamidinophenyl)thiazole dihydrochloride (7a)—Free base: yield, (41%), pale yellow solid, mp 186 °C. 1H NMR (400 MHz, DMSO) δ : 3.77(s, 3H), 3.78(s, 3H), 6.14(s, 2H), 6.17(s, 2H), 7.76(s, 4H), 7.82(d, 2H, $J = 8.8$ Hz), 7.98(d, 2H, $J = 8.4$ Hz), 8.41(s, 1H). ^{13}C NMR (400MHz, DMSO) δ : 61.2, 61.3, 126.3, 126.6, 127.0, 134.0, 141.0, 150.8.

Salt: Yield, (90%), pale yellow solid, mp >230 °C. 1H NMR (400 MHz, DMSO) δ : 3.83(s, 3H), 3.84(s, 3H), 7.83(d, 2H, $J = 8.4$ Hz), 7.87(d, 2H, $J = 8.0$ Hz), 7.89(d, 2H, $J = 7.2$ Hz), 8.07(d, 2H, $J = 8.4$ Hz), 8.53(s, 1H). ^{13}C NMR (100MHz, DMSO) δ : 61.9, 62.8, 119.1, 127.8, 129, 130.5, 134.4, 135.7, 140.7, 142.1, 147.9, 150.9, 155.5, 166.2, 168. Anal. Calcd for $C_{19}H_{19}N_5O_2S \cdot 2HCl \cdot 1.2H_2O$: C, 47.94; H, 4.95; N, 14.71. Found: C, 48.26; H, 4.85; N, 14.33.

5-(4-*N*-hydroxyamidinophenyl)-2-(2-*N*-hydroxyamidinopyridin-5-yl)thiazole dihydrochloride (6b)—Free base: yield, (90%), mp 242 °C. 1H NMR (400 MHz, DMSO) δ : 5.95(s, 2H), 5.93(s, 2H), 7.78(q, 4H, $J_1 = 8.8$, $J_2 = 14.8$ Hz), 8.00(d, 1H, $J = 8.4$ Hz), 8.33(dd, 1H, $J_1 = 2.4$, $J_2 = 8.4$ Hz), 8.47(s, 1H), 9.14(dd, 1H, $J_1 = 0.8$, $J_2 = 2.4$ Hz), 9.77(s, 1H), 10.14(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 120.3, 126.7, 129.4, 131.2, 133.9, 134.3, 139.8, 141.1, 146.0, 149.6, 150.6, 151.5, 163.1.

Salt: yield, (93%), yellow solid, mp >220 °C. 1H NMR (400 MHz, DMSO) δ : 7.89(d, 2H, $J = 8.8$ Hz), 8.00(d, 2H, $J = 8.4$ Hz), 8.27(d, 1H, $J = 8.4$ Hz), 8.57(dd, 1H, $J_1 = 2.4$, $J_2 = 8.4$ Hz), 8.69(s, 1H), 9.18(bs, 2H), 9.29(dd, 1H, $J_1 = 0.4$, $J_2 = 2.0$ Hz). ^{13}C NMR (100 MHz, DMSO) δ : 119.3, 123.37, 124.3, 131.5, 135.9, 137.7, 138.7, 143.8, 144.9, 147.6, 149.9, 153.8, 163.9, 168.5. Anal. Calcd for $C_{16}H_{14}N_6O_2S \cdot 2HCl \cdot 1.95H_2O$: C, 41.55; H, 4.33; N, 18.17. Found: C, 41.88; H, 4.01; N, 17.83.

5-(4-*N*-methoxyamidinophenyl)-2-(2-*N*-methoxyamidinopyridin-5-yl)thiazole dihydrochloride (7b)—Free base: yield (62.5%), yellow solid, mp 211 °C. 1H NMR (400 MHz, DMSO) δ : 3.77(s, 3H), 3.84(s, 3H), 6.15(s, 2H), 6.19(s, 2H), 7.78(s, 4H), 7.99(d, 1H, $J = 8$ Hz), 8.35(d, 1H, $J = 8$ Hz), 8.48(s, 1H), 9.15(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 61.2, 61.7, 120.7, 126.7, 127.0, 129.7, 131.6, 133.1, 134.5, 139.8, 141.3, 146.1, 149.45, 150.8, 163.0.

Salt: yield (93%), yellow solid, mp >240 °C. 1H NMR (400 MHz, DMSO) δ : 3.85(s, 3H), 3.87(s, 3H), 7.88(d, 2H, $J = 8$ Hz), 7.93(d, 2H, $J = 7.2$ Hz), 8.05(d, 1H, $J = 8.4$ Hz), 8.39(d, 1H, $J = 8.4$ Hz), 8.61(s, 1H), 9.18(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 62.2, 63.4, 120, 125.2, 127.1, 129, 129.9, 135, 139.2, 142.1, 143.6, 148.4, 151.8, 167.9, 169.9. Anal. Calcd for $C_{18}H_{18}N_6O_2S \cdot 2HCl \cdot 0.4C_2H_5OH$: C, 47.66; H, 4.76; N, 17.74. Found: C, 47.94; H, 4.66; N, 18.02.

5-(4-*N*-hydroxyamidinophenyl)-2-(3-*N*-hydroxyamidinopyridin-6-yl)thiazole dihydrochloride (6c)—Free base: yield (88%), yellow solid, mp 225 °C. 1H NMR (400 MHz, DMSO) δ : 5.89(s, 2H), 6.08(s, 2H), 7.78(s, 4H), 8.15(d, 1H, $J = 8.0$ Hz), 8.20(dd, 1H, $J_1 = 2.0$, $J_2 = 8.4$ Hz), 8.45(s, 1H), 8.92(d, 1H, $J = 2.0$ Hz), 9.77(s, 1H), 10.03(s, 1H). ^{13}C NMR (400 MHz, DMSO) δ : 119.0, 126.6, 129.7, 130.5, 131.6, 134.7, 141.0, 141.3, 147.2, 149.0, 150.7, 150.8, 167.3

Salt: yield (86%), yellow solid, mp >220 °C. 1H NMR (400 MHz, DMSO) δ : 7.89(d, 2H, $J = 8.4$ Hz), 8.04(d, 2H, $J = 8.4$ Hz), 8.32(d, 1H, $J = 8.4$ Hz), 8.37(dd, 1H, $J_1 = 2.4$, $J_2 = 8.4$ Hz), 8.70(s, 1H), 9.02(d, 1H, $J = 1.2$ Hz), 9.33(brs, 2H). ^{13}C NMR (100 MHz, DMSO) δ : 123.2,

126, 127.3, 129.6, 131.5, 135.2, 135.3, 139.7, 142.8, 146.9, 154.2, 159, 163.5, 167.3. Anal. Calcd for $C_{16}H_{14}N_6O_2S \cdot 2HCl \cdot 2H_2O$: C, 41.47; H, 4.35; N, 18.13. Found: C, 41.79; H, 3.95; N, 17.74.

5-(4-*N*-methoxyamidinophenyl)-2-(3-*N*-methoxyamidinopyridin-6-yl)thiazole dihydrochloride (7c)—Free base: yield (25%), yellow solid, mp 203 °C. 1H NMR (400 MHz, DMSO) δ : 3.77(s, 3H), 3.80(s, 3H), 6.14(s, 2H), 6.35(s, 2H), 7.79(q, 4H, $J_1 = 6.4$, $J_2 = 8.4$ Hz), 8.16(d, 1H, $J = 8.4$ Hz), 8.19(dd, 1H, $J_1 = 2$, $J_2 = 6.4$ Hz), 8.48(s, 1H), 8.90(d, 1H, $J = 2$ Hz). ^{13}C NMR (100MHz, DMSO) δ : 61.1, 61.2, 119.0, 126.7, 127.0, 129.9, 132.0, 132.9, 135.2, 141.1, 141.5, 147.4, 149.2.

Salt: yield (87%), yellow solid, mp >200 °C. 1H NMR (400 MHz, DMSO) δ : 3.81(s, 3H), 3.99(s, 3H), 7.81(d, 2H, $J = 8.4$ Hz), 7.95(d, 2H, $J = 8$ Hz), 8.19(d, 1H, $J = 8$ Hz), 8.22(dd, 1H, $J_1 = 2$, $J_2 = 8.4$ Hz), 8.57(s, 1H), 8.92(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 61.9, 64.3, 119, 122.9, 124.4, 128.2, 128.6, 129.9, 135.8, 141.1, 146.4, 147.7, 150.2, 155.4, 164.2, 165.8. Anal. Calcd for $C_{18}H_{18}N_6O_2S \cdot 2HCl \cdot 1.8H_2O \cdot 0.2C_2H_5OH$: C, 44.46, H, 5.03; N, 16.90. Found: C, 44.55; H, 4.73; N, 16.59.

2-(4-*N*-hydroxyamidinophenyl)-5-(2-*N*-hydroxyamidinopyridin-5-yl)thiazole dihydrochloride (6d)—Free base: yield (82%), yellow solid mp 249 °C. 1H NMR (400 MHz, DMSO) δ : 5.58(s, 2H), 5.93(s, 2H), 7.83(d, 2H, $J = 8.4$ Hz), 7.94(d, 1H, $J = 8.4$ Hz), 8.00(d, 1H, $J = 8$ Hz), 8.15(dd, 1H, $J_1 = 2.4$ $J_2 = 8$ Hz), 8.95(d, 1H, $J = 2$ Hz), 9.84 (s, 1H), 10.05 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 120.2, 126.4, 126.7, 127.5, 133.4, 134.6, 135.5, 135.7, 142.0, 146.0, 149.6, 150.0, 150.6, 167.0.

Salt: yield (81%), yellow solid, mp >350 °C. 1H NMR (400 MHz, DMSO) δ : 7.92(d, 2H, $J = 8.4$ Hz), 8.18(d, 1H, $J = 8.4$ Hz), 8.22(d, 1H, $J = 8.4$ Hz), 8.42(dd, 1H, $J_1 = 2.4$, $J_2 = 8.4$ Hz), 8.77(s, 1H), 8.73(s, 1H), 9.14(d, 1H, $J = 1.6$ Hz). ^{13}C NMR (100 MHz, DMSO) δ : 123.5, 127, 129.7, 130.4, 135.5, 135.8, 136.8, 143.8, 144, 147.2, 154.9, 158.9, 166.7, 167. Anal. Calcd for $C_{16}H_{14}N_6O_2S \cdot 3HCl \cdot 1.0H_2O$: C, 39.88; H, 3.97; N, 17.44. Found: C, 40.06; H, 4.19; N, 17.04.

2,5-Bis-(2-*N*-hydroxyamidinopyridin-5-yl)thiazole dihydrochloride (6e)—Free base: yield (93%), yellow solid, mp >200 °C. 1H NMR (400 MHz, DMSO) δ : 5.90(s, 2H), 5.94 (s, 2H), 7.94(d, 2H, $J = 8.4$ Hz), 8.00(d, 2H, $J = 8.4$ Hz), 8.16(d, 1H, $J = 8$ Hz), 8.17(d, 1H, $J = 8$ Hz), 8.58(s, 1H), 8.97(s, 1H), 9.15(s, 1H), 10.07(s, 1H), 10.16(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 120.2, 120.4, 127.3, 129.2, 134.7, 136.3, 142.2, 146.1, 146.1, 149.6, 150.2, 151.7, 164.1.

Salt: yield (50%), yellow solid, mp >280 °C. 1H NMR (400 MHz, DMSO) δ : 8.19–8.21(m, 2H), 8.44(dd, 1H, $J_1 = 2.0$, $J_2 = 8.0$ Hz), 8.56(dd, 1H, $J_1 = 2.0$, $J_2 = 8.4$ Hz), 8.77(s, 1H), 9.15 (s, 1H), 9.29(s, 1H). ^{13}C NMR (100 MHz, DMSO) δ : 119.5, 121.4, 122, 123.5, 130.3, 131.5, 136, 136.6, 137, 144, 147, 147.3, 155, 165.4, 166.8. Anal. Calcd for $C_{15}H_{13}N_7O_2S \cdot 4HCl \cdot 0.7H_2O \cdot 0.2C_2H_5OH$: C, 35.36; H, 3.77; N, 18.76. Found: C, 35.64; H, 3.98; N, 18.47.

2,5-Bis(2-*N*-methoxyamidinopyridin-5-yl)thiazole dihydrochloride (7e)—Free base: yield (39%), yellow solid, mp 191 °C. 1H NMR (400 MHz, DMSO) δ : 3.83(s, 3H), 3.84 (s, 3H), 6.16(brs, 2H), 6.20(brs, 2H), 7.94(d, 1H, $J = 8.4$ Hz), 8.00(d, 1H, $J = 8.4$ Hz), 8.18(dd, 1H, $J_1 = 2.4$, $J_2 = 8.4$ Hz), 8.36(dd, 1H, $J_1 = 2$, $J_2 = 8.0$ Hz), 8.60(s, 1H,), 8.98(d, 1H, $J = 2.0$ Hz), 9.16(d, 1H, $J = 2$ Hz). ^{13}C NMR (DMSO, 100 MHz) δ : 61.6, 61.7, 120.6, 120.8, 127.7, 129.5, 134.6, 134.8, 136.3, 142.4, 146.2, 149.2, 149.4, 150.8, 164.0.

Salt: yield, (84%), yellow solid, mp >280 °C. ¹H NMR (400 MHz, DMSO) δ: 3.83(s, 3H), 3.84 (s, 3H), 7.99(d, 1H, *J* = 8.0 Hz), 8.04(d, 1H, *J* = 8.4 Hz), 8.22(dd, 1H, *J*₁ = 2.4, *J*₂ = 8.4 Hz), 8.40(dd, 1H, *J*₁ = 2, *J*₂ = 8.4 Hz), 8.64(s, 1H), 9.01(d, 1H, *J* = 2.4 Hz), 9.18(d, 1H, *J* = 2.4 Hz). ¹³C NMR (100 MHz, DMSO) δ: 61.9, 62.7, 121, 127, 128.5, 129.3, 130, 133.8, 134.6, 139.4, 141.9, 146.2, 150, 150.4, 155, 163.7, 164.1. Anal. Calcd for C₁₇H₁₇N₇O₂S·2HCl·1.0H₂O: C, 43.04; H, 4.46; N, 20.67. Found: C, 43.27; H, 4.62; 20.67.

2,5-Bis(3-*N*-hydroxyamidinopyridin-6-yl)thiazole dihydrochloride (6g)—Free base: yield, (97%), yellow solid, mp >250 °C. ¹H NMR (400 MHz, DMSO) δ: 6.03(s, 2H), 6.07 (s, 2H), 8.10–8.21(m, 4H), 8.90(d, 1H, *J* = 1.6 Hz), 8.94(d, 1H, *J* = 2 Hz), 9.94(s, 1H), 10.03 (s, 1H). ¹³C NMR (100MHz, DMSO) δ: 111.3, 119.2, 119.8, 128.7, 130.6, 134.3, 134.7, 142.3, 142.8, 147.2, 149.0, 150.4, 150.9, 169.3.

Salt: yield, (78%), yellow solid, mp >300 °C. ¹H NMR (400 MHz, DMSO) δ: 8.26(dd, 1H, *J*₁ = 2.0, *J*₂ = 8.4 Hz), 8.33(s, 2H), 8.91(s, 1H), 8.95 (d, 1H, *J* = 2.0 Hz), 9.00(s, 1H). ¹³C NMR (100 MHz, DMSO) δ: 118.4, 120.2, 120.5, 122.8, 126.3, 128.6, 129.4, 131.6, 144.3, 144.5, 156.7, 160.3, 160.6, 165.8, 168. Anal. Calcd for C₁₅H₁₃N₇O₂S·2HCl·0.3H₂O·0.2C₂H₅OH: C, 41.76; H, 3.82; N, 22.13. Found: C, 41.86; H, 4.03; 22.01.

2,5-Bis(3-*N*-methoxyamidinopyridin-6-yl)thiazole dihydrochloride (7g)—Free base: yield (67%), yellow solid mp >280 °C. ¹H NMR (400 MHz, DMSO) δ: 3.79(s, 3H), 3.80 (s, 3H), 6.30(s, 2H), 6.35(s, 2H), 8.12(d, 2H, *J* = 1.6 Hz), 8.19–8.22(m, 2H), 8.71(s, 1H), 8.87 (s, 1H), 8.92(s, 1H). ¹³C NMR (100 MHz, DMSO) δ: 61.3, 61.4, 119.2, 119.8, 127.9, 129.9, 134.7, 135.2, 142.3, 147.5, 149.2, 149.3, 150.7, 150.9, 151.3, 163.7, 169.4.

Salt yield: (83%), yellow solid, mp >300 °C. ¹H NMR (400 MHz, DMSO) δ: 3.80(s, 3H), 3.98 (s, 3H), 8.14–8.21(m, 2H), 8.74(s, 1H), 8.86(s, 1H), 8.93(s, 1H). ¹³C NMR (100 MHz, DMSO) δ: 62.9, 63.3, 119.3, 120, 123, 125.8, 135.6, 136.9, 140.5, 142.1, 143.7, 148.0, 148.3, 151.9, 152.8, 167.8, 169.6. Anal. Calcd for C₁₇H₁₇N₇O₂S·2HCl·0.8H₂O·0.2C₂H₅OH: C, 43.54; H, 4.57; N, 20.43. Found: C, 43.26; H, 4.45; 20.25.

Acknowledgments

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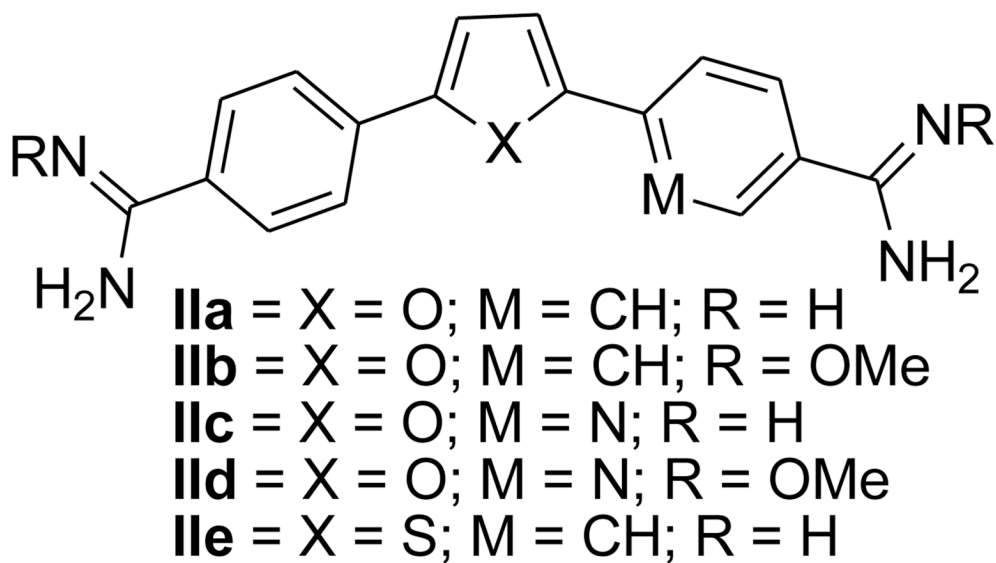
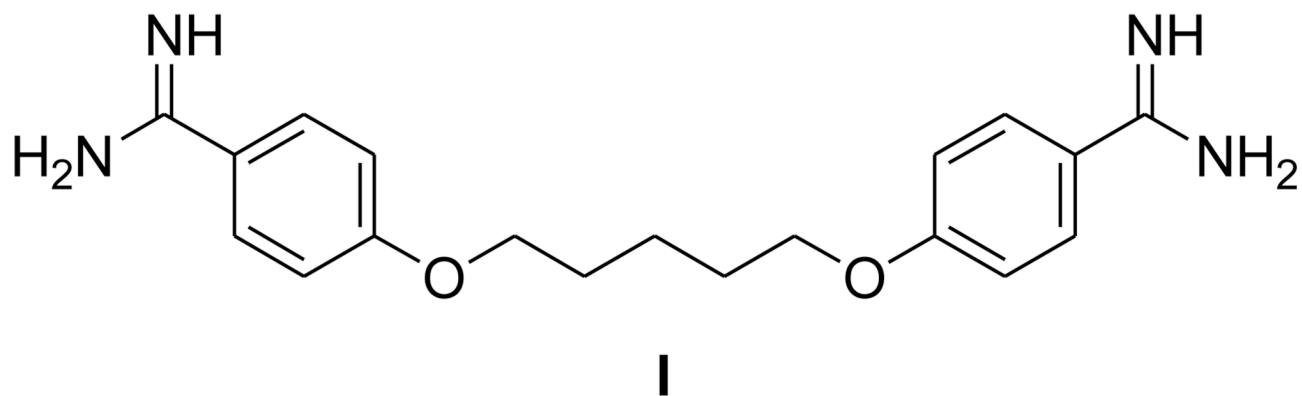


Figure 1.
Important antiparasitic compounds.

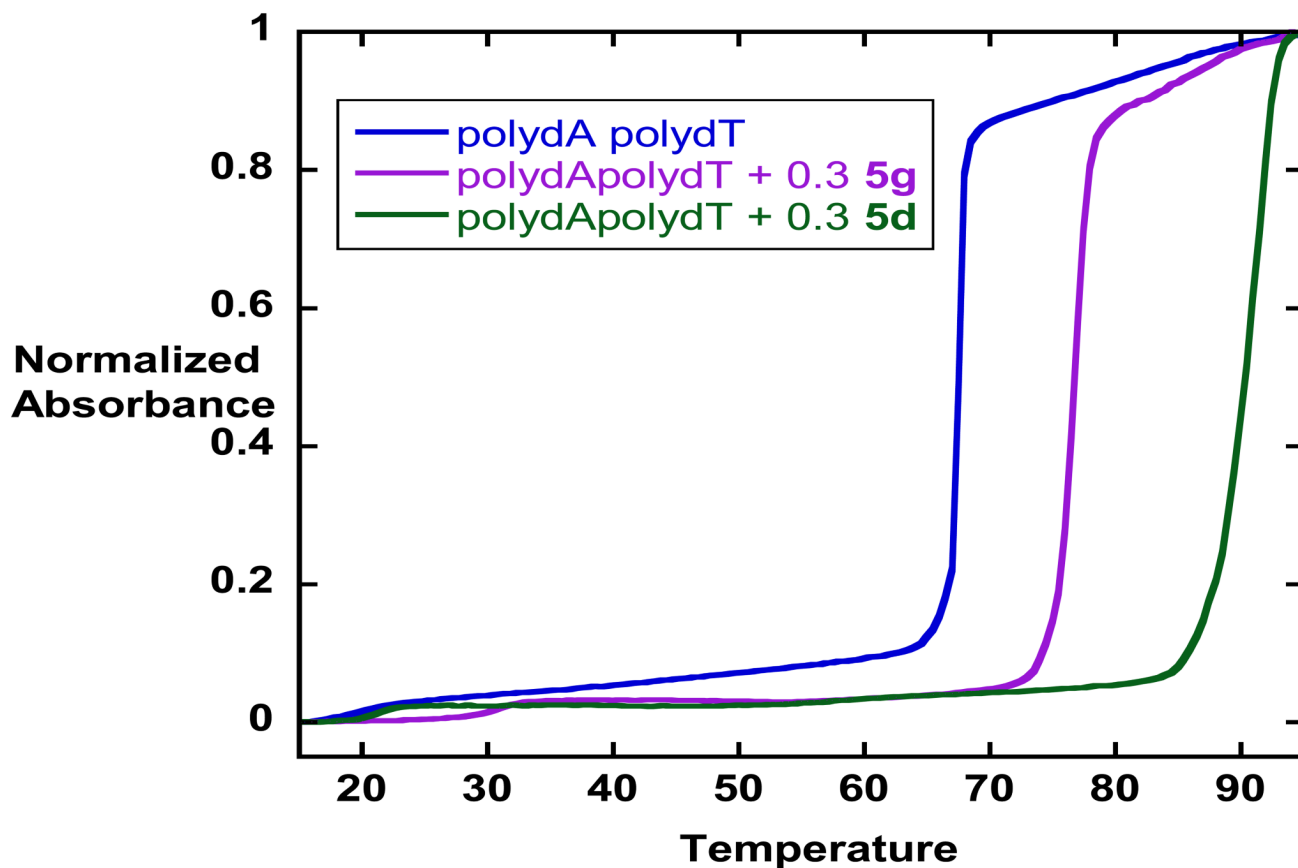


Figure 2. Thermal melting curves (Absorbance at 260nm versus temperature in degrees C) are shown for poly dA•dT and its complexes with **5g** and **5d**. ΔT_m values were determined from the peak in first derivative plots (dA_{260}/dT) and are collected in Table 1.

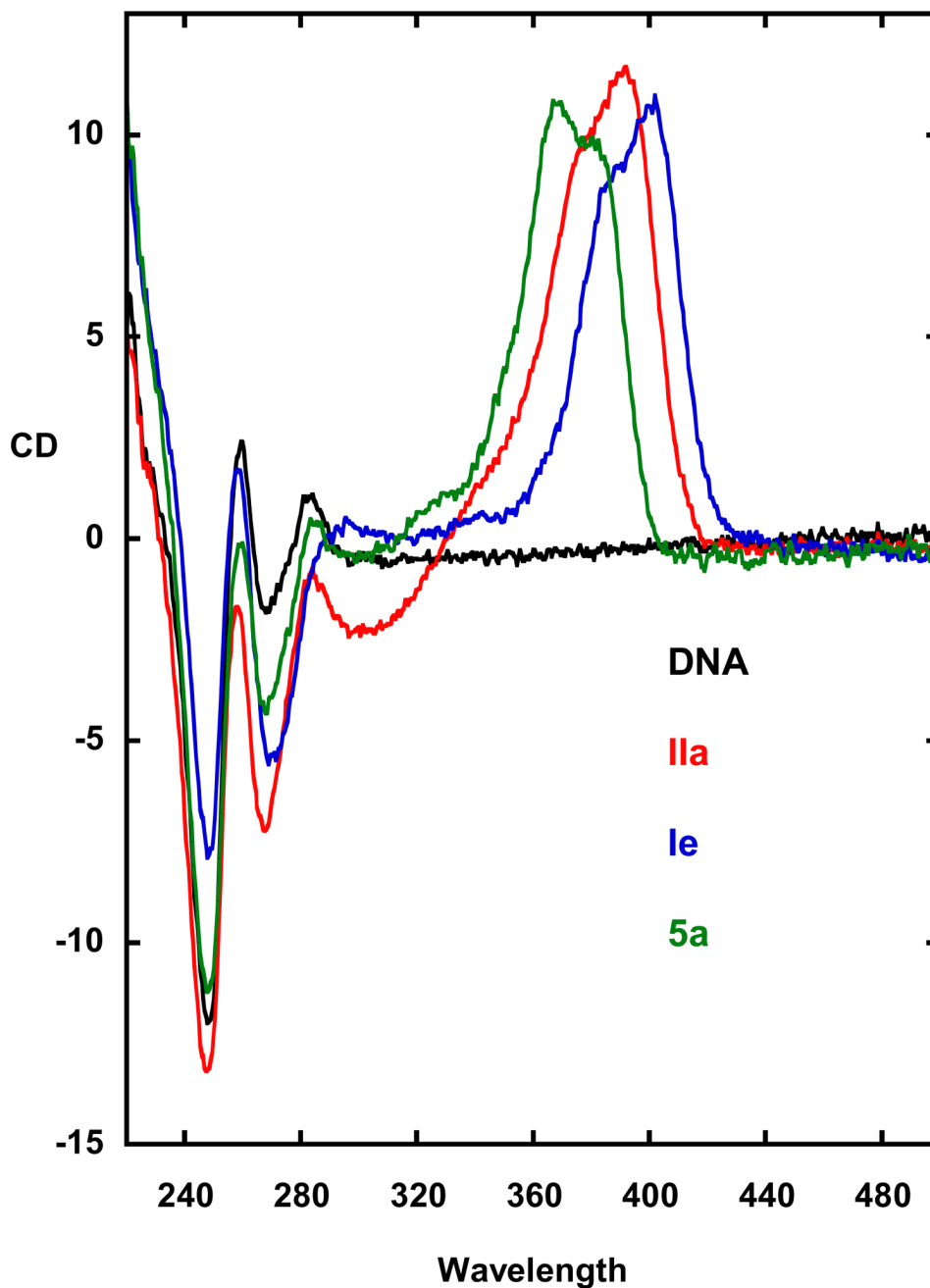
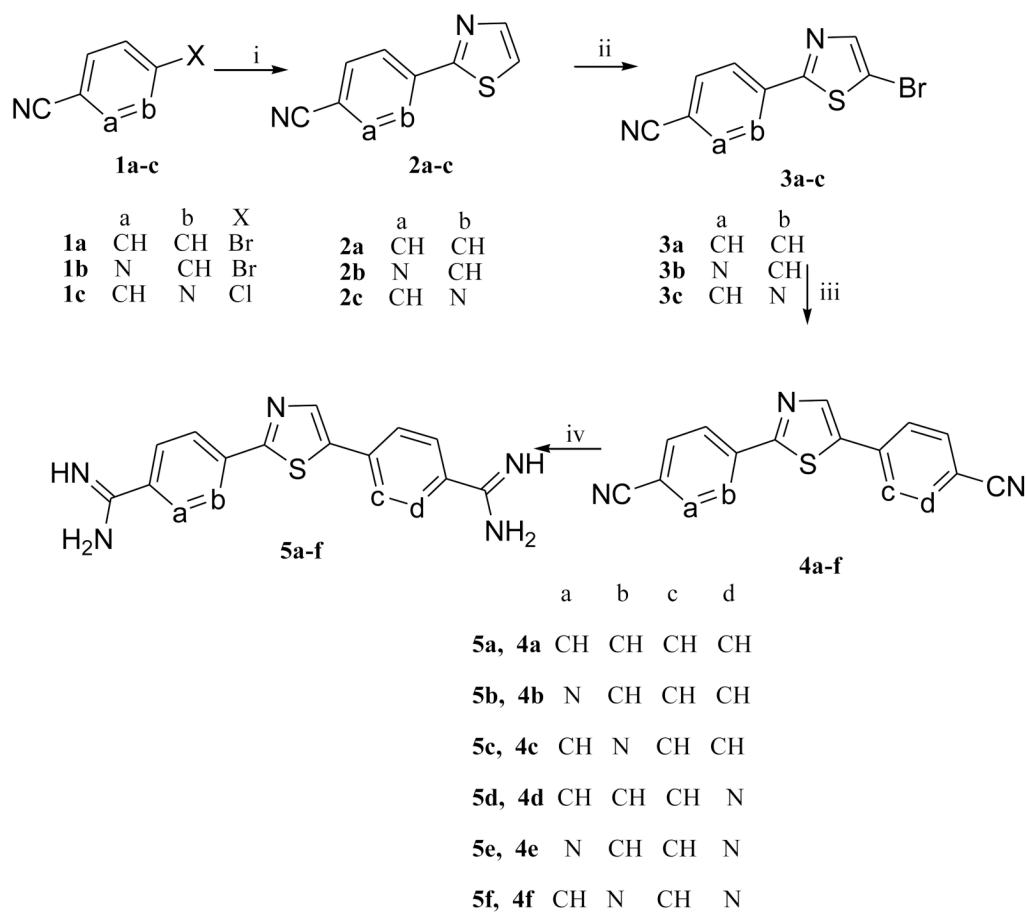
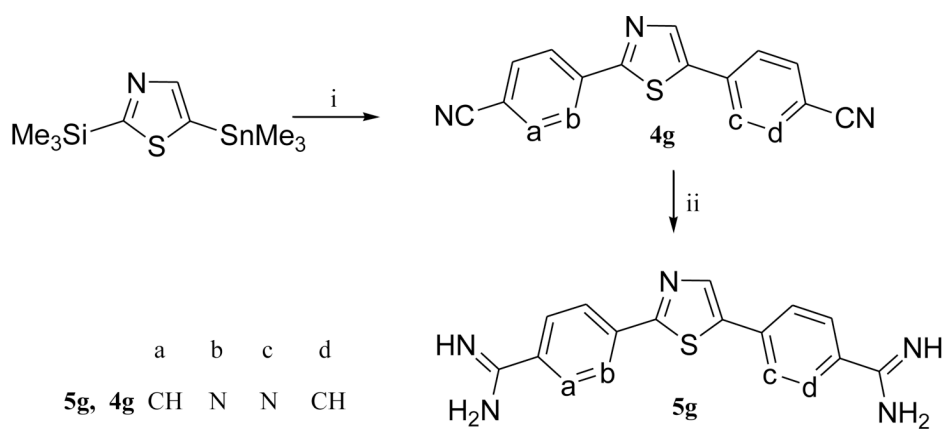


Figure 3. Circular dichroism plots (CD versus wavelength) are shown for poly dA•dT (DNA) and its complexes with **IIa**, **IIe** and **5a**. DNA without bound compound does not have any CD signal above 300 nm while the unbound compounds are not chiral. On binding to DNA the compounds exhibit a large positive CD spectrum above 300 nm that is characteristic of minor groove binding. The changes below 300 nm are due to induced changes in the compound CD as well as slight changes in the DNA spectrum.



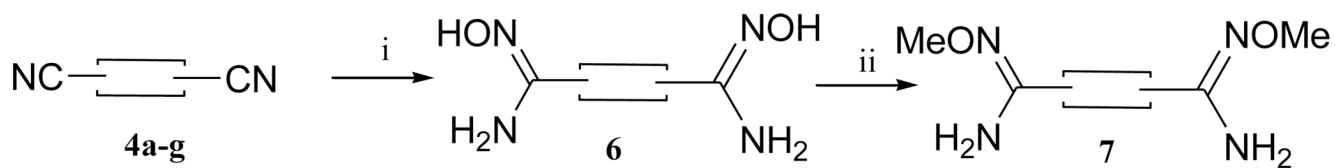
Reagents and conditions: i) 2-tributyltin thiazole, Pd(PPh₃)₄, ii) NBS, DMF, iii) 4-cyanophenyl boronic acid or 5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-2-cyanopyridine Pd(PPh₃)₄ iv) 1) LiN[Si(CH₃)₃]₂, THF 2) NaOH/H₂O, 3) EtOH/HCl

Scheme 1.
Synthesis of bis-amidines **5a-f**.



Reagents and conditions: i) 2-bromo-5-cyanopyridine, $\text{Pd}(\text{PPh}_3)_4$ ii) 1) $\text{LiN}[\text{Si}(\text{CH}_3)_3]_2$, THF 2) $\text{NaOH}/\text{H}_2\text{O}$, 3) EtOH/HCl

Scheme 2.
Synthesis of bis-amidine **5g**.

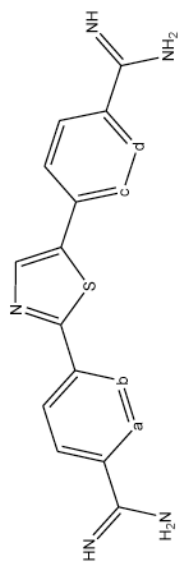


Reagents and conditions: i) $\text{NH}_2\text{OH}\cdot\text{HCl}$, $\text{KO}\cdot\text{t-Bu}$, r. temp. ii) Me_2SO_4 , $\text{LiOH}\cdot\text{H}_2\text{O}$

Scheme 3.
Synthesis of bis-amidoxime prodrugs.

Table 1

DNA affinities and in vitro antiprotozoan activity for thiazole analogs.



Code	a	b	c	d	DNA affinity $\Delta T_m(^{\circ}C)^a$	<i>T. b. r.</i> $IC_{50}(nM)^b$	<i>P. f. cytotox</i> $IC_{50}(\mu M)^c$
5a	CH	CH	CH	CH	21.1	4	36.7
5b	N	CH	CH	CH	19.2	4	1.8
5c	CH	N	CH	CH	13.9	16	102.5
5d	CH	CH	CH	N	22.9	6	40.2
5e	N	CH	CH	N	18.2	6	1.4
5f	CH	N	CH	N	12.8	38	37.4
5g	CH	N	N	CH	10.0	176	>195.0
IIa					25	3.2	6.5
IIc					19.3	7.0	77.9
IIe					25	3	51.7
I					12.6	2.8	46.6

^aIncrease in thermal melting of poly(dA-dT)_n²¹

^bThe *T. b. r.* (*Trypanosoma brucei rhodesiense*) strain was STIB900 and the *P. f.* (*Plasmodium falciparum*) strain was K1. The values are the average of duplicate determinations; 10,28

^cCytotoxicity was evaluated using cultured L6 rat myoblast cells; 28

Table 2

Antitrypanosomal evaluation of the thiazole analogues in the STIB900 mouse model.

Code	Dosage route ^b	Dosage(4 × mg/kg)	Cures ^c	Survival (days ^d)
5a	ip	5	1/4	>44.5
6a	po	25	0/4	35
7a	po	25	0/4	41
5b	ip	5	0/4	44.5
6b	po	25	0/4	37.5
7b	po	25	0/4	42.75
5c	ip	5	0/4	30.25
6c	po	25	0/4	24
7c	po	25	0/4	35
5d	ip	5	0/4	>36
6d	po	25	0/4	36.75
5e	ip	5	0/4	50.5
6e	po	25	1/4	>37.5
7e	po	25	1/4	>47.25
5f	ip	5	0/4	29.25
5g	ip	5	0/4	21.5
6g	po	25	0/4	9
7g	po	25	0/4	9
I	ip	5	2/4	.45
IIa	ip	5	1/4	>46
IIb	po	25 5	4/4 0/4	>60 30
IIc	ip	5	3/4	>54.5
IId	po	25 5	4/4 4/4	>60 >60
IIe	ip	5	2/4	>48.75

^a See refs 10 and 29 for details of STIB900 model.

^b ip = intraperitoneal; po = oral.

^c Number of mice that survive and are parasite free for 60 days.

^d Average days of mice survival; untreated control animals expire between day 7 and 10 post-infection.