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Synthesis and Antiprotozoal Activity of Dicationic 2, 6-Diphenylpyrazines and Aza-Analogues

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Abstract

Dicationic 2,6-diphenylpyrazines, aza-analogues and prodrugs were synthesized; evaluated for DNA affinity, activity against *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*) in vitro, efficacy in *T. b. r.* STIB900 acute and *T. b. brucei* GVR35 CNS mouse models. Most diamidines gave poly(dA-dT)$_2$ ΔTm values greater than pentamidine, IC_{50} values: *T. b. r.* (4.8 to 37 nM) and *P. f.* (10 to 52 nM). Most diamidines and prodrugs gave cures for STIB900 model (**11**, **19a** and **24b** 4/4 cures); **12** 3/4 cures for GVR35 model. Metabolic stability half-life values for *O*-methylamidoxime prodrugs did not correlate with STIB900 results.

1. Introduction

Tropical protozoan diseases, such as malaria and human African trypanosomiasis (HAT), affect millions of people in large parts of the world.[1] Malaria caused by *Plasmodium falciparum* (*P. f.*) leads to 665,000 deaths each year.[2] HAT (or sleeping sickness), another devastating disease, is caused by *Trypanosoma brucei rhodesience* (*T. b. r.*) and *Trypanosoma brucei gambiense* (*T. b. g.*), which is eventually fatal without treatment. There are below 10,000 reported cases and 30,000 estimated cases of HAT a year mostly in sub-Saharan Africa.[3, 4] HAT has two stages: an early stage in which the parasites reside and proliferate in the hemolymphatic system and a second stage in which the parasites cross the blood-brain barrier (BBB) and infect the central nervous system (CNS). [5] The drugs currently in use against both malaria and HAT are far from satisfactory; most drugs suffer from poor oral bioavailability and severe side effects. For example, the second stage HAT drug melarsoprol causes a reactive encephalopathy which leads to death in 5% of treated patients.[6] Furthermore, for both diseases the cases of treatment failure because of drug resistance have increased in recent years. For second stage HAT, there are only two drugs melarsoprol and eflornithine available for monotherapy and NECT a combination therapy

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with niturtimox and eflornithine.[7] Therefore, there is an urgent need for development of more effective, orally available and less toxic drugs against these tropical protozoan diseases.

Although numerous DNA binding aromatic diamidines exhibit potent antiprotozoal activity against these tropical diseases, pentamidine **1** (Fig. 1) is the only one which has seen significant clinical use in humans.[8] Pentamidine has been used to treat first stage *T. b. g.* HAT, antimony-resistant leishmaniasis, and *Pneumocystis jiroveci* pneumonia. Because the amidine groups are protonated at physiological pH, pentamidine has low oral availability and requires parenteral administration, which makes its clinical use difficult in remote regions. Furamidine **2a** (Fig. 1), a diphenyl furan diamidine analogue, is the active metabolite of the di-*O*-methylamidoxime prodrug pafuramidine **2c** (Fig. 1) which reached phase III clinical trials against first-stage HAT and *P. jiroveci* pneumonia, and phase II clinical trials against malaria.[1, 5, 8, 9] Due to hepatic and renal toxicity of pafuramidine in humans observed in an additional safety study paralleling the phase III trials, the further development of pafuramidine has been terminated. Introduction of a nitrogen atom into one or both of the terminal phenyl rings of furamidine resulted in aza-analogues of furamidine **3a** and **4a** (Fig. 1), which exihibited more potent in vivo activities against HAT than pentamidine and furamidine.[10, 11] The *O*-methylamidoxime prodrugs of azafuramidines **3c** and **4c** (Fig. 1) were found to be quite effective in the second stage HAT GVR35 CNS mouse model [5] and **3c** was effective in a vervet monkey model of second stage HAT.[12] As yet, only very few compounds have shown good activity in the GVR35 CNS mouse model. Since the aza-analogue **4c** is better tolerated than **3c** in toxicity studies in vervet monkeys,[5] it is under further evaluation as a possible preclinical candidate for treatment of the second stage HAT. Unexpectedly, the parent diamidine **4a** also showed potent activity in the CNS mouse model on intraperitoneal injection; therefore, **4a** may have potential to become a treatment option for second stage HAT.[5]

Due to the promising results from the furamidine series, a large number of furamidine related diamidines have been synthesized.[8, 13] Recently, we have described a series of linear terphenyl diamidine **5** (Fig. 1) and their aza-analogues (e.g. **6**), which showed significant DNA minor groove binding affinity and low nanomolar antiprotozoal activity against *T. b. r.* and *P. f.*[14–16] The in vivo efficacy for three of those aza-analogues in the *T. b. r.* STIB900 acute mouse model is much superior to that of furamidine, and comparable to the azafuramidines. Unfortunately, the di-amidoximes and di-*O*-methylamidoxime prodrugs of the terphenyl dicationic analogues showed poor bioconversion and were not effective on oral administration.[14] In this study we have explored a series of novel curved dicationic 2,6-diarylpyrazines, their aza-analogues and their prodrugs which are isomeric with their linear 2,5-pyrazines.[14–16] Herein, we describe the synthesis, DNA binding affinity, in vitro activities against *T. b. r.* and *P. f.* and in vivo activities in the *T. b. r.* STIB900 acute mouse model and *T. b. brucei* GVR35 CNS mouse model for these curved 2,6-diarylpyrazines.

2. Chemistry

The synthesis of the parent dicationic 2,6-diphenylpyrazine **10** begins with Suzuki coupling of 2,6-dichloropyrazine (**7)** with 4-cyanophenylboronic acid (**8)** to yield the diphenylpyrazine di-nitrile **9** (Scheme 1).[14–16] The di-nitrile **9** was converted to the diamidine **10** by the action of lithium trimethylsilylamide [$LiN(TMS)_2$] in THF. The di-amidoxime prodrug **11** was obtained by reaction of the di-nitrile **9** with hydroxylamine and followed by *O*-methylation with dimethylsulfate in the presences of lithium hydroxide to yield the corresponding di-*O*-methylamidoxime prodrug **12**. [10]

Employing the related Stille coupling process starting with 2,6-di(tri-*n*-butylstannyl)pyrazine the symmetrical di-nitriles **14a** and **14b** were made in one step (Scheme 2).[17] The di-nitriles **14a–b** were converted to the diamidines **15a–b** using $LiN[TMS]_2$ as previously mentioned.

The dissymmetric mono-aza analogues **19a–b** were made as outlined in Scheme 3. The pyridyl rings are introduced in the first step by performing Stille coupling between 2-chloro-6-(tri-n-butylstannyl)pyrazine and the appropriate bromocyanopyridines **16a–b**.[17] Subsequently, a Suzuki reaction between the 6-(pyridyl)-2-chloropyrazines **17a–b** and 4-cyanophenylboronic acid yields the dissymmetric di-nitriles **18a–b.** The di-nitriles were converted into the corresponding diamidines **19a–b**, the amidoxines **20a,b** and the *O*-methylamidoximes **21a–b** as discussed previously for Scheme 1.

The synthesis of the symmetrical di-aza analogues is presented in Scheme 4. It this case the needed di-nitriles **22a–b** are made directly by Stille coupling of the bromocyanopridines **16a–b** with 2,6-di(tri-*n*-butylstannyl)pyrazine. The di-nitriles were converted into the corresponding diamidines **23a–b**, the amidoxines **24a–b** and the *O*-methylamidoximes **25a–b** as discussed previously for Scheme 1.

3. Biology

The results for the evaluation of the dicationic 2,6-diarylpyrazine analogues and their prodrugs against *T. b. r.* and *P. f.* and their DNA binding affinities are shown in Table 1. For comparative purposes, the analogous data for pentamidine (**1)**, furamidine (**2a)**, azafuramidines **3a**, **4a** and 2,5-diphenyl pyrazine diamidine (**6)** are also included in Table 1. [10, 16]

The interaction of diamidines with nuclear and kinetoplast DNA has been shown to be an important part of their mode of antiparasitic action.[8f] The ΔTm values of these dicationic 2,6-diphenylpyrazines and their aza-analogues range from high values of 15.1 °C to low ones of 5.1 °C, as shown in Table 1. The parent diamidine **10** showed a ΔTm value of 15.1 °C, which is lower than that of furamidine **(2a**) (ΔTm = 25 °C) and higher than that of pentamidine (**1)** (ΔTm = 12.6 °C). In comparison to **2a** the ΔTm value of **10** is consistent with its increased hydrophilic property as a result of the additional two nitrogen atoms in the central pyrazine ring of **10**. This result may further suggest that the hydrophobic component is important for minor groove DNA binding. The ΔTm value (15.1°C) for **10** is higher than that of the linear 2,5-isomer **6** (ΔTm = 8.0°C). One possible explanation for this result may be that compound **10** presents an approximately crescent shape which more closely fits the curvature of the DNA minor groove, similar to furamidine; and the linear isomer **6** needs incorporation of a water molecule in the complex to simulate the curved structure of DNA minor groove.[20] The compounds **15a** and **15b**, methyl and fluorine substituted analogues, showed much lower ΔTm values, which may be due to their twisted shape. Introduction of a nitrogen atom *meta* to the amidine group in one or both of the phenyl rings in the parent compound **10** leads to decreased DNA binding affinity: compound **19a** with one nitrogen resulted in a 2 °C decrease in ΔTm value and compound **23a** with two nitrogen atoms gave a ΔTm value with a 3.9 °C decrease. However, their corresponding *ortho*-isomers **19b** and **23b** showed a smaller influence on the DNA binding affinity: compound **19b**, with one nitrogen atom, showed the same ΔTm value as that of the parent compound **10** and compound **23b**, with two nitrogen atoms, showed a ΔTm value reduced by 3.0 °C. These results are consistent with the effect of nitrogen substitution relationships found in the previous study of the aza-analogues of furamdine (**2a)**.[10] It is also noteworthy that the compounds which exhibit the higher ΔTm values showed the higher antitrypanosomal activity whereas weaker binding compounds show lower activity (see below) which is

consistent with previous observations that while a threshold level of binding appears essential for activity a direct correlation between DNA affinity and in vitro activity is neither expected nor found.[8f] A similar trend was not found for the antiplasmodial activity.

These 2,6-diarylpyrazine diamidines exhibited significant in vitro antitrypanosomal and antiplasmodial activity at the low nanomolar level. The parent dicationic 2,6-diphenylpyrazine **10** showed an IC_{50} value of 6 nM against *T. b. r.*, comparable to that of pentamidine and furamidine. The antiplasmodial activity of **10** (IC_{50} = 10 nM) is approximately five-fold more active than pentamidine and slightly less active than furamidine. The antiprotozoal activity of **10** is similar to that of the azafuramidine **3a**. Compared to the isomer 2,5-dicationic diphenylpyrazine **6**, there are significant differences: the 2,6-isomer **10** was three times more active than 2,5-isomer **6** against *T. b. r.*; conversely, **10** was 25-fold less potent than **6** against *P. f.* The 2,6-isomer **10** lost the good selectivity for *P. f.* versus *T. b. r.* for which the 2,5-isomer **6** showed 45-fold selectivity for *P. f.*, compared to *T. b. r.* These differences may be due, in part, to the fact that the 2,6-diphenyl pyrazine diamidine **10** is an approximately crescent shaped molecule which more closely fits the curvature of the DNA minor groove, much similar to furamidine;[8, 19] however, its dicationic isomer 2,5-diphenylpyrazine **6** is a linear molecule which presumably requires the incorporation of a water molecule into the recognition complex to simulate the curved structure of DNA minor groove.[20] The introduction of a methyl group or fluorine atom into the *meta*-position to the amidine group on both of the phenyl rings yielded compounds **15a** and **15b** which showed more than a sixty- and four-fold loss of their antiprotozoal activities against *T. b. r.* and *P. f.*, respectively. Compound **19a** and **23a**, in which a nitrogen atom has been placed *meta* to the amidine group in one or both of the phenyl rings, showed a two- and six-fold decrease in potency compared to the parent diamidine **10** against *T. b. r.* and a ten- and six-fold decreased potency against *P. f.* The two analogues 1**9b** and **23b**, in which the nitrogen atoms are *ortho* to the amidine, exhibited equivalent potency against *T. b. r.* and a similar or eight-fold decrease in activity against *P. f.*, compared to the parent compound **10**. It is noted that the compounds 1**9b** and **23b** in which the nitrogen atoms are *ortho* to the amidine exhibited higher activity against *T. b. r.* than the corresponding *meta*-isomers **19a** and **23a**. However, a similar trend was not found for *P. f.* activity. Ten potential di-amidoxime and di-*O*-methylamidoxime prodrugs of the dicationic 2,6-diphenylpyrazine and aza-analogues were prepared. As expected, these amidoximes and *O*-methylamidoxime prodrugs showed low antiprotozoal activity when tested in vitro due to the absence of metabolizing enzymes.[10]

Given the promising in vitro *T. b. r.* activity of these new diamidines, except the methyl analogue **15a** which showed only moderate antitrypanosomial activity, we have evaluated them and their prodrugs in the stringent STIB900 acute mouse model for *T. b. r.* which mimics first stage disease.[5, 9, 18] Since the diamidines exhibit quite high pKa values (10–11) and therefore unlikely to cross the intestinal barrier they were administered intraperitoneally ; the prodrugs were designed to enhance oral bioavailability and hence were given orally. The results are shown in Table 2 (diamidines) and Table 3 (prodrugs).

For comparative purposes Table 2 and Table 3 also contain in vivo data for the dicationic analogues pentamidine (**1**), furamidine (**2a**), azafuramidines **3a** and **4a,** the dicationic 2,5-diphenylpyrazine **6** and the prodrugs **2b**, **2c**, **3c** and **4c** in the same mouse model. [10, 14, 16, 23] On intraperitoneal dosing at 5 mg/kg all of the tested dications show a significant increase in survival time for the treated animals compared to untreated controls. The parent compound **10** gave 3/4 cures at a dose of 5 mg/kg, which is superior to that of pentamidine (**1**) and furamidine (**2a**) (1/4 cure), and is as effective as the azafuramidines **3a** and **4a** (3/4 cure). The linear 2,5-isomer **6** was less effective and gave no cure but did show an increase in mean survival time. The fluorine substituted analogue **15b** gave also no cures in the in vivo

model which is consistent with its lower in vitro activity against *T. b. r.* The four aza-analogues **19a**, **19b**, **23a** and **23b** exhibited identical or better results to that for furamidine at a dose of 5 mg/kg. The best result obtained was for compound **19a**, which showed 4/4 cures at a dose of 5 mg/kg. It is noteworthy that although compounds **19a** and **23a**, in which the nitrogen atom is *meta* to the amidine group, exhibited a significant loss of in vitro potency against *T. b. r.* compared to their isomeric compounds **19b** and **23b**, in which the nitrogen atom is *ortho* to the amidine, the in vivo efficacy of **19a** (4/4 cure) and **23a** (2/4 cure) was superior to the *ortho* isomers **19b** (2/4 cure) and **23b** (1/4 cure). This result may be due to pharmacokinetic differences between the *ortho* and *meta* isomers and/or the differential involvement of transporters. In general, the results for the aza-pyrazine analogues are consistent with those observed for the aza-furamidine system.[10]

Although previous studies of di-amidoxime and di-*O*-methylamidoxime prodrugs of the linear terphenyl diamidine analogues showed poor bioconversion and were not curative on oral administration,[14] all the di-amidoxime and di-*O*-methylamidoxime prodrugs of the dicationic 2,6-diphenylpyrazine and aza-analogues showed activity when they were administered orally to the mice during this study. In vitro metabolic stability studies using mouse liver microsomes showed that di-*O*-methylamidoxime prodrugs were biotransformed at different rates, with **12**, **21b,** and **25b** showing shorter half-life than **21a** and **25a** (Table 4). However, this difference did not translate into activity in the STIB900 mouse model, as the latter generally gave more cures, with the exception of **12** (Table 3). This disconnect is likely due to a recent finding that intrahepatic binding and efflux of diamidines formed in the hepatocytes, rather than enzymatic biotransformation of prodrugs, determined the disposition of active diamidine metabolites.[22] It should also be noted that there was a marked interspecies difference in the metabolic stability of di-*O*-methylamidoxime prodrugs, as liver microsomes derived from humans metabolized the prodrugs much faster than those from mice (Table 4). This could be due to species differences in the enzyme activity and expression level of CYP4F/cyp4f enzymes, which were shown to be responsible for catalyzing the primary *O*-demethylation of the di-*O*-methylamidoxime pafuramidine in the human liver and intestinal microsomes.[23]

Very interestingly, treatment with the amidoxime produgs **11** and **24b** resulted in cures of all mice at the oral dose of 25 mg/kg, superior to the corresponding *O*-methylamidoxime prodrugs **12** (3/4 cure) and **25b** (1/4 cure) at the same dosage. Both of the amidoximes **11** and **24b** are more potent than the amidoxime prodrug **2b** of furamidine which gave no cure at an oral dose of 100 mg/kg (Table 3).[24] These results do not parallel those observed in the furamidine series, which showed that the *O*-methylamidoximes are more effective than the amidoximes.[10] The *O*-methylamidoxime prodrug **12** of the parent compound **10** gave 3/4 cures at the oral dose of 10 mg/kg. To evaluate the bioconversion of the di-amidoxime prodrugs, mouse liver S9 fractions were used as they contain cytochrome b_5 and cytochrome b_5 reductase, which are likely required to reduce amidoxime to amidine.[25] All di-amidoxime prodrugs examined in this study were efficiently metabolized (half-lives ranged from 2 to 51 min; Table 4), supporting their potential as prodrugs to generate active diamidine metabolites in vivo, but failed to explain the superiority of oral **24b** over other di-amidoximes (except **11**) in the STIB900 mouse model. As discussed above, this observation underscores the role of intrahepatic binding and efflux from hepatocytes, rather than bioconversion, in determining the disposition and activity of active diamidine metabolites in vivo.

Given the potent in vivo activity found in the *T. b. r*. STIB900 acute mouse model **11** and **12** were selected for study in the *T. b. brucei* GVR35 mouse model for second stage disease.[5] In sharp contrast to the results found in the STIB900 model, **11** was not effective at a dosage of 100 mg/kg for five days in the GVR35 model providing no cures and only a modest increase

(69 days) in survival time (Table 5). This shows that compound **11** is only able to remove trypanosomes from the hemolymphatic compartment. However, at the same dosage **12** gave 2/5 cures. To achieve cures in the CNS mouse model drugs must cross the blood brain barrier and reach trypanocidal levels in the CSF and CNS. These additional barriers often result in CNS drug levels lower than that in blood. Hence, to attempt to compensate for this circumstance where possible we further test with increased doses. When the dosing of **12** was extended to 10 days 3/4 cures were noted showing that this compound is penetrating into the brain in sufficient concentration to cure CNS infection. The activity of **12** in the GVR35 CNS model, with 2/5 mice cured at dosage of 100 mg/kg for five days compares favorably with that for pafuramidine **2c** which showed 3/5 cured at the same dosage with the five day regimen. The efficacy of **12** is somewhat reduced from that of the *O*-methylamidoxime prodrug **4c** for treatment of second stage HAT which gave 5/5 cures at the same dosage (Table 5).[5] Nevertheless, prodrug **12** is one of only a very few compounds which have shown good activity in this CNS model. The compounds **24b** and **25a** were also selected for study in the T. b. brucei GVR35 CNS mouse model. Both compounds were not curative at the oral dosage of 100 mg/kg for five days but they extended the survival time of mice similarly to control mice treated with diminazine (at 40mg/kg ip single dose) which is a diamidine curing only first stage disease. The results for the amidoxime and *O*-methylamidoxime prodrugs of this series of dications provides stimulation for further evaluation of their efficacy and toxicity.

4. Conclusions

A series of dicationic 2,6-diphenylpyrazines and aza analogues have been prepared which exhibited DNA binding which is consistent with a role in their mode of action, showed potent in vitro activity against both *T. b. r.* and *P. f.*, and gave promising results on intraperitoneal administration in the stringent *T. b. r.* STIB900 mouse model. The diamidines **10** and **19a** exhibited in vivo efficacy (3/4 or 4/4 cures at 5 mg/kg dosage, ip) in the STIB900 model, superior to that of furamidine (**2a)**, and comparable to or better than the azafuramidines **3a** and **4a**. Eight of the ten prodrugs of the dicatonic 2,6-diphenylpyrazine and aza-analogues showed good oral activity, giving cures in the STIB900 acute mouse model. The potent *O*-methylamidoxime prodrug **12** also showed good in vivo oral efficacy in the GVR35 second stage mouse model. This series of dicationic 2,6-diphenylpyrazine analogues and their prodrugs merit further evaluation for treatment of both stages of HAT.

5. Experimental Section

5.1 Biology

5.1.1 Efficacy Studies—The in vitro assays[13] with *T. b. r.* STIB 900 and *P. f.* K1 strain as well as the efficacy studies in an acute mouse model for *T. b. r.* STIB 900[5] were carried out as previously reported. The studies in the *T. b. brucei* GVR35 mouse model for second stage disease were performed as previously described.[5] All protocols and procedures for the mouse models used in the current study were reviewed and approved by the local veterinary authorities of Canton Basel-Stadt, Switzerland. The data was generated at the time the determination of survival was still accepted by the authorities.

5.1.2 Tm Measurements—Thermal melting experiments were conducted with a Cary 300 spectrophotometer. Cuvettes for the experiment were mounted in a thermal block and the solution temperatures monitored by a thermistor in the reference cuvette. Temperatures were maintained under computer control and increased at 0.5 °C/min. The experiments were conducted in 1 cm path length quartz curvettes 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 its absorbance at 260 nm. A ratio of 0.3 moles

compound per mole of DNA was used for the complex and DNA alone was used as a control.[14] ΔTm values were determined by the peak in first derivative curves (dA/dT).[26]

5.1.3 In vitro metabolic stability assays—The procedures used were similar to a reported method.[23a] Substrate stock solutions were prepared in DMSO. DMSO content was kept at 0.5% (v/v) in final incubations. Incubation mixtures (final volume 0.25 ml) consisted of 10 μM substrate and 0.5 mg/ml pooled liver microsomes from human or mouse (XenoTech, Lenexa, KS) for *O*-methylamidoxime prodrugs, or liver S9 fraction from mouse for amidoxime prodrugs. Reactions were carried out in 100 mM phosphate buffer (pH 7.4) containing 3.3 mM $MgCl_2$. Mouse liver S9 fractions were prepared from male Swiss Webster mice (25–30 g) as previously reported. [27] Briefly, four volumes of 0.25 M sucrose containing 0.1 M KCl and 1 mM EDTA was added to mouse liver and homogenized on ice with a sonic dismembrator (Fisher Scientific, Fair Lawn, NJ). The homogenate was centrifuged at 9,000×*g* for 20 min at 4°C and then the supernatant fraction (S9 fraction) was collected and aliquoted before storing at −78°C. After a 5-min pre-equilibration period at 37°C, the metabolic stability reactions (in triplicate) were initiated by adding the cofactor (1 mM β-NADPH for microsomal incubations or a cocktail of 1 mM β-NADPH, 1 mM NADH, and 3.3 mM UDPGA for incubations with S9 fractions) and kept at 37°C. Aliquots (100 μl) of the reaction mixtures were removed at 0, 15, 30, and 60 min and individually mixed with 100 μl of ice-cold acetonitrile. The mixtures were vortex-mixed, and precipitated protein was removed by centrifugation at 1,400×*g* for 15 min. The supernatant fractions were analyzed immediately by HPLC/UV. [23a] In vitro half-lives were obtained using the one-phase exponential decay model with plateau set at zero (GraphPad Prism® 5.0, San Diego, CA).

5.2 Chemistry

5.2.1 General materials and methods—Melting points were determined on a Mel-Temp 3.0 melting point apparatus, and are uncorrected. TLC analysis was carried out on silica gel 60 F254 precoated aluminum sheets using UV light for detection. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 300 MHz or Bruker 400 MHz spectrometer using indicated solvents. Mass spectra was obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Elemental analysis were performed by 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 other solvents; in each case ^1H NMR spectra was consistent with the analysis. All chemicals and solvents were purchased from Aldrich Chemical Co., VWR International, or Combi-Blocks, Inc.

5.2.2 2,6-Di-(4′-cyanophenyl)pyrazine (9)—To a stirred solution of 2,6-dichloropyrazine (**7**, 2.0 g, 13.4 mmol) in toluene (56 mL) under nitrogen atmosphere at 80 °C was added 27 mL of 2 M aqueous solution of Na_2CO_3 followed by 4-cyanophenylboronic acid **8** (4.34 g, 29.5 mmol) in 30 mL of methanol. After 30 minutes, tetrakis(triphenylphosphine) palladium (1.34 g, 1.16 mmol) was added to the reaction mixture. The reaction mixture was stirred overnight at 80 °C. After cooling to room temperature, water was added to the mixture. The solution was filtered, and the precipitate was washed with water and MeOH. The crude product was purified by recrystallization to afford the title compound **9** (2.92 g, 77% yield); mp 298.5–299 °C. ^1H NMR (DMSO-d_6): δ 8.04 (d, J = 8.1 Hz, 4H), 8.47 (d, J = 8.1 Hz, 4H), 9.41 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 148.8, 142.1, 139.9, 133.0, 127.7, 118.6, 112.6. MS: m/z 282 (M^+). Anal. Calc. for $C_{18}H_{10}N_4$: C, 76.58; H, 3.57; N, 19.85. Found: C, 76.33; H, 3.55; N, 19.76.

5.2.3 2,6-Di-(4′-amidinophenyl)pyrazine hydrochloride (10)—The above dinitrile **9** (0.14 g, 0.48 mmol), suspended in freshly distilled THF (4 mL), was treated with lithium trimethylsilylamide (1 M solution in THF, 2.5 mL, 2.5 mmol), and the reaction was allowed to stir overnight. The reaction mixture was then cooled to 0 °C and to which was added HCl saturated ethanol (3 mL), whereupon a precipitate started forming. The mixture was allowed to stir overnight, after which it was diluted with ether, and the precipitate was filtered. The diamidine was purified by neutralization with 1 N NaOH followed by filtration of the resultant solid and washing with water. Finally, the free base was stirred with ethanolic HCl overnight and diluted with ether, and the solid formed was filtered and dried to give the diamidine salt **10** (0.14 g, 76% yield); mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 8.05 (d, J = 7.6 Hz, 4H), 8.53 (d, J = 7.6 Hz, 4H), 9.31 (s, 4H), 9.48 (s, 2H), 9.57 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 165.1, 149.1, 141.6, 140.5, 129.0, 128.9, 127.2. Anal. Calc. for $C_{18}H_{16}N_6$-2.0HCl-2.3H_2O: C, 50.19; H, 5.29; N, 19.51. Found: C, 50.31; H, 4.92; N, 19.18.

5.2.4 2,6-Di-(4′-*N*-hydroxyamidinophenyl)pyrazine hydrochloride (11)—A mixture of hydroxylamine hydrochloride (4.9 g, 70.8 mmol) in anhydrous DMSO (60 mL) was cooled to 5 °C under nitrogen. Potassium *tert*-butoxide (7.95 g, 70.8 mmol) was added in portions and the mixture was stirred for 30 minutes. To this mixture was added the above dinitrile **9** (1.0 g, 3.54 mmol) and the mixture was stirred overnight. The reaction mixture was poured slowly into a beaker with ice water and stirred for 15 minutes. The white precipitate was filtered and washed with water to afford the free base of **11**. The free base was stirred with ethanolic HCl overnight and diluted with ether, and the precipitate which formed was collected by filtration to give the title compound HCl salt **11** in 87% yield; mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 6H), 4.64 (s, 2H), 7.96 (d, J = 8.4 Hz, 4H), 8.51 (d, J = 8.4 Hz, 4H), 9.25 (s, 4H), 9.43 (s, 2H), 11.42 (s, 2H), 13.13 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 158.8, 149.1, 141.8, 140.0, 128.9, 127.3, 126.7. Anal. Calc. for $C_{18}H_{16}N_6O_2$-2.2HCl-2.0H_2O: C, 46.53; H, 4.82; N, 18.09. Found: C, 46.59; H, 4.45; N, 17.80.

5.2.5 2,6-Di-(4′-*N*-methoxyamidinophenyl)pyrazine hydrochloride (12)—A solution of lithium hydroxide monohydrate (0.24 g, 5.74 mmol) in water (4 mL) was added dropwise to the mixture of the free base of the above di-amidoxime **11** (0.43 g, 1.44 mmol) in DMF (26 mL) at room temperature. The reaction mixture was stirred for 30 minutes at room temperature. Dimethylsulfate (0.45 g, 3.59 mmol) was added to the reaction mixture and the mixture was stirred at room temperature overnight. The reaction mixture was poured slowly into a beaker with ice water and stirred for 15 minutes. The precipitate was filtered and washed with water to afford the free base of **12**. The free base was stirred with ethanolic HCl overnight and diluted with ether, and the precipitate which formed was collected by filtration to give the title compound **12** in 58% yield; mp 244–246 °C. ^{1}H NMR (DMSO-d_6): δ 3.49 (s, 8H), 3.83 (s, 6H), 7.90 (d, J = 8.4 Hz, 4H), 8.38 (d, J = 8.4 Hz, 4H), 9.33 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 156.7, 149.2, 141.6, 139.3, 128.5, 128.2, 127.1, 63.1. Anal. Calc. for $C_{20}H_{20}N_6O_2$-2.0HCl-1.5H_2O: C, 50.43; H, 5.29; N, 17.64. Found: C, 50.28; H, 5.17; N, 17.46.

5.2.6 2,6-Di-(4′-amidino-2′-methylphenyl)pyrazine hydrochloride (15a)—A solution of 2,6-di(tri-*n*-butylstannyl)pyrazine (77% purity)[17] (4.04 g, 4.68 mmol), 4-bromo-3-methylbenzonitrile **13a** (2.02 g, 10.30 mmol), tetrakis(triphenylphosphine) palladium (0.56 g, 0.56 mmol) in degassed xylene (80 mL) was heated at 120 °C under nitrogen atmosphere for 24 h. After cooling to room temperature, the mixture was filtered and the precipitate was washed with xylene and ether. The crude product was purified by recrystallization (DMF) to afford compound **14a** in 62% yield **[**mp 239–141 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 6H), 7.74 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H), 7.89 (s,

2H), 8.95 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 151.8, 143.5, 140.8, 137.8, 134.4, 131.0, 129.9, 118.5, 111.8, 19.8] and it was used directly in the next step without further characterization.

The same procedure described for the preparation of **10** was used starting with the above dinitrile **14a**; 52% yield; mp 250–252 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 6H), 7.80 (d, J = 8.1 Hz, 2H), 7.84 (d, J = 8.1 Hz, 2H), 7.89 (s, 2H), 8.98 (s, 2H), 9.32 (s, 4H), 9.52 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 165.2, 152.1, 143.4, 141.2, 137.1, 130.6, 130.6, 128.6, 125.9, 20.2. Anal. Calc. for $C_{20}H_{20}N_6$-2.5HCl-1.0H_2O: C, 52.96; H, 5.44; N, 18.53. Found: C, 52.96; H, 5.31; N, 18.26.

5.2.7 2,6-Di-(4′-amidino-2′-fluorophenyl)pyrazine hydrochloride (15b)—The same procedure described above for the preparation of **14a** was used starting with 4-bromo-3-fluorobenzonitrile **13b**; to give **14b** 62% yield [mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 8.12–8.16 (m, 2H), 8.37 (d, J = 8.4 Hz, 2H), 8.49 (d, J = 10.4 Hz, 2H), 9.48 (s, 2H). HRMS: m/z 319.0800 (M+1) (calculated for $C_{18}H_9N_4F_2$, 319.0795)] and it was used directly in the next step without further characterization.

The same procedure described for the preparation of **10** was used starting with the above dinitrile **14b**; 60% yield; mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 7.89–7.92 (m, 2H), 8.37 (d, J = 8.4 Hz, 2H), 8.44 (d, J = 12.4 Hz, 2H), 9.46 (s, 4H), 9.49 (s, 2H), 9.63 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 161.9, 159.2 (d, J = 248.2 Hz), 147.9 (d, J = 2.3 Hz), 142.0, 141.6 (d, J = 8.6 Hz), 130.7, 122.8 (d, J = 2.9 Hz), 118.3 (d, J = 13.7 Hz), 114.4 (d, J = 23.5 Hz). HRMS: *m/z* 353.1311 (M+1) (calculated for $C_{18}H_{15}N_6F_2$, 353.1326). Anal. Calc. for $C_{18}H_{14}F_2N_6$-2.0HCl-1.3H_2O: C, 48.18; H, 4.18; N, 18.73. Found: C, 48.37; H, 4.11; N, 18.67.

5.2.8 2-Chloro-6-(5′-cyanopyridin-2′-yl)pyrazine (17a)—A solution of 2-chloro-6-(tri-*n*-butylstannyl)pyrazine[17] (3.62 g, 9.0 mmol), 2-bromo-5-cyanopyridine **16a** (1.67 g, 9.0 mmol), tetrakis(triphenylphosphine) palladium (0.52 g, 0.45 mmol) in degassed xylene (20 mL) was heated at 120 °C under nitrogen atmosphere overnight. After cooling to room temperature, the mixture was filtered. Most of the solvent was removed under reduced pressure; the precipitate which formed was filtered and washed with xylene and ether. The crude product was purified by column chromatography on silica gel (eluent hexane/ethyl acetate (5/1)) to give the title compound **17a** in 67% yield; mp 148–150 °C. ^{1}H NMR (DMSO-d_6): δ 8.41 (d, J = 8.4 Hz, 1H), 8.51 (dd, *J* = 2.0, 8.4 Hz, 1H), 8.97 (s, 1H), 9.20 (d, *J* = 2.0 Hz, 1H), 9.51 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 154.9, 152.6, 148.5, 147.9, 145.7, 141.7, 141.0, 121.2, 116.7, 110.1. Anal. Calc. for $C_{10}H_5ClN_4$: C, 55.44; H, 2.33; N, 25.86. Found: C, 55.55; H, 2.28; N, 25.83.

5.2.9 2-(4′-cyanophenyl)-6-(5′-cyanopyridin-2′-yl)pyrazine (18a)—The same procedure described for 2,6-di-(4′-cyanophenyl)pyrazine **9** was used by employing 2-chloro-6-(5′-cyanopyridin-2′-yl)pyrazine **17a** and 4-cyanophenylboronic acid **8** to furnish the compound **18a** in 71% yield; mp 293–295 °C. ^{1}H NMR (DMSO-d_6):): δ 8.04 (d, J = 8.4 Hz, 2H), 8.50 (d, J = 8.4 Hz, 2H), 8.53 (dd, *J* = 2.0, 8.4 Hz, 1H), 8.71 (d, J = 8.4 Hz, 1H), 9.19 (d, *J* = 2.0 Hz, 1H), 9.48 (s, 1H), 9.57 (s, 1H). HRMS: *m/z* 284.0930 (M+1) (calculated for $C_{17}H_{10}N_5$, 284.0936). Anal. Calc. for $C_{17}H_9N_5$: C, 72.08; H, 3.20; N, 24.75. Found: C, 71.85; H, 3.17; N, 24.64.

5.2.10 2-(4′-amidinophenyl)-6-(5′-amidinopyridin-2′-yl)pyrazine hydrochloride (19a)

The same procedure described for the preparation of **10** was used starting with the above dinitrile **18a**; 90% yield; mp 296–298 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.05 (d, J = 8.4 Hz, 2H), 8.46 (dd, J = 2.0, 8.4 Hz, 1H), 8.58 (d, J = 8.4 Hz, 2H), 8.77 (d, J = 8.4 Hz, 1H), 9.17

(d, J = 2.0 Hz, 1H), 9.27 (s, 2H), 9.40 (s, 2H), 9.56 (s, 2H), 9.56 (s, 1H), 9.62 (s, 1H), 9.73 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 165.1, 163.7, 157.2, 149.0, 148.9, 148.2, 143.5, 142.1, 140.1, 137.9, 129.2, 128.9, 127.4, 125.2, 120.9. Anal. Calc. for $C_{17}H_{15}N_7$-3.0HCl-1.7H_2O: C, 44.64; H, 4.72; N, 21.44. Found: C, 44.67; H, 4.52; N, 21.44.

5.2.11 2-(4′-*N*-hydroxyamidinophenyl)-6-(5′-*N*-hydroxyamidinopyridin-2′-yl)pyrazine hydrochloride (20a)—The same procedure described for the preparation of **11** was used starting with the above dinitrile **18a**; yield 89%; mp 283–285 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.43 (s, 8H), 7.95 (d, J = 8.4 Hz, 2H), 8.36 (dd, J = 2.0, 8.4 Hz, 1H), 8.52 (d, J = 8.4 Hz, 2H), 8.68 (d, J = 8.4 Hz, 1H), 9.08 (d, J = 2.0 Hz, 1H), 9.47 (s, 1H), 9.57 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 158.8, 156.9, 156.8, 149.0, 148.7, 148.2, 143.4, 142.0, 139.8, 137.9, 128.9, 127.5, 126.8, 122.9, 121.1. Anal. Calc. for $C_{17}H_{15}N_7O_2$-3.0HCl-0.8H_2O: C, 43.16; H, 4.18; N, 20.72. Found: C, 43.15; H, 4.22; N, 20.62.

5.2.12 2-(4′-*N*-methoxyamidinophenyl)-6-(5′-*N*-methoxyamidinopyridin-2′-yl)pyrazine hydrochloride (21a)—The same procedure described for the preparation of **12** was used starting with the above compound **20a;** 29% yield; mp 213–215 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.43 (s, 8H), 3.83 (s, 3H), 3.85 (s, 3H), 7.92 (d, J = 8.4 Hz, 2H), 8.29 (dd, J = 2.0, 8.4 Hz, 1H), 8.39 (d, J = 8.4Hz, 2H), 8.57 (d, J = 8.4 Hz, 1H), 9.03 (d, J = 2.0 Hz, 1H), 9.38 (s, 1H), 9.52 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 157.5, 155.1, 152.7, 149.0, 148.4, 147.7, 143.0, 141.8, 139.5, 136.6, 128.7, 127.4, 127.3, 126.1, 121.0, 63.4, 62.3. Anal. Calc. for $C_{19}H_{19}N_7O_2$-3.0HCl-0.7H_2O: C, 45.70; H, 4.72; N, 19.63. Found: C, 45.91; H, 4.67; N, 19.29.

5.2.13 2-Chloro-6-(2′-cyanopyridin-5′-yl)pyrazine (17b)—The same procedure described for 2-chloro-6-(5′-cyanopyridin-2′-yl)pyrazine **17a** was used by employing 2-chloro-6-(tri-*n*-butylstannyl)pyrazine [17] and 2-bromo-5-cyanopyridine **16b** to furnish the title compound **17b** in 52% yield; mp 149–151 °C. ^{1}H NMR (DMSO-d_6): δ 8.24 (d, J = 8.4 Hz, 1H), 8.71 (dd, *J* = 2.0, 8.4 Hz, 1H), 8.91 (s, 1H), 9.45 (d, *J* = 2.0 Hz, 1H), 9.46 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 149.4, 148.1, 147.7, 144.7, 141.3, 136.0, 133.5, 133.4, 129.2, 117.3. Anal. Calc. for $C_{10}H_5ClN_4$: C, 55.44; H, 2.33; N, 25.86. Found: C, 55.67; H, 2.30; N, 25.60.

5.2.14 2-(4′-amidinophenyl)-6-(2′-amidinopyridin-5′-yl)pyrazine hydrochloride (19b)—The same procedure described for 2,6-di-(4′-cyanophenyl)pyrazine **9** was used by employing 2-chloro-6-(2′-cyanopyridin-5′-yl)pyrazine **17b** and 4-cyanophenylboronic acid **8** to furnish the compound **18b** in 88% yield [mp 271–273 °C. ^{1}H NMR (DMSO-d_6): δ 8.06 (d, J = 8.4 Hz, 2H), 8.26 (d, J = 8.4 Hz, 1H), 8.51 (d, J = 8.4 Hz, 2H), 8.90 (dd, *J* = 2.0, 8.4 Hz, 1H), 9.48 (s, 1H), 9.50 (s, 1H), 9.63 (d, *J* = 2.0Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 149.5, 149.0, 147.0, 142.6, 142.4, 139.6, 135.9, 134.6, 133.3, 133.0, 129.2, 127.8, 118.6, 117.4, 112.7] and it was used directly in the next step without further characterization.

The same procedure described for the preparation of **10** was used starting with the above dinitrile **18b;** yield 87%; mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 8.8 Hz, 2H), 8.52 (d, J = 8.4 Hz, 1H), 8.55 (d, J = 8.8 Hz, 2H), 9.02 (dd, J = 2.0, 8.4 Hz, 1H), 9.21 (s, 2H), 9.46 (s, 2H), 9.51 (s, 2H), 9.51 (s, 1H), 9.54 (s, 1H), 9.65 (d, J = 2.0 Hz, 1H), 9.70 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 165.1, 161.6, 149.3, 148.1, 147.2, 144.7, 142.6, 142.4, 140.2, 136.4, 135.3, 129.3, 128.9, 127.4, 123.6. Anal. Calc. for $C_{17}H_{15}N_7$-2.0HCl-1.55H_2O: C, 48.83; H, 4.84; N, 23.44. Found: C, 49.15; H, 4.78; N, 23.07.

5.2.15 2-(4′-*N*-hydroxyamidinophenyl)-6-(2′-*N*-hydroxyamidinopyridin-5′-yl)pyrazine hydrochloride (20b)—The same procedure described for the preparation of **11** was used starting with the above dinitrile **18b**; yield 87%; mp 291–293 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.44 (s, 4H), 7.95 (d, J = 8.4 Hz, 2H), 8.24 (d, J = 8.4 Hz, 1H), 8.53 (d, J = 8.8 Hz, 2H), 8.87 (dd, J = 2.0, 8.4 Hz, 1H), 9.21 (s, 2H), 9.45 (s, 1H), 9.49 (s, 1H), 9.56 (d, J = 2.0 Hz, 1H), 11.01 (s, 1H), 11.20 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 158.7, 154.7, 149.2, 147.9, 147.4, 145.0, 142.3, 142.1, 139.7, 136.0, 134.4, 128.9, 127.4, 126.8, 123.0. Anal. Calc. for $C_{17}H_{15}N_7O_2$-2.0HCl-1.4H_2O: C, 45.63; H, 4.46; N, 21.91. Found: C, 45.99; H, 4.40; N, 21.56.

5.2.16 2-(4′-*N*-methoxyamidinophenyl)-6-(2′-*N*-methoxyamidinopyridin-5′-yl)pyrazine hydrochloride (21b)—The same procedure described for the preparation of **12** was used starting with the above compound **20b**; yield 37%; mp 160–162 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H), 3.88 (s, 3H), 3.89 (s, 8H), 7.96 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 8.4 Hz, 1H), 8.46 (d, J = 8.0 Hz, 2H), 8.87 (dd, J = 2.0, 8.4 Hz, 1H), 9.41 (s, 1H), 9.43 (s, 1H), 9.47 (d, J = 2.0 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 157.3, 150.7, 149.3, 148.3, 147.9, 147.2, 141.8, 141.7, 139.5, 135.5, 132.8, 128.7, 127.6, 127.2, 121.1, 63.4, 62.0. Anal. Calc. for $C_{19}H_{19}N_7O_2$-2.0HCl-2.0H_2O: C, 46.92; H, 5.18; N, 20.16. Found: C, 46.98; H, 5.09; N, 19.93.

5.2.17 2,6-Di-(5′-cyanopyridin-2′-yl)pyrazine (22a)—The same procedure described for 2,6-di-(4′-cyano-2′-methylphenyl)pyrazine **14a** was used by employing 2,6-di(tri-*n*-butylstannyl)pyrazine and 2-bromo-5-cyanopyridine **16a** to furnish the title compound **22a** in 58% yield; mp 264–266 °C (dec).^{1}H NMR (DMSO-d_6): δ 8.69 (dd, J = 2.1, 8.4 Hz, 2H), 8.80 (d, J = 8.4 Hz, 2H), 9.23 (s, 2H), 9.67 (d, J = 2.1 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 155.9, 152.6, 147.8, 143.8, 141.6, 121.4, 116.9, 110.0. Anal. Calc. for $C_{16}H_8N_6$: C, 67.60; H, 2.84; N, 29.56. Found: C, 67.41; H, 2.73; N, 29.31.

5.2.18 2,6-Di-(5′-amidinopyridin-2′-yl)pyrazine hydrochloride (23a)—The same procedure described for the preparation of **10** was used starting with the above dinitrile **22a**; 50% yield; mp 277–279 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.48 (dd, J = 2.4, 8.4 Hz, 2H), 8.86 (d, J = 8.1 Hz, 2H), 9.18 (d, *J* = 2.4 Hz, 2H), 9.41 (s, 4H), 9.72 (s, 2H), 9.74 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 163.7, 156.9, 149.0, 148.1, 143.6, 138.0, 125.2, 121.1. Anal. Calc. for $C_{16}H_{14}N_8$-2.0HCl-2.5H_2O: C, 44.05; H, 4.85; N, 25.68. Found: C, 44.08; H, 4.67; N, 25.39.

5.2.19 2,6-Di-(5′-*N*-hydroxyamidinopyridin-2′-yl)pyrazine hydrochloride (24a)—The same procedure described for the preparation of **11** was used starting with the above dinitrile **22a** in 97% yield; mp 274–276 °C (dec).^{1}H NMR (DMSO-d_6-D_2O): δ 3.52 (s, 6H), 8.32 (dd, J = 2.0, 8.4 Hz, 2H), 8.74 (d, J = 8.4 Hz, 2H), 9.03 (d, J = 2.0 Hz, 2H), 9.65 (s, 2H), 11.08 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 156.4, 156.2, 148.6, 148.1, 143.4, 137.6, 123.5, 121.2. Anal. Calc. for $C_{16}H_{14}N_8O_2$-2.0HCl-2.1H_2O: C, 41.68; H, 4.42; N, 24.30. Found: C, 41.71; H, 4.30; N, 24.30.

5.2.20 2,6-Di-(5′-*N*-methoxyamidinopyridin-2′-yl)pyrazine hydrochloride (25a)—The same procedure described for the preparation of **12** was used starting with the above compound **24a**; yield 58%; mp 216–218 °C (dec). ^{1}H NMR (DMSO-d_6): δ 3.82 (s, 6H), 4.00 (s, 6H), 6.70 (s, 2H), 8.27 (dd, J = 1.8, 8.4 Hz, 2H), 8.64 (d, J = 8.4 Hz, 2H), 9.04 (d, J = 1.8 Hz, 2H), 9.61 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 154.5, 151.4, 148.4, 147.4, 142.8, 135.9, 127.2, 121.1, 61.9. Anal. Calc. for $C_{18}H_{18}N_8O_2$-3.0HCl-2.3H_2O: C, 40.85; H, 4.88; N, 21.17. Found: C, 41.01; H, 4.89; N, 21.07.

5.2.21 2,6-Di-(2′-cyanopyridin-5′-yl)pyrazine (22b)—The same procedure described for 2,6-di-(4′-cyano-2′-methylphenyl)pyrazine **14a** was used by employing 2,6-di(tri-*n*-butylstannyl)pyrazine and 5-bromo-2-cyanopyridine **16b** to furnish the title compound **22b;** 70% yield; mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 8.27 (d, J = 8.0 Hz, 2H), 8.94 (dd, J = 2.0, 8.0 Hz, 2H), 9.54 (s, 2H), 9.66 (d, J = 2.0 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ149.6, 147.2, 143.0, 136.1, 134.4, 133.4, 129.2, 117.4. Anal. Calc. for $C_{16}H_8N_6$: C, 67.60; H, 2.84; N, 29.56. Found: C, 67.34; H, 2.73; N, 29.28.

5.2.22 2,6-Di-(2′-amidinopyridin-5′-yl)pyrazine hydrochloride (23b)—The same procedure described for the preparation of **10** was used starting with the above dinitrile **22b**; 83% yield; mp 249–251 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.58 (d, J = 8.1 Hz, 2H), 9.06 (dd, J = 1.2, 8.1 Hz, 2H), 9.59 (s, 4H), 9.61 (s, 2H), 9.68 (d, *J* = 1.2 Hz, 2H), 9.78 (s, 4H). ^{13}C NMR (DMSO-d_6): δ 151.3, 147.9, 147.6, 147.3, 142.0, 135.6, 133.0, 121.5. Anal. Calc. for $C_{16}H_{14}N_8$-2.0HCl-1.4H_2O: C, 46.14; H, 4.55; N, 26.91. Found: C, 46.42; H, 4.52; N, 26.52.

5.2.23 2,6-Di-(2′-*N*-hydroxyamidinopyridin-5′-yl)pyrazine hydrochloride (24b)—The same procedure described for the preparation of **11** was used starting with the above dinitrile **22b**; 69% yield; mp 280–282 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.40 (dd, J = 2.1, 8.4 Hz, 2H), 8.80 (d, J = 8.4 Hz, 2H), 8.82 (s, 2H), 9.10 (d, J = 2.1 Hz, 2H), 9.68 (s, 2H), 11.28 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 155.0, 148.0, 147.6, 144.8, 142.7, 136.2, 134.4, 123.2. Anal. Calc. for $C_{16}H_{14}N_8O_2$-3.0HCl-0.75H_2O: C, 40.61; H, 3.94; N, 23.68. Found: C, 40.81; H, 4.18; N, 23.35.

5.2.24 2,6-Di-(2′-*N*-methoxyamidinopyridin-5′-yl)pyrazine hydrochloride (25b)—The same procedure described for the preparation of **12** was used starting with the above compound **24b**; 50% yield; mp 89–91 °C. ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 6H), 8.07 (d, J = 8.4 Hz, 2H), 8.72 (dd, J = 2.4, 8.4 Hz, 2H), 9.43 (s, 2H), 9.46 (d, J = 2.4 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 150.1, 149.0, 148.3, 146.9, 141.5, 135.0, 131.8, 119.9, 61.2. Anal. Calc. for $C_{18}H_{18}N_8O_2$-2.0HCl-0.7H_2O: C, 46.60; H, 4.65; N, 24.15. Found: C, 46.80; H, 4.79; N, 23.95.

Acknowledgments

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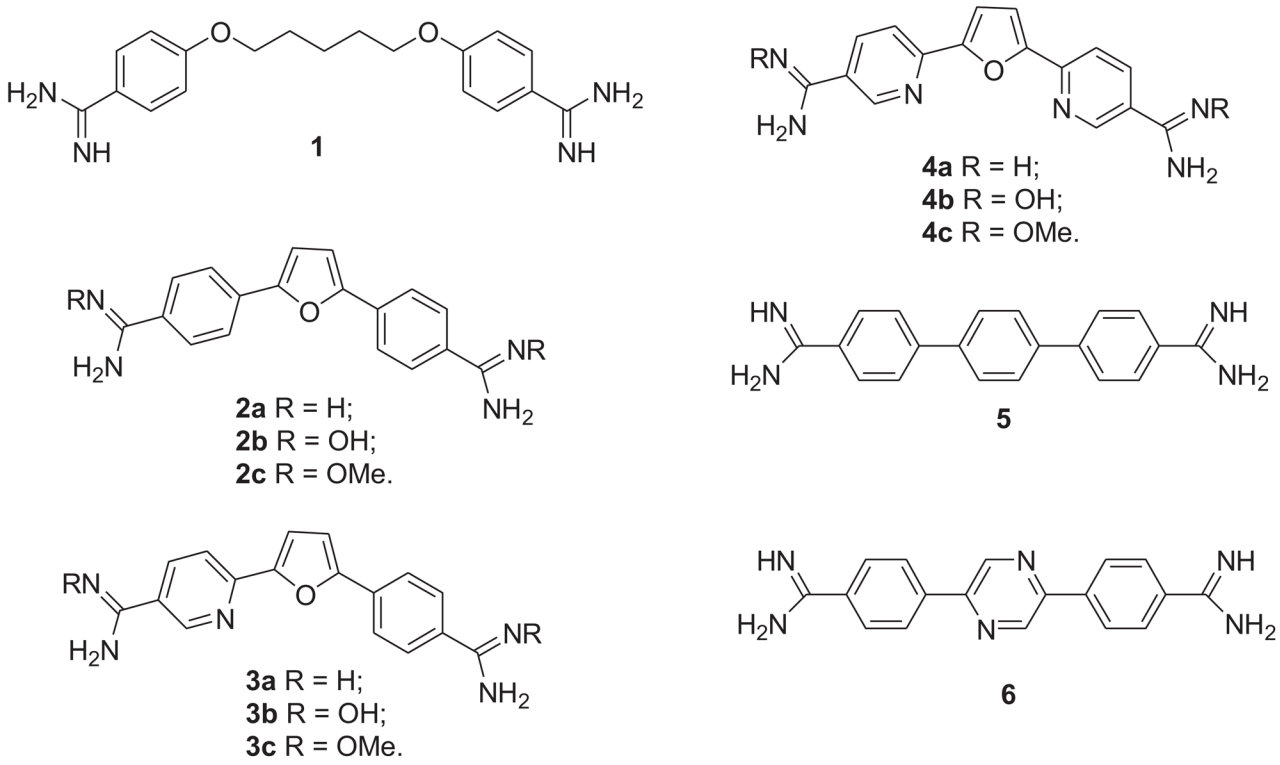
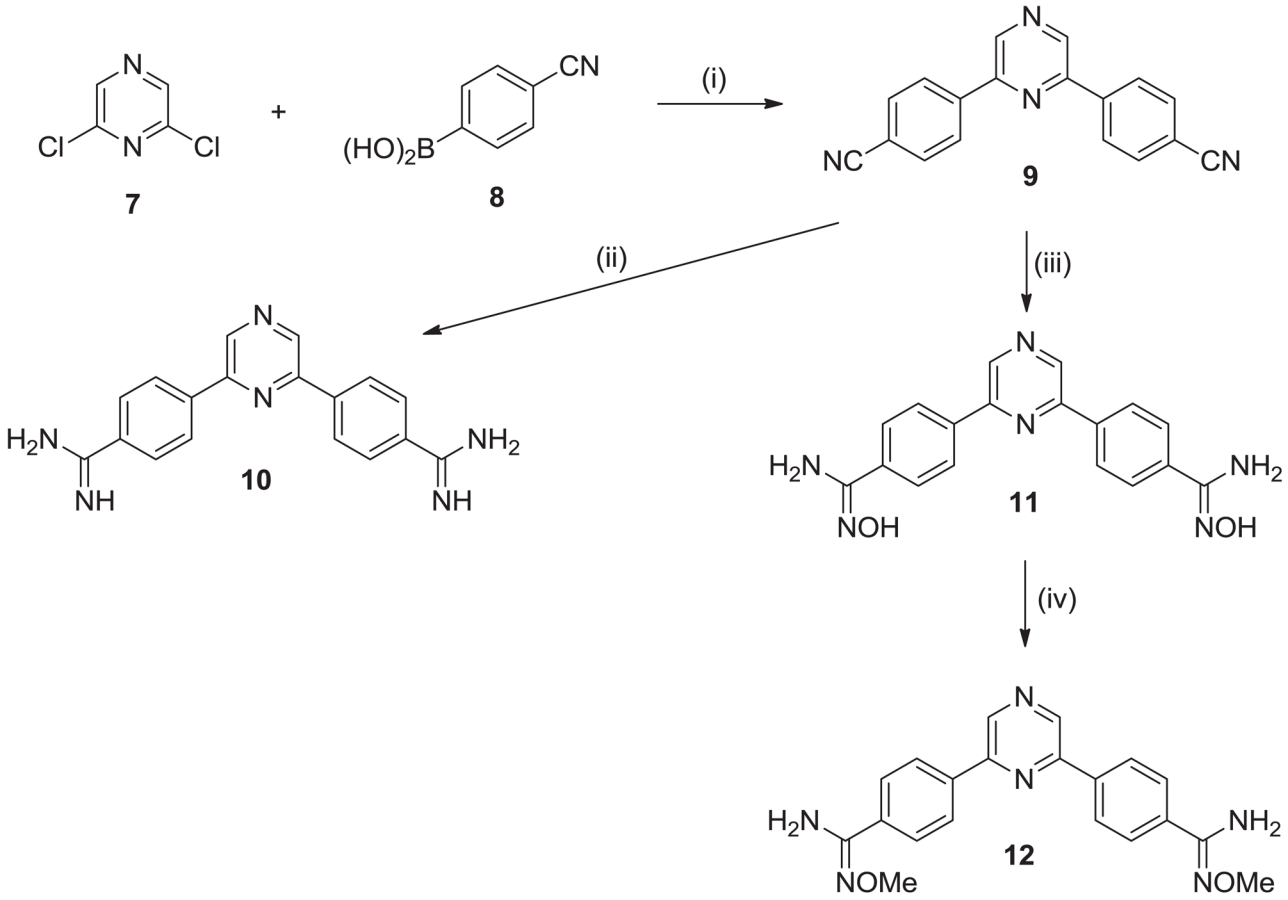
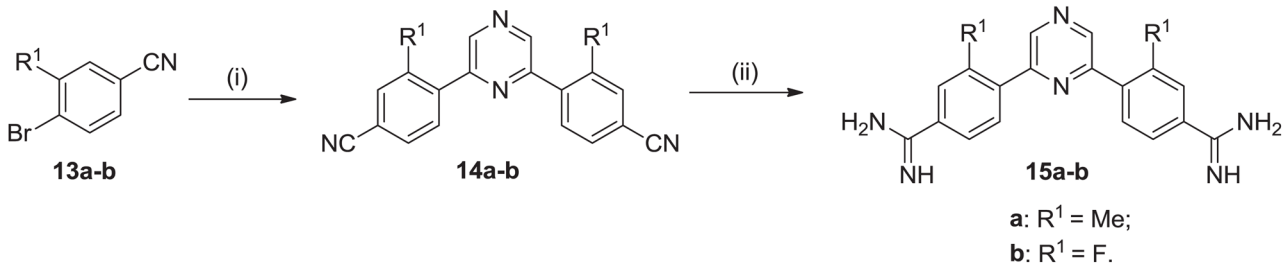


Figure 1.
Aromatic diamidine antiprotozoal agents.



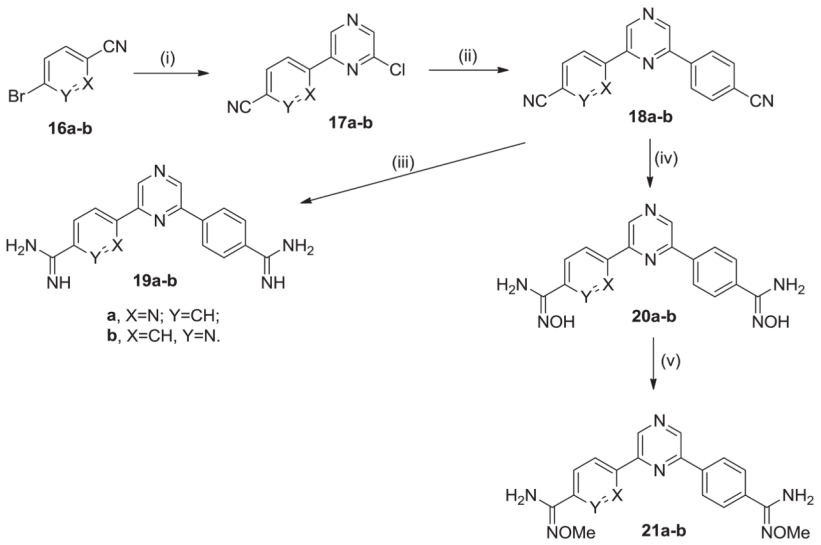
Scheme 1. Reagents and conditions

(i) $Pd(PPh_3)_4$, Na_2CO_3, toluene, 80 °C; (ii) (a) $LiN(TMS)_2$, THF; (b) HCl(gas), EtOH; (iii) NH_2OH-HCl, KO*t*-Bu, DMSO; (iv) LiOH, $(CH_3)_2SO_4$, DMF.



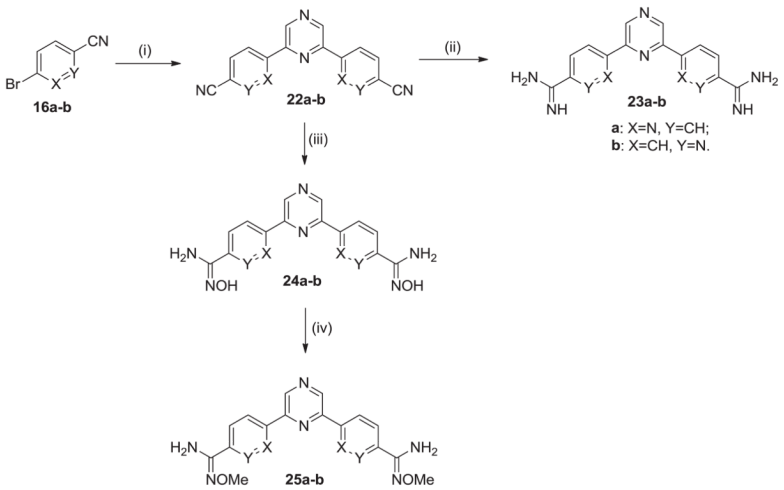
Scheme 2. Reagents and conditions

(i) 2,6-bis(tri-*n*-butylstannyl)pyrazine, $Pd(PPh_3)_4$, xylene, 120 °C; (ii) (a) $LiN(TMS)_2$, THF; (b) HCl(gas), EtOH.



Scheme 3.

Regents and conditions: (i) 2-chloro-6-(tri-*n*-butylstannyl)pyrazine, $Pd(PPh_3)_4$, xylene, 120 °C; (ii) $Pd(PPh_3)_4$, Na_2CO_3, toluene, 80 °C; (iii) (a) $LiN(TMS)_2$, THF; (b) HCl(gas), EtOH; (iv) NH_2OH-HCl, KO*t*-Bu, DMSO; (v) LiOH, $(CH_3)_2SO_4$, DMF.

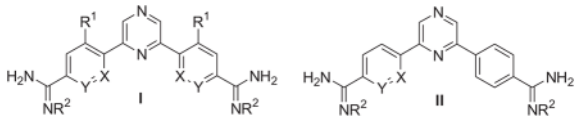


Scheme 4. Reagents and conditions

(i) 2,6-bis(tri-*n*-butylstannyl)pyrazine, $Pd(PPh_3)_4$, xylene, 120 °C; (ii) (a) $LiN(TMS)_2$, THF; (b) HCl(gas), EtOH; (iii) NH_2OH-HCl, KO*t*-Bu, DMSO; (iv) LiOH, $(CH_3)_2SO_4$, DMF.

Table 1

DNA affinities and antiprotozoan activity for 2,6-diarylpyrazine diamidines.



Code	Structure type	X	Y	R^1	R^2	ΔTm (°C) [a]	*T. b. r.* IC_{50} (nM) [b]	*P. f.* IC_{50} (nM) [b]	Cytotoxicity IC_{50} (nM) [c]
1 pentam idne	/	/	/	/	/	12.6	2.2	46.4	2,100
2a	/	/	/	/	/	25	4.5	15.5	6,400
3a	/	/	/	/	/	19.3	6.5	6.5	77,900
4a	/	/	/	/	/	15.5	21	83	83,000
6	/	/	/	/	/	8.0	18	0.4	42,500
10	I	CH	CH	H	H	15.1	5.8	10	80,800
11	I	CH	CH	H	OH	/	7,950	870	2,900
12	I	CH	CH	H	OMe	/	18,900	410	58,700
15a	I	CH	CH	Me	H	5.1	462	323	117,000
15b	I	CH	CH	F	H	8.2	27	27	29,800
19a	II	N	CH	/	H	13.1	14	52	117,000
20a	II	N	CH	/	OH	/	7,780	8,400	24,600
21a	II	N	CH	/	OMe	/	25,300	5,600	>180,000
19b	II	CH	N	/	H	15.1	6.0	10	34,100
20b	II	CH	N	/	OH	/	10,800	453	5,000
21b	II	CH	N	/	OMe	/	194,000	453	>185,000
23a	I	N	CH	H	H	11.2	37	31	139,000
24a	I	N	CH	H	OH	/	90,600	7,010	>196,000
25a	I	N	CH	H	OMe	/	119,600	7,390	20,100
23b	I	CH	N	H	H	12.1	4.8	85	24,400
24b	I	CH	N	H	OH	/	95,400	785	>190,000
25b	I	CH	N	H	OMe	/	7,870	328	>212,000

[a] Increase in thermal melting of poly(dA-dT)2; see refs 14 and 26.

[b] The *T. b. r.* (*Trypanosoma brucei rhodesiense*) strain was STIB900, and the *P. f.* (*Plasmodium falciparum*) strain was K1. The IC50 values are the mean of two independent assays. Individual values differed by less than 50% of the mean see ref 17.

[c] Cytotoxicity was evaluated using cultured L6 rat myoblast cells; see refs 14 and 18.

Table 2

In vitro and in vivo anti-trypanosomal activity of 2,6-diarylpyrazine diamidines in the STIB900 mouse model.[a]

Code	T. b. r. IC_{50}(nM)	Dosage (ip, mg/kg) [b]	Cures [c]	Survival (days) [d]
1	2.2	20	2/4	>57.5
		5	1/4	>38
2a	4.5	20	3/4	>57.75
		5	1/4	>46
3a	6.5	20	4/4	>60
		5	3/4	>54.5
4a	21	20	4/4	>60
		5	3/4	>49.5
6	18	5	0/4	36.5
10	5.8	5	3/4	>53.5
15a	462	/	/	/
15b	27	5	0/4	>41
19a	14	5	4/4	>60
19b	6	5	2/4	>60
23a	37	5	2/4	>53.75
23b	4.8	5	1/4	>36.5

[a] See Refs. 5 and 18 for details of STIB900 mouse model.

[b] Daily dosage was administered for 4 days; ip, intraperitoneal.

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

[d] Average days of survival; untreated control expires between day 7 and 9 post-infection.

Table 3

In vivo anti-trypanosomal activity of 2,6-diarylpyrazine diamidine prodrugs in the STIB900 mouse model.[a]

Code	Dosage (po, mg/kg)[c]	Cures [d]	Survival (days)[e]
2b(2a)	100	0/4	50
2c(2a)	25	4/4	>60
	10	4/4	>60
3c(3a)	25	4/4	>60
4c(4a)	25	2/4	>42
11(10)	25	4/4	>60
	10	0/4	22.5
12(10)	25	3/4	>53.5
	10	3/4	>56.5
20a(19a)	25	2/4	>45.75
21a(19a)	25	3/4	>57.5
	10	1/4	>49
20b(19b)	25	2/4	>50
21b(19b)	25	2/4	>50
24a(23a)	25	0/4	23.75
25a(23a)	25	2/4	>38.75
24b(23b)	25	4/4	>60
	10	1/4	>33.75
25b(23b)	25	1/4	>43.25

[a] See Refs. 5 and 18 for details of STIB900 mouse model.

[b] Code for parent of prodrug in parenthesis.

[c] Daily dosage was administered for 4 days.

[d] Number of mice that survive and are parasite free for 60 days.

[e] Average days of survival; untreated control expires between day 7 and 9 post-infection.

Table 4

In vitro metabolic stability of 2,6-diarylpyrazine diamidine prodrugs

Code	Mouse $t_{1/2}$ (min)[a]	Human $t_{1/2}$ (min)[a]
2b	29 ± 10[#]	ND[b]
2c	150 ± 10[c]	6.8 ± 2[c]
11	1.9 ± 0.1[#]	ND
12	26 ± 6	8.6 ± 0.9
20a	30 ± 4[#]	ND
21a	210 ± 100	7.6 ± 0.3
20b	36 ± 5[#]	ND
21b	35 ± 6	6.5 ±1.2
24a	20 ± 0.4[#]	ND
25a	200 ± 70	70 ± 23
24b	51 ± 1.4[#]	ND
25b	59 ± 10	14 ± 6

[#]Bis-amidoxime prodrugs were incubated with mouse liver S9 fraction, instead of liver microsomes.

[a]Mean ± standard deviations of triplicate determinations.

[b]ND, not determined.

[c]Substrate concentration for **2c** was 3 μM and its $t_{1/2}$ was shown as mean and range of duplicate determinations.

Table 5

In vivo anti-trypanosomal activity of 2,6-diarylpyrazine diamidine prodrugs in the GVR35 CNS mouse model.[a]

Code[b]	Dosage (po, mg/kg)/no. of days administered	No. of mice cured [c]	MSD[d]
2c[e](2a)	100/5	3/5	>167.8
3c[e](3a)	100/5	5/5	>180
4c[e](4a)	100/5	5/5	>180
11(10)	100/5	0/5	69
12(10)	100/5	2/5	>173.8
	100/10	3/4	>180.0
24b(23b)	100/5	0/5	67.2
25a(23a)	100/5	0/5	95.2

[a] See Ref. 5 for details of GVR35 CNS mouse model.

[b] Code for parent of prodrug in parenthesis.

[c] Cure defined as survival for more than 180 days after infection without showing a parasitemia relapse.

[d] MSD (mean survival days) was determined for mice with and without parasitemia relapse.

[e] Data from Ref. 5