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Synthesis, DNA binding and antileishmanial activity of low molecular weight bis-arylimidamides

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Abstract

The effects of reducing the molecular weight of the antileishmanial compound DB766 on DNA binding affinity, antileishmanial activity and cytotoxicity are reported. The bis-arylimidamides were prepared by the coupling of aryl *S*-(2-naphthylmethyl)thioimidates with the corresponding amines. Specifically, we have prepared new series of bis-arylimidamides which include **3a, 3b, 6, 9a, 9b, 9c, 13**, and **18**. Three compounds **9a, 9c**, and **18** bind to DNA with similar or moderately lower affinity to that of DB766, the rest of these compounds either show quite weak binding or no binding at all to DNA. Compounds **9a, 9c**, and **13** were the most active against *L. amazonensis* showing IC_{50} values of less than 1 μM, so they were screened against intracellular *L. donovani* showing outstanding activity with IC_{50}.values of 25–79 nM. Despite exhibiting little in vitro cytotoxicity these three compounds were quite toxic to mice.

Keywords

Leishmaniasis; arylimidamides; DB766; antileishmanial agents

1. Introduction

In rural developing countries protozoan parasitic diseases, which have plagued mankind for centuries, continue to cause significant public health problems. Leishmaniasis, a potentially fatal protozoal tropical disease, is caused by parasites of the genus *Leishmania* which include as many as 20 species that are pathogenic for humans [1]. The three main clinical manifestations of leishmaniasis are cutaneous, mucocutaneous, and visceral leishmaniasis, with symptoms ranging from skin and mucosal ulceration to a systemic infection that is fatal if left untreated [2]. An estimated 12 million people are currently infected with *Leishmania*

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and up to 350 million people in 88 countries are at risk of infection [3]. Approximately 2 million new cases of leishmaniasis are believed to occur annually, 0.5 million involving visceral leishmaniasis and 1.5 million with cutaneous leishmaniasis. A number of drugs have been used to treat leishmaniasis including pentostam, glucantime, amphotericin B, miltefosine, and pentamidine, but all of these have one or more limitations, including the development of resistance, cost, parenteral administration, long treatment regimens, and various toxicities [4–8]. From this brief survey of the current status of chemotherapy for this disease, it is clear that an urgent need exists for development of new effective and safe therapies for treating leishmaniasis.

In our efforts to find promising antileishmanial agents from aromatic diamidines and their analogues, we identified a new class of molecules, arylimidamides (I , Figure 1, AIAs; previously referred to as “reversed” amidines [9, 10], that exhibited submicromolar 50% inhibitory concentrations (IC_{50}s) in the axenic *Leishmania donovani* amastigote assay and the *L. donovani* infected macrophage assay [9]. Sub 100-nanomolar IC_{50} values were found in a few cases against *L. amazonensis* intracellular parasites [11] and nanomolar IC_{50}.values were observed against promastigotes of *L. infantum, L. major* and *L. tropica* [12,13]. DB766 (Figure 1), a relatively high molecular weight compound, is among the most potent antileishmanial AIAs, with an IC_{50}.value < 0.1 μM. This in vitro activity is similar to that of amphotericin B and substantially greater than miltefosine and paromomycin [14]. The AIAs, including DB766, also have significant activity against *T. cruzi* 15]. Recently, anti-inflammatory and anticancer activity of this class of compounds has been described [16].

AIAs significantly differ from classical diamidines (pentamidine/furamidine analogues) as they are much less basic (pKs in the 7–8 range as opposed to 10–12 for amidines) and this property is thought to give rise to their enhanced oral bioavailability [14]. Little is known about the mode of action of AIAs, despite the fact that they were originally conceived as DNA minor groove binders [10]. Their DNA affinity has been found to be highly variable with structure, ranging from quite weak to moderately strong [10,14,17]. It has been shown that the *T. cruzi* activity of AIAs is not correlated with their binding to *T. cruzi* kinetoplast DNA [17]. Given the potent antiprotozoal activity and promising pharmacokinetic properties of AIAs, despite their relatively high molecular weights (e.g. DB766), we decided to embark upon SAR studies of this class of compounds by exploring low molecular weight AIAs. In this study, we have prepared four different types of low molecular weight AIAs including (i) replacement of the diphenylfuran moiety of DB766 with only a phenyl group (**3a, 3b, 6**), (ii) removing the furan ring of DB766 (**9a, 13**), (iii) replacement of the furan ring of DB766 with a single atom (**9b, 9c**), (iv) replacement of the furan ring of DB766 with a 3-atom linker which can approximate the geometry of the furan ring of DB766 (**18**).

2. Results and Discussion

2. 1. Chemistry

Scheme 1 outlines our approach to the synthesis of the target compounds **3a,b**. Compounds **3a,b** were prepared by stirring the diamine **1** with 2.2 equivalents of *S*-(2-naphthylmethyl)thioimidate hydrobromide **2a,b** [9,10] in anhydrous ethanol/acetonitrile mixture at room temperature to give the hydrobromide salt. Purification was achieved by conversion to the free base followed by making the dihydrochloride salts of **3a,b**.

Scheme 2 outlines the synthesis of target compound **6**. 2-Aminopyridine **5** was allowed to react with dicyanobenzene **4** in the presence of sodium bis(trimethylsilyl)amide, a strong non-nucleophilic base, in tetrahydrofuran at −80°C [18] followed by addition of ethanolic hydrochloride to form the hydrochloride salt of compound **6**.

Scheme 3 outlines the synthesis of target compounds **9a–c**. The commercial dinitro compounds **7a–c** were reduced through reaction with a mixture of hydrazine and Raney nickel in methanol [19] to give the corresponding diamines **8a–c** in very good yield (87–95%). These diamines were allowed to react with *S*-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide **2a** to give the corresponding arylimidamides **9a–c** as discussed before.

Scheme 4 outlines the synthesis of the target compound **13**. The dinitro biaryl compound **11** was achieved by a one-pot Suzuki-Miyaura homocoupling process [20] using the bromo compound **10**, bis(pinacolato)diboron, $PdCl_2(dppf)_2$, and potassium acetate in dimethylformamide as solvent. The dinitro compound **11** was reduced to the diamine **12** using hydrazine and Raney nickel as discussed before. The diamine was conveniently converted to the arylimidamide **13** through reaction with *S*-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide **2a**.

Scheme 5 outlines the synthesis of the target compound **18**. The dinitro compound **16** was prepared through alkylation of 4-nitrobenzyl alcohol **14** with p-nitrobenzyl bromide **15** using sodium hydride as the base and a catalytic amount of sodium iodide in dimethylformamide as solvent. The diamine **17** was obtained in good yield through reduction of the dinitro compound **16** using hydrazine and Raney nickel as discussed before. The arylimidamide **18** was conveniently prepared through reaction of the diamine **17** with *S*-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide **2a.**

2. 2. Biology

Table 1 contains the DNA binding affinities for the new lower molecular weight AIAs as well as the in vitro activity for these compounds against*Leishmania spp.* For comparative purposes, similar data for DB766 are included. The thermal melting increase ΔTm (Tm of complex – Tm of free DNA) is a rapid and reliable method for ranking binding affinities for large numbers of aryldiamidines and arylimidamides [21]. The ΔTm values for the complexes between poly (dA-dT) and the new analogues range from 0 to 6.0°C. The three smallest AIAs (**3a, 3b, 6**) essentially do not bind to DNA. The parent biphenyl analogue **9a** binds with the same affinity as DB766,however, the bis-*i*-propoxybiphenyl analogue **13** essentially does not bind to DNA, likely due to the quite twisted biphenyl unit. The two analogues with one atom linkers between the two phenyl groups **9b** and **9c** give ΔTm values of 2.1 and 5.2 °C, respectively. The ΔTm differences are also likely to be due to differences in the twist of the two molecules. The molecule with a three atom linker **18** exhibits modest DNA binding with a ΔTm value of 4.0°C.

Also in Table 1, the activity of the new AIAs against *L. amazonensis* in vitro and their cytotoxicity to L6 rat myoblast cells is presented. The lowest molecular weight analogues in this study **3a, 6**, and **3b** are, in general, the least active against *L. a.*, giving IC_{50}.values ranging from 5 to > 10 μM. The biphenyl analogues **9a** and **13** show promising activity with IC_{50}.values of less than 1 μM. The two analogues **9c** and **9b**, which have the two AIA separated by a single atom linker, show unexpectedly divergent IC_{50}.values of 0.67 and >10 μM. The compound **18**, with a 3-atom linker which can approximate the geometry of the furan ring of DB766, gives an IC_{50}.value of 1.1 μM. The selectivity indices (SI = IC_{50}cytotox / IC_{50}*L. d*.) of the three most active compounds **9a**, **9c**, and **13** are 594, 178 and 916, respectively. To further evaluate the antileishmanial potential of low molecular weight AIAs, the three most active compounds (**9a**, **13**, **9c**) with submicromolar IC_{50}.values against *L. amazonensis* were screened against intracellular *L. donovani* (see Table 1). These compounds apparently enter macrophages rather well as the IC_{50}.values for (**9a**, **13**, **9c**) are 79, 25 and 28 nM, respectively. Given this activity and selectivity, these compounds (**9a, 13**,

9c) were advanced to animal studies. Unfortunately, in preliminary toxicity evaluations all three of these compounds caused severe tremors in uninfected animals 10 minutes after administered intraperitoneally at a dose of 30 mg/kg. Considering the potency of these compounds against *L. donovani* in vitro we tested one of these compounds (**9a**) for its toxicity at a dose of 10 mg/kg, again by the intraperitoneal route. However, similar adverse reactions were also observed 40 minutes after administration of this lower dose of **9a**. Due to their high in vivo toxicity, these compounds could not be evaluated in the murine visceral leishmaniasis model.

3. Conclusion

Four small series of low molecular weight AIAs derived from DB766 have been synthesized and three compounds from two of the series were found to have excellent activity versus intracellular *L. donovani* and acceptable selectivity indices. Our studies show that relatively low molecular weight AIAs can retain high activity against *Leishmania sp*. However, these molecules were highly toxic to mice and additional efforts are required to determine if structural modifications can be found which retain antileishmanial activity and eliminate toxicity.

4. Experimental

4.1. Biology

4.1.1. In vitro Efficacy Studies—The antileishmanial efficacy of the compounds against intracellular *Leishmania amazonensis* parasites was measured as described by Delfin *et al* 22]. Efficacy of the compounds against intracellular *Leishmania donovani* was assessed by the method of Zhu *et al* 23].

4.1.2. Cytotoxicity Measurements—The in vitro cytotoxicity of the compounds was determined as described by Bakunov *et al* 24].

4.1.3. 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 10 mM, EDTA 1 mM, NaCl 100 mM 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 [21]. ΔTm values were determined by the peak in first derivative curves (dA/dT).

4.2 Chemistry

All commercial solvents and reagents were used without purification. 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. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using the indicated solvents. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or other solvents; in each case ^{1}H NMR spectra were consistent with the presence of water or organic solvent(s).Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

General procedure for the synthesis of the arylimidamides dihydrochloride: 3a,b, 6, 9a-c, 13,18

S-(2-Naphthylmethyl)-2-pyridyl or pyrimidylthioimidate hydrobromide **2a,b** (2.2 mmol) was added to a cooled solution of the diamine (1 mmol) in a mixture of dry ethanol (30 mL) and dry acetonitrile (10 mL) in an ice bath. The reaction mixture was allowed to come to room temperature and was stirred overnight. After the disappearance of the starting material, the organic solvent was evaporated under reduced pressure to yield a crude oil product. Dry ether (50 mL) was added to the crude material and the mixture was stirred at room temperature for 4 h. The red precipitate was filtered and washed with dry ether. The solid was dissolved in ethanol (5 ml); the solution was cooled to 0 °C in an ice bath and 10% NaOH was added until the pH reached approximately 10. The free base was extracted with ethyl acetate (3 ×100 mL). The organic layer was washed with distilled water, dried over dry K_2CO_3, filtered and concentrated under reduced pressure. The resulting suspension was crystallized by adding dry ether and then filtered. The free base was suspended in dry ethanol (10 mL) and cooled to 0 °C in an ice bath. Freshly prepared anhydrous HCl-ethanol (2 mL) was added to the suspension and the mixture was stirred at room temperature overnight. The resulting red solution was concentrated under reduced pressure. The red crude solid was crystallized from a dry ethanol / ether mixture and filtered.

N,N′-(1,4-phenylene)dipicolinimidamide hydrochloride (3a)

White solid, yield: 68%; mp 200–202 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.65 (brs, 4H), 7.89 (dd, J = 4.0, 8.0 Hz, 2H), 8.23-8.26 (m, 2H), 8.53 (d, J = 8.0 Hz, 2H), 8.93 (d, J = 4.0 Hz, 2H), 9.41 (s, 2H), 10.24 (s, 2H), 12.01 (s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 124.7, 129.1, 129.2, 135.4, 139.2, 144.6, 150.3, 160.8; ESI-MS: m/z calc. for $C_{18}H_{16}N_6$: 316.36, found: 316.20 (base M_+); Anal. Calc. for $C_{18}H_{16}N_6$. 2 HCl. 1.55 H_2O. 0.1 Et_2O: C, 52.04; H, 5.24; N, 19.79. Found: C, 51.68; H, 4.98; N, 19.44.

N,N′-(1,4-phenylene)dipyrimidine-2-carboximidamide hydrochloride (3b)

White solid, yield: 59%; mp 220–222 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.64–7.65 (m, 4H), 8.00-8.06 (m, 2H), 9.19–9.24 (m, 4H), 9.53 (s, 2H), 10.25 (s, 2H), 12.20 (s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 125.5, 129.0, 135.4, 153.5, 158.5, 158.9; ESI-MS: m/z calc. for $C_{16}H_{14}N_8$: 318.34, found: 319.20 (base M^++ H_+); Anal. Calc. for $C_{16}H_{14}N_8$. 2 HCl. 1.75 H_2O. 0.25 EtOH: C, 45.63; H, 4.87; N, 25.80. Found: C, 45.54; H, 4.66; N, 25.47.

N ^{1},*N* ^{4}-di(pyridin-2-yl)terephthalimidamide hydrochloride (6)

Sodium bis(trimethylsilyl)amide 1M solution in tetrahydrofuran (1.58 g, 9.48 ml, 8.59 mmol) was added dropwise to a solution of 2-aminopyridine (0.8 g, 8.59 mmol) in tetrahydrofuran (10 mL) at −80 °C. Stirring was continued for 1 h, then 1,4-dicyanobenzene (0.5 g, 3.9 mmol) in tetrahydrofuran was added dropwise at −80 °C. The reaction mixture was allowed to come to room temperature and was stirred overnight. The reaction mixture was then cooled to 0 °C and HCl saturated ethanol (3 ml) was added. The mixture was stirred for 5 h, diluted with ether and the resultant solid was collected by filtration. Purification was via free base formation by neutralization with 1*N* sodium hydroxide solution followed by filtration of the resultant solid that was washed with water. Finally, the dry free base was stirred with saturated ethanolic HCl for 8 h, diluted with ether, and the solid which formed was filtered and dried to give the target compound as hydrochloride salt.

White solid, yield: 42%; mp 190–192 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.43-7.46 (m, 2H), 7.83 (d, J = 8.0 Hz, 2H), 8.08 (d, J = 8.0 Hz, 2H), 8.25 (br s, 4H), 8.55-8.56 (br s, 2H), 11.30 (s, 2H), 11.95 (s, 2H), 12.85 (s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 124.7, 127.6,

129.3, 135.4, 139.2, 144.6, 149.7, 163.1; ESI-MS: m/z calc. for $C_{18}H_{16}N_6$: 316.36, found: 316.20 (base M_+); Anal. Calc. for $C_{18}H_{16}N_6$. 3 HCl. 1 H_2O: C, 48.71; H, 4.76; N, 18.93. Found: C, 48.71; H, 4.63; N, 18.58.

General procedure for the reduction of the dinitro compounds: 8a–c, 12, 17

The commercially available or synthesized dinitro compounds (1 mmol) were dissolved in methanol and 4 equivalents of hydrazine were added followed by a pinch of Raney nickel and the mixture was heated to 50 °C. A vigorous evolution of hydrogen gas ensued and the solution slowly turned colorless in 2–3 h time. It was cooled and passed through a pad of celite and washed with ethyl acetate, dried and concentrated to obtain the diamine which was subsequently washed with hexane to obtain sufficiently pure compounds to use directly in the next step as judged from ^{1}H NMR.

Benzidine (8a)

Yield: 95%; mp 128–130 °C; ^{1}H NMR ($CDCl_3$), δ (ppm): 3.56 (br s, 4H), 6.68 (d, J = 8.2 Hz, 4H), 7.21 (d, J = 8.2 Hz, 4H); ^{13}C NMR ($CDCl_3$), δ (ppm): 115.2, 122.4, 132.2, 143.1; ESI-MS: Calc. for $C_{12}H_{12}N_2$: 184.1, found 185.2 ($M+H^+$).

4,4'-Methylenedianiline (8b)

Yield: 93%; mp 97–99 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 3.54 (br s, 4H), 3.79 (s, 2H), 6.61 (d, J = 8.2 Hz, 4H), 6.99 (d, J = 8.2 Hz, 4H); ^{13}C NMR (DMSO-d_6), δ (ppm): 40.0, 115.1, 129.4, 131.7, 144.1; ESI-MS: Calc. for $C_{13}H_{14}N_2$: 198.1, found 199.2 ($M+H^+$).

4,4'-Oxydianiline (8c)

Yield: 87%, mp 190–191 °C; ^{1}H NMR ($CDCl_3$), δ (ppm): 6.86 (d, J = 8.8 Hz, 4H), 6.97 (br s, 4H), 7.41 (d, J = 8.8 Hz, 4H) ^{13}C NMR ($CDCl_3$), δ (ppm): 119.9, 120.7, 152.4, 154.1; ESI-MS: Calc. for $C_{12}H_{12}N_2O$: 200.1, found 201.2 ($M+H^+$).

N,N'-([1,1'-biphenyl]-4,4'-diyl)dipicolinimidamide hydrochloride (9a)

Yield: 97%; mp 210–212 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 6.89 (d, J = 8.2 Hz, 4H), 7.84–7.87 (m, 2H), 7.89 (d, J = 8.2 Hz, 4H), 8.22–8.25 (m, 2H), 8.68 (br s, 2H), 8.89 (d, J = 4.0 Hz, 2H), 10.21 (br s, 2H), 11.41 (br s, 2H), 12.05 (br s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 114.8, 123.1, 132.8, 138.3, 142.8, 144.5, 148.1, 149.9, 153.4, 159.2; ESI-MS: Calc. for $C_{24}H_{20}N_6$: 392.2, found 393.3 (base $M+H^+$); Anal. Calc. for $C_{24}H_{20}N_6$. 3.5 HCl. 1 H_2O: C, 53.57; H, 4.77; N, 15.61. Found: C, 53.20; H, 4.66; N, 15.61.

N,N'-(methylenebis(4,1-phenylene))dipicolinimidamide hydrochloride (9b)

Yield: 98%; mp 220–223 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 3.74 (s, 2H), 6.78 (d, J = 8.0 Hz, 4H), 7.70 (d, J = 8.0 Hz, 4H), 7.84 (br s, 2H), 8.05–8.06 (m, 2H), 8.42 (d, J = 7.6 Hz, 2H), 8.91 (d, J = 4.0 Hz, 2H), 9.37 (br s, 2H), 10.07 (br s, 2H), 11.73 (br s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 39.6, 114.3, 128.5, 132.9, 137.8, 138.3, 144.2, 145.3, 149.3, 155.0, 158.8; ESI-MS: Calc. for $C_{25}H_{22}N_6$: 406.2, found 407.3 (base $M+H^+$); Anal. Calc. for $C_{25}H_{22}N_6$. 4 HCl. 0.5 H_2O: C, 53.49; H, 4.84; N, 14.97. Found: C, 53.15; H, 4.90; N, 14.58.

N,N'-(oxybis(4,1-phenylene))dipicolinimidamide hydrochloride (9c)

Yield: 87%; mp 280 °C (dec.); ^{1}H NMR (DMSO-d_6), δ (ppm): 7.01 (d, J = 8.1 Hz, 4H), 7.56 (d, J = 8.1 Hz, 4H), 7.86 (br s, 2H), 8.21–8.23 (m, 2H), 8.43 (d, J = 7.6 Hz, 2H), 8.90 (d, J = 4.0 Hz, 2H), 9.39 (br s, 2H), 10.27 (br s, 2H), 11.71 (br s, 2H); ^{13}C NMR (DMSO-d_6), δ (ppm): 119.4, 123.1, 149.7, 150.3, 153.4, 153.9, 155.0, 156.8, 159.4; ESI-MS (+):

Calc. for $C_{24}H_{20}N_6O$: 408.2, found 409.3 (base M+H$^+$); Anal. Calc. for $C_{24}H_{20}N_6O$. 3.5 HCl. 1.4 H_2O: C, 51.35; H, 4.72; N, 14.97. Found: C, 51.60; H, 4.67; N, 14.47.

2,2'-Di-isopropoxy-4,4'-dinitro-1,1'-biphenyl (11)

1-Bromo-2-isopropoxy-4-nitrobenzene (2 g, 7.69 mmol) was dissolved in 20 mL of dry DMF and potassium acetate (1.5 g, 15.38 mmol), bis(pinacolato)diboron (1.95 g, 7.69 mmol) and $PdCl_2(dppf)_2$ (5 mol %) was added and heated for 8 h at 90 °C. The black solution was cooled, passed through celite and washed with ethyl acetate. The ethyl acetate filtrate was washed with ice cold water, concentrated and the desired biphenyl compound was purified from it as a light yellow solid by column chromatography with hexane-ethyl acetate as the eluent.

Yield: 46%; mp 156–158 °C; ^{1}H NMR ($CDCl_3$), δ (ppm): 1.34 (d, *J* = 6.4 Hz, 12H), 4.63 (septet, *J* = 6.4 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 2.0 Hz, 2H), 7.80 (dd, *J* = 8.1 Hz, 2.0 Hz, 2H); ^{13}C NMR ($CDCl_3$), δ (ppm): 22.0, 71.7, 109.1, 116.1, 129.2, 130.9, 147.6, 155.2. ESI-MS: Calc. for $C_{18}H_{20}N_2O_6$: 360.1, found 361.2 (M+H$^+$). This compound was used in the next step without further characterization.

2,2'-Di-isopropoxy-[1,1'-biphenyl]-4,4'-diamine (12)

Yield: 91%; mp 123–125 °C; ^{1}H NMR ($CDCl_3$), δ (ppm): 1.23 (d, *J* = 6 Hz, 12H), 3.63 (br s, 4H), 4.34 (septet, *J* = 6.0 Hz, 2H), 6.31 (d, *J* = 2.2 Hz, 2H), 6.35 (dd, *J* = 8.0 Hz, 2.2 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$), δ (ppm): 22.3, 71.0, 102.8, 108.2, 113.4, 122.6, 146.7, 148.1, 150.8, 151.7, 152.9, 155.9, 156.1, 159.2; ESI-MS: Calc. for $C_{18}H_{24}N_2O_2$: 300.2, found 301.3 (M+H$^+$). This compound was used in the next step without further characterization.

N,N'-(2,2'-di-isopropoxy-[1,1'-biphenyl]-4,4'-diyl)dipicolinimidamide hydrochloride (13)

Yield: 95%; mp 261–264 °C; ^{1}H NMR (DMSO-*d*$_6$), δ (ppm): 1.28 (d, *J* = 6.0 Hz, 12H), 4.41 (septet, J = 6.0 Hz, 2H), 6.68 (d, *J* = 2.2 Hz, 2H), 6.71 (dd, *J* = 8.0 Hz, 2.2 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.78 (m, 2H), 8.24 (m, 2H), 8.66 (d, *J* = 7.8 Hz, 2H), 8.91 (d, *J* = 4.0 Hz, 2H), 10.32 (br s, 2H), 11.36 (br s, 2H), 12.01 (br s, 2H); ^{13}C NMR (DMSO-*d*$_6$), δ (ppm): 23.1, 72.1, 105.9, 111.8, 116.5, 124.2, 137.2, 138.8, 145.2, 147.2, 150.3, 156.2, 157.1, 159.3; ESI-MS: Calc. for $C_{30}H_{32}N_6O_2$ (base): 508.3, found 509.4 (M+H$^+$); Anal. Calc. for $C_{30}H_{32}N_6O_2$. 2 HCl. 1.4 H_2O: C, 59.45; H, 6.12; N, 13.87. Found: C, 59.55; H, 6.05; N, 13.64.

4,4'-(Oxybis(methylene))bis(nitrobenzene) (16)

4-Nitrobenzyl alcohol (1.2 g, 7.84 mmol) was dissolved in 5 mL of dry DMF, cooled in an ice bath and NaH (60 % dispersion in mineral oil) (0.41 g, 10.2 mmol) was added. After 10–15 min, a solution of 4-nitrobenzyl bromide (1.54 g, 7.13 mmol) in 2 mL of DMF was added followed by a catalytic amount of NaI. After allowing the reaction mixture to warm to room temperature over a period of 4 h, it was quenched by the addition of water to obtain a yellow solid which was washed with ether and recrystallized from ethanol.

Yield: 52%; mp 231–233 °C; ^{1}H NMR (DMSO-*d*$_6$), δ (ppm): 4.51 (s, 4H), 7.84 (d, *J* = 8.0 Hz, 2H), 8.10 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (DMSO-*d*$_6$), δ (ppm), 74.1, 119.4, 126.1, 143.1, 144.5; ESI-MS: Calc. for $C_{14}H_{12}N_2O_5$: 288.1, found 289.2 (M+H$^+$). This compound was used in the next step without further characterization.

4,4'-(Oxybis(methylene))dianiline (17)

Yield: 92%; mp 178–180 °C; ^{1}H NMR (DMSO-d_6), δ (ppm): 3.45 (br s, 4H), 4.59 (s, 4H), 6.93 (d, J = 8.1 Hz), 7.14 (d, J = 8.1 Hz); ^{13}C NMR (DMSO-d_6), δ (ppm): 75.2, 116.3, 126.9, 128.9, 146.1; ESI-MS: Calc. for $C_{14}H_{16}N_2O$: 228.1, found 229.2 ($M+H^+$); This compound was used in the next step without further characterization.

N,N'-((Oxybis(methylene))bis(4,1-phenylene))dipicolinimidamide hydrochloride (18)

Yield: 73%; mp 280–282 °C (dec); ^{1}H NMR (DMSO-d_6), δ (ppm): 4.51 (s, 4H), 7.01 (d, J = 8.2 Hz, 4H), 7.42 (d, J = 8.2 Hz, 4H), 7.81 (m, 2H), 8.24 (dd, J = 7.8, 6.8 Hz, 2H), 8.39 (d, J = 7.8 Hz, 2H), 8.89 (d, J = 4.0 Hz, 2H), 10.26 (br s, 2H), 11.38 (br s, 2H), 12.02 (br s, 2H). ^{13}C NMR (DMSO-d_6), δ (ppm): 74.4, 118.2, 126.2, 132.4, 144.1, 147.6, 150.7, 152.3, 152.8, 158.2, and 159.5; ESI-MS: Calc. for $C_{26}H_{24}N_6O$ (base): 436.2, found 437.3 (free base $M+H^+$); Anal. Calc. for $C_{26}H_{24}N_6O$. 3.5 HCl. 0.5 H_2O: C, 54.48; H, 5.01; N, 14.66. Found: C, 54.35; H, 5.04; N, 14.44.

Acknowledgments

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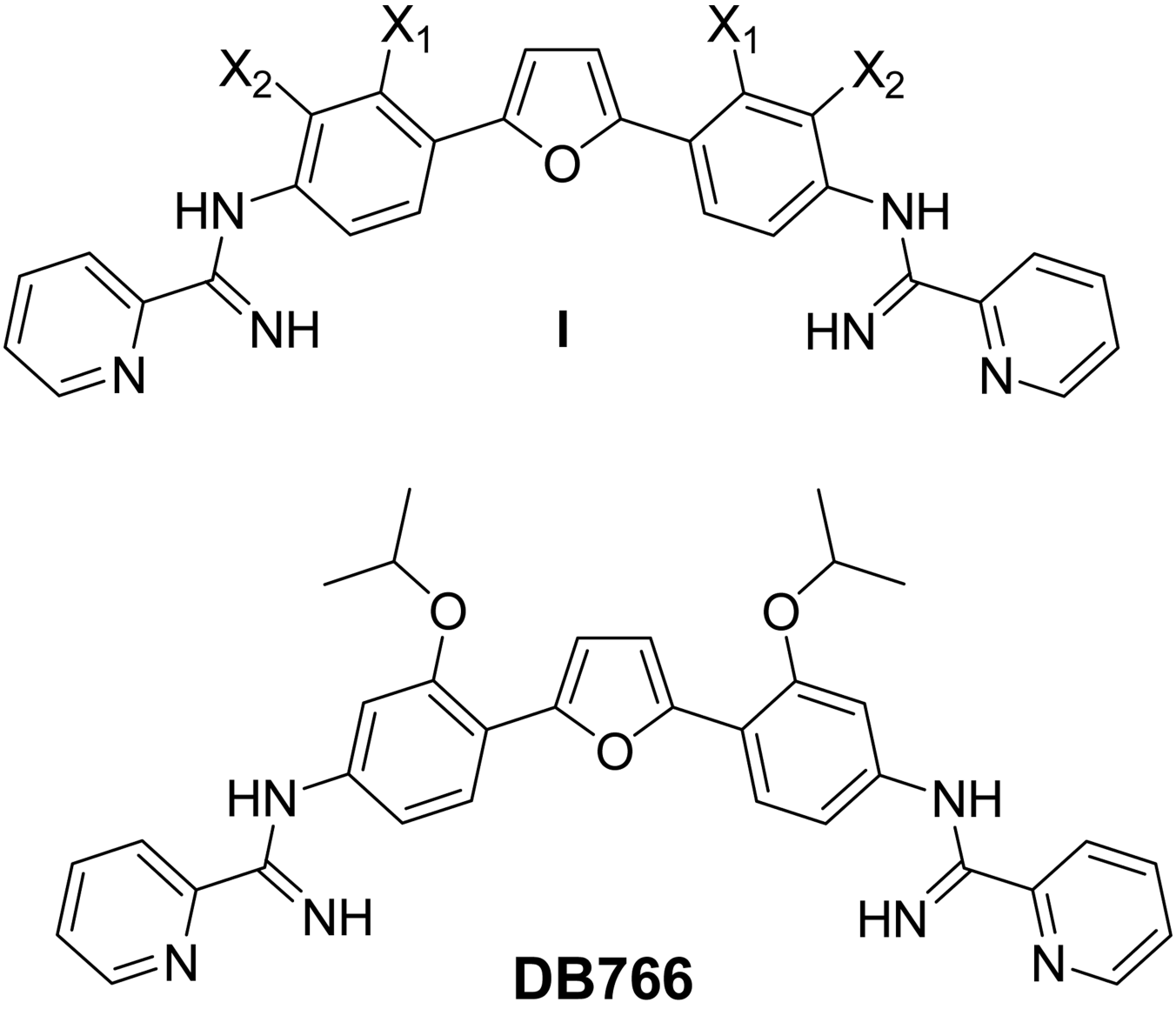
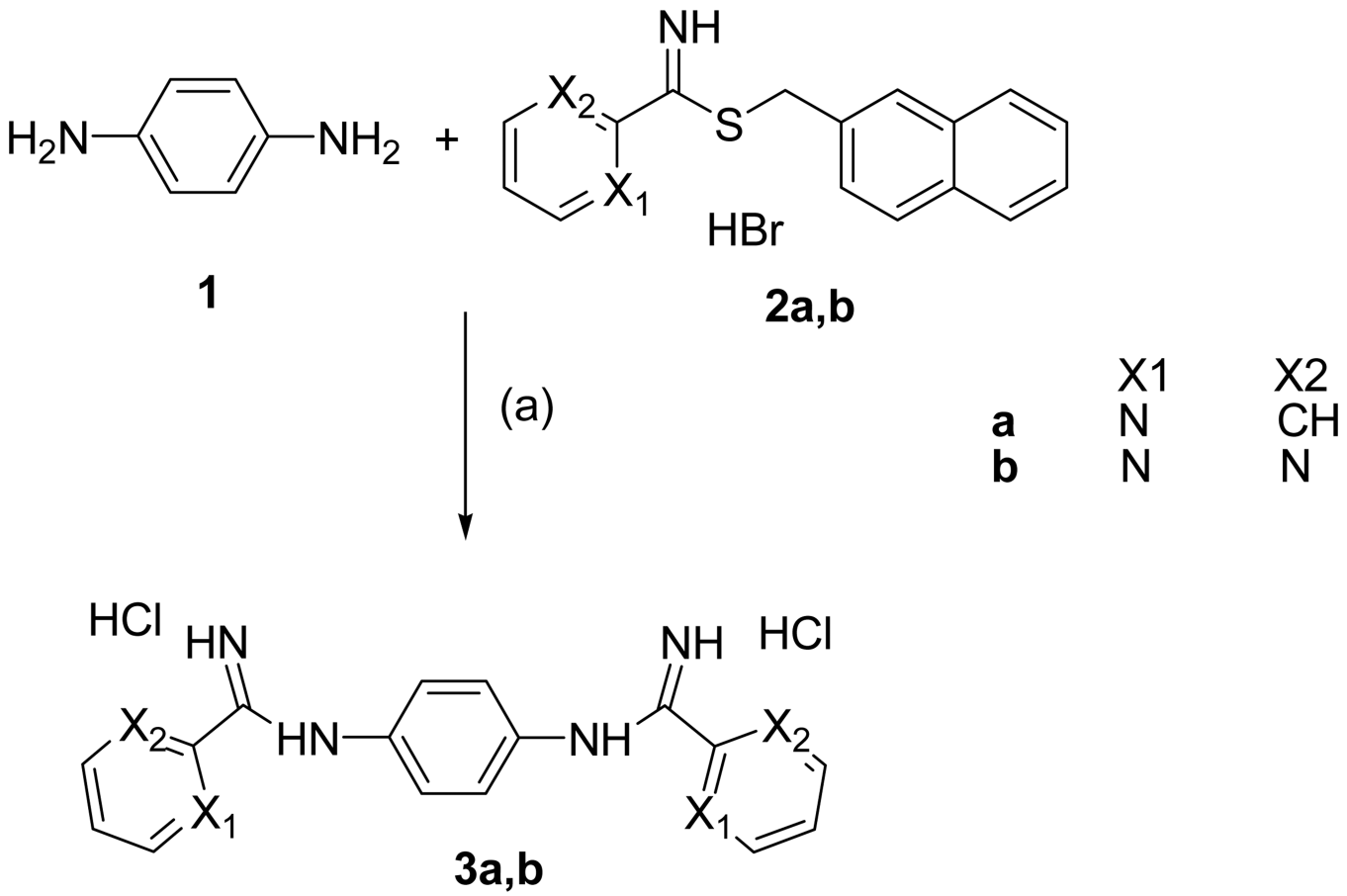
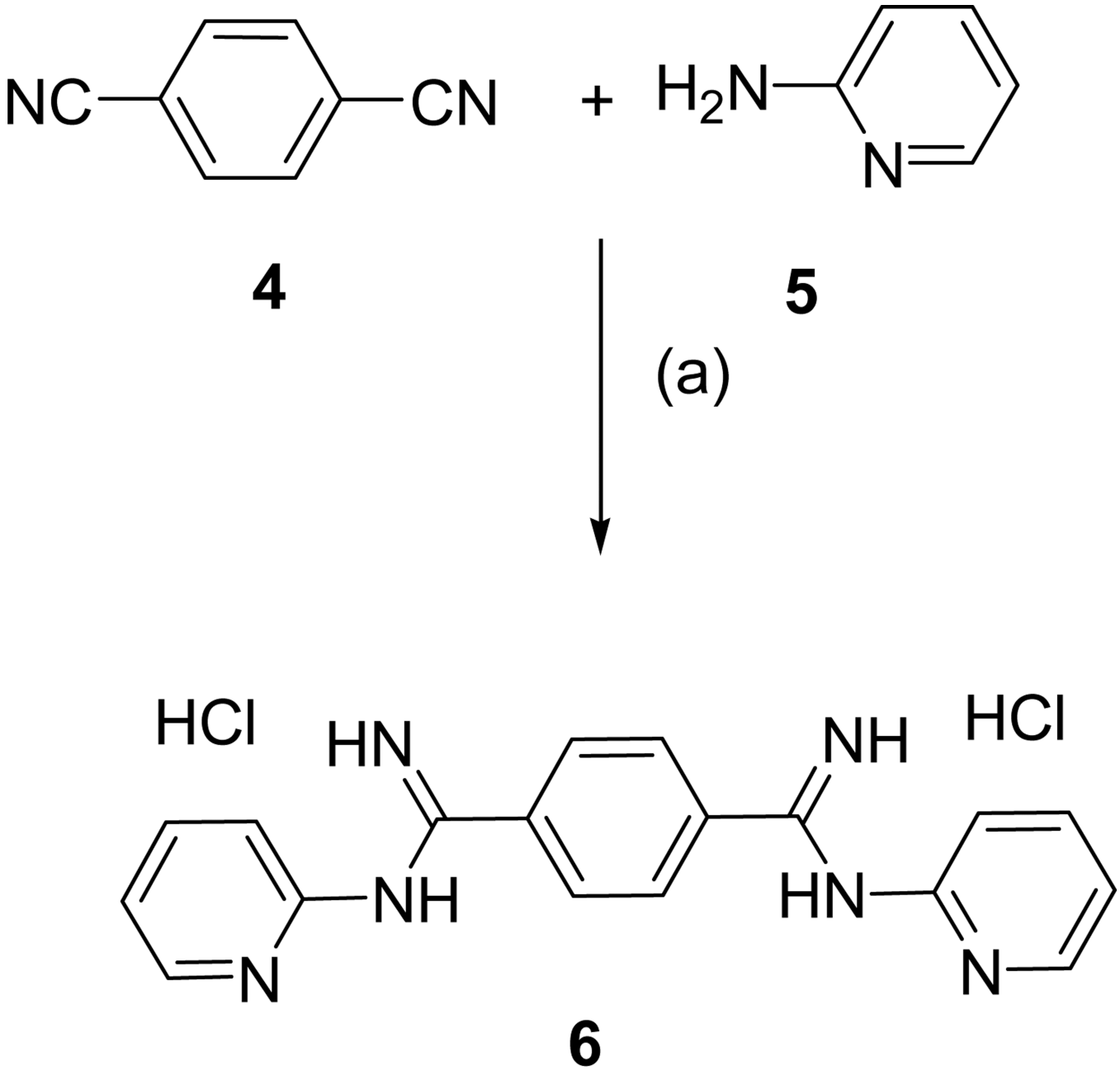


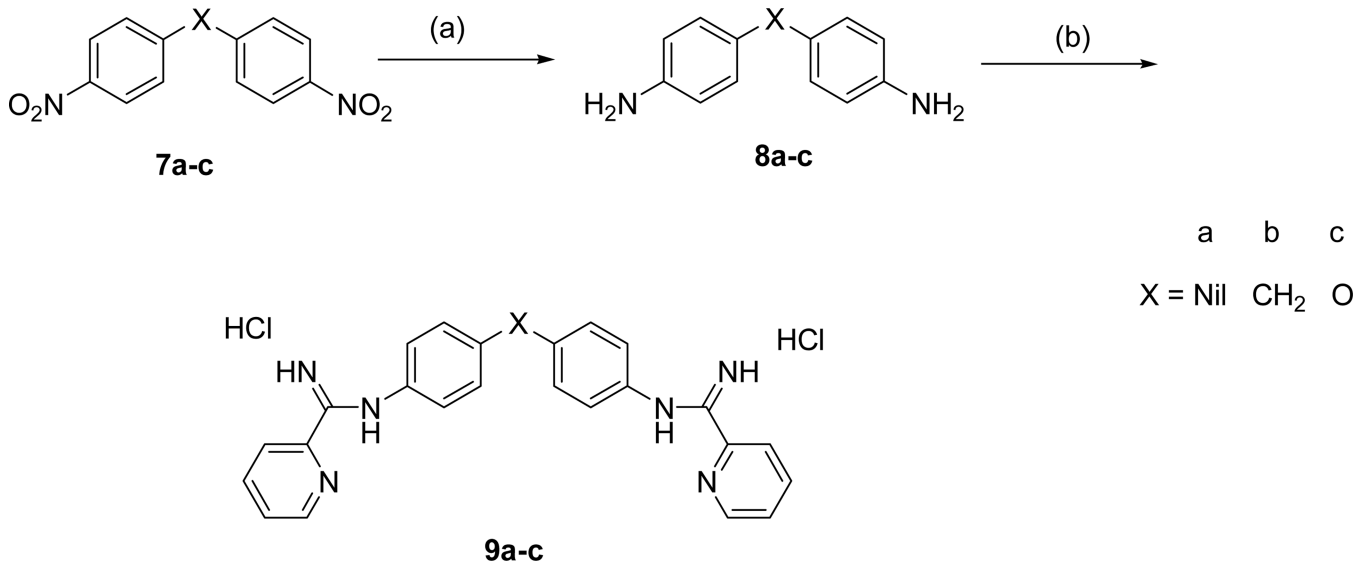
Fig. 1.
Lead bis-arylimidamides.



Scheme 1.
Reagents and conditions (a) i-EtOH, CH_3CN, NaOH; ii-EtOH/HCl.

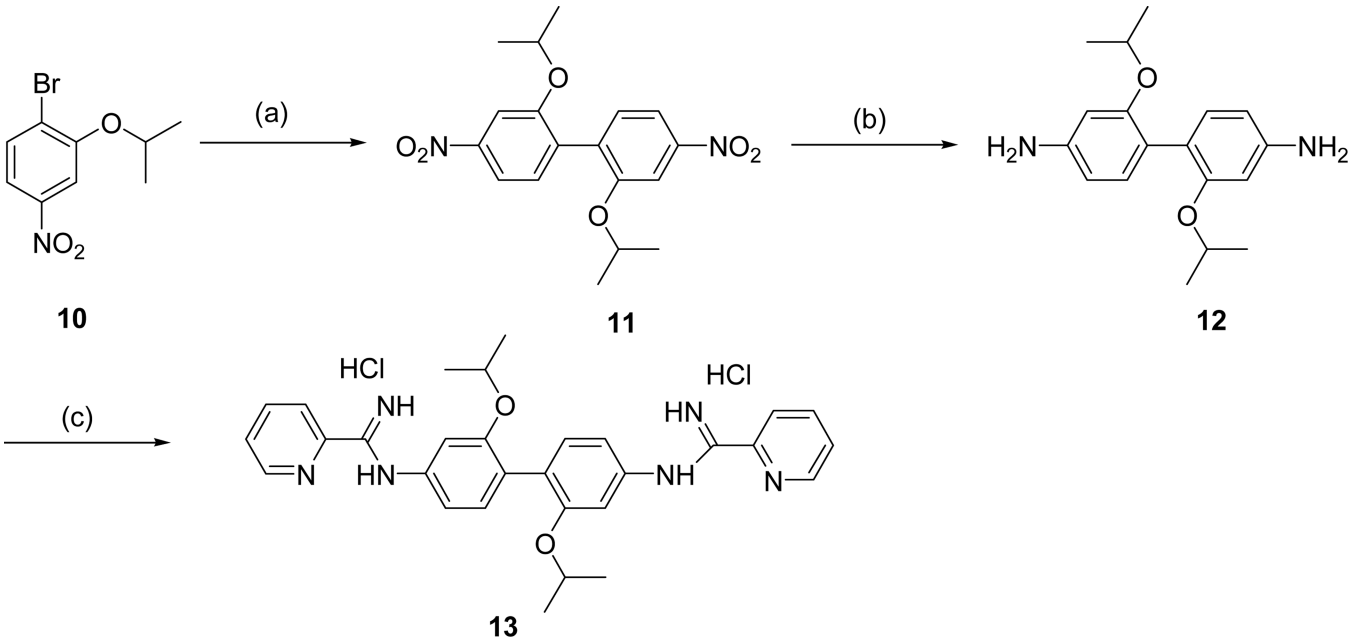


Scheme 2.
Reagents and conditions (a) i) $NaN(TMS)_2$, THF, −80 °C, ii) EtOH/HCl.



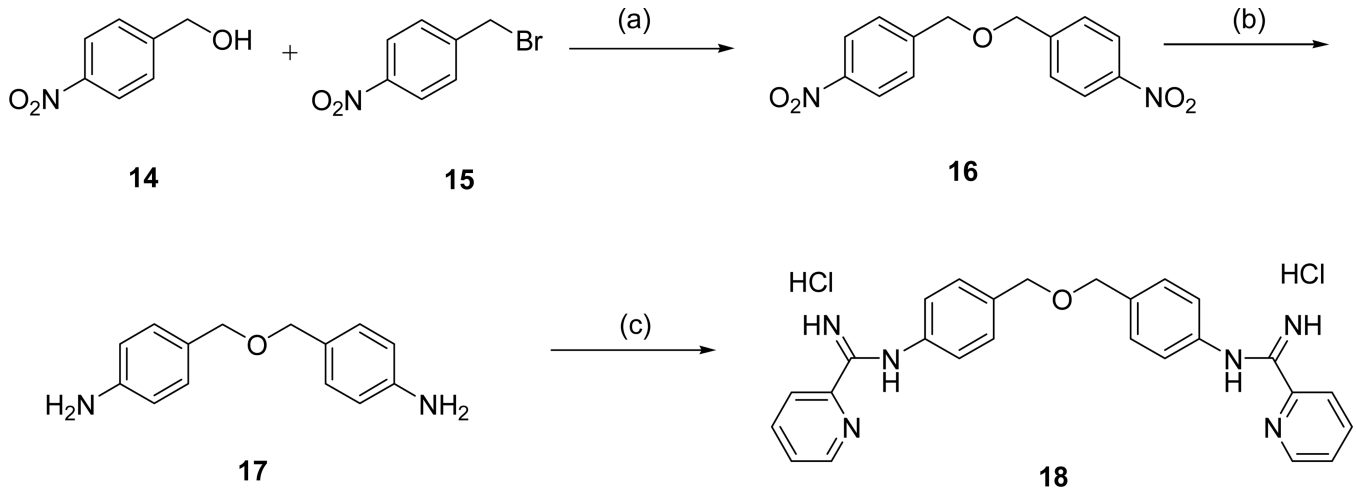
Scheme 3.

Reagent and conditions (a) Raney Ni, NH_2NH_2, MeOH; (b) i) S-(2-naphthylmethyl)-2-pyridylthioimidate HBr, EtOH, CH_3CN, NaOH; ii) EtOH/HCl.



Scheme 4.

Reagents and conditions (a) Bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMF; (b) Raney Ni, NH_2NH_2, MeOH; (c) i) *S*-(2-naphthylmethyl)-2-pyridylthioimidate HBr, EtOH, CH_3CN, NaOH; ii) EtOH/HCl.



Scheme 5.

Reagents and conditions (a) NaH, NaI, DMF; (b) Raney Ni, NH_2NH_2, MeOH; (c) i) *S*-(2-naphthylmethyl)-2-pyridylthioimidate HBr, EtOH, CH_3CN, NaOH; ii) EtOH/HCl.

Table 1

DNA binding, in vitro antileishmanial and cytotoxicity data molecular weight arylimidamides.

code	IC_{50}[a] *L. a. (μM)*	IC_{50}[b] *L. d. (μM)*	IC_{50}[c] Cytotox (μM)	Δ Tm[d] (°C)
DB766	0.087[e]	0.036[e]	3.0	6.0
3a	>10	ND[f]	>212	0.0
3b	ND	ND	>207	0.0
6	5.1	ND	>202	0.5
9a	0.76	0.079	46.9	6.0
9b	>10	ND	93.5	2.1
9c	0.67	0.028	5.0	5.2
13	0.14	0.025	22.9	0.1
18	1.1	ND	21.6	4.0

[a] IC_{50} values obtained against intracellular *L. a.* [14].

[b] IC_{50} values obtained against intracellular *L. d.* [14].

[c] Cytotoxicity (IC_{50}) was evaluated using cultured L6 rat myoblast cells [24,25].

[d] Increase in thermal melting of poly(dA-dT)n in °C [21].

[e] Values taken from [14].

[f] ND, not determined.

Low MW arylimidamides as analogues of DB 766 were prepared
Arylimidamides are synthesized from aryl thioimidates and corresponding diamines
New arylimidamides showed *L. d.* IC_{50} values of 25–79 nM and acceptable cytotoxicity
DNA binding affinities and in vivo toxicity reported