Supplemental Material

Title: In vitro and In vivo Biological Effect of Novel Arylimidamides Derivatives Against *Trypanosoma cruzi*

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Running title: Anti-*T.cruzi* effect of novel arylimidamides

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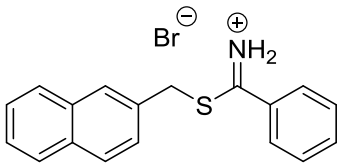
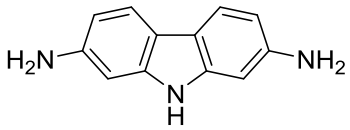
General experimental

Uncorrected melting points were measured on a Thomas–Hoover Capillary melting point apparatus. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian Gemini 2000 300 MHz or a Varian Inova 400 MHz spectrometer. ^{13}C NMR spectra were recorded in DMSO-d_6 on a Varian Gemini 2000 spectrometer operating at 75 MHz. Organic starting materials were prepared by established procedures as noted, or purchased from Aldrich Chemical Co., Milwaukee, WI, as were anhydrous solvents in Sure-seal® containers, which were used without further purification. Reaction mixtures were monitored by reverse phase HPLC. Organic layers of extraction mixtures were neutralized as necessary with acidic or basic washes, washed with saturated NaCl solution and dried over $MgSO_4$ or Na_2SO_4 before being evaporated under reduced pressure. Final products were dried under high vacuum over P_2O_5. Analytical HPLC chromatograms were recorded on an Agilent 1200 chromatograph using a Zorbax Rx C8 column (4.6 × 75 mm, 3.5 µm) maintained at 40 °C and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. Area % values are reported at the wavelengths where the strongest signals of the products were observed. Mobile phases consisted of mixtures of acetonitrile (0–75%) or methanol (0–95%) in water containing formic acid (80 mM), ammonium formate (20 mM) and triethylamine (15 mM). Samples were eluted at appropriate gradients at a flow rate of 1.5 mL/min. Preparative reverse phase HPLC was performed on a Varian ProStar Chromatography Workstation configured with two PS-215 pumps fitted with 50 mL pump heads, a Dynamax Microsorb C18 (60 Å) column (41.4 × 25 cm, 8 µm), PS-320 variable wavelength UV–Vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of acetonitrile (0–75%) in water containing a buffer (formic acid, 40 mM and ammonium formate, 10 mM) or 0.1% trifluoroacetic acid. Flow rates were maintained at 40 mL/min. Select fractions were analyzed for purity by analytical HPLC as described above. Pooled purified fractions were evaporated under reduced pressure, reconstituted in water, and lyophilized on a VirTis BenchTop or 6K lyophilizer. Low resolution ESI mass spectra were recorded on an Agilent Technologies 1100 Series LC/MSD Trap mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ± 0.4% of calculated values. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or other solvents; in each case ^{1}H NMR spectra was consistent with the analysis.



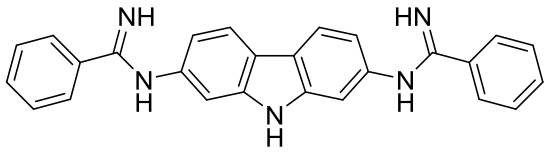
S-(2-Naphthyl)methylbenothioimidate hydrobromide[1]

To a solution of thiobenzamide (2.09 g, 15.2 mmol) in dry $CHCl_3$ was added 2-(bromomethyl)naphthalene (3.39 g, 15.3 mmol). The mixture was refluxed for 1 hour. The resulting precipitate (3.82 g, 70%) was filtered off, dried, and used without further purification in the next step.



2,7-Diaminocarbazole

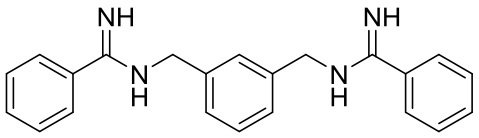
A suspension of 2,7-dinitrocarbazole (prepared in two steps from 4,4'-dinitro-2-biphenylamine,[2] 5.14 g, 20.0 mmol) in EtOH (500 mL) was brought to reflux. Hydrazine monohydrate (50 mL, 1.03 mol) and 5% ruthenium on carbon (0.75 g, 0.37 mmol) were added, and the mixture was refluxed for 1 hour. The cooled reaction mixture was gravity filtered, concentrated to ca. 250 mL, and diluted with water (50 mL) to give light tan crystals (2.84 g, 72%), mp 256-260 °C (lit.[2] 248 °C). ^{1}H NMR (300 MHZ, DMSO-d_6) δ 10.25 (s, 1H), 7.43 (d, J = 7.9 Hz, 2H), 4.48 (s, 2H), 6.33(d, J = 7.9 Hz, 2H), 4.89 (s, 4H); HPLC 100 area % (254 nM).



16DAP002 (dihydrochloride salt)

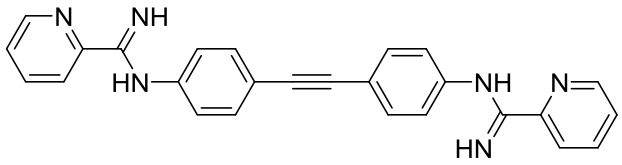
To a chilled (ice bath) mixture of 2,7-diaminocarbazole (0.50 g, 2.55 mmol) in dry CH_3CN (30 mL) and dry EtOH (20 mL) *S*-(2-naphthyl)methylbenothioimidate hydrobromide (1.90 g, 5.30 mmoL) was added. The ice bath was removed and the mixture was stirred overnight. The mixture was concentrated to an oily residue, which was diluted with ether to give a precipitate

(1.64 g). The solid was nearly completely dissolved in hot water (150 mL). The mixture was gravity filtered and basified with NaOH solution. The resulting precipitate was filtered off, suspended in water, and acidified with 1H HCl solution to give a white solid (0.36 g, 71%): mp > 230 °C (dec); ^{1}H NMR (300 MHZ, DMSO-d_6) δ 12.13 (br s, 1H), 11.73 (br s, 2H), 9.89(br s, 2H), 9.10 (br s, 2H), 8.38 (d, J = 8.2 Hz, 2H), 8.00 (d, J = 7.8 Hz, 4H), 7.81 (t, J = 7.3 Hz, 2H), 7.70 (m, 6H), 7.30 (d, J = 8.6 Hz, 2H); ESI-MS m/z 404.2 ($[M + 1]^{+}$), 202.6 ($[M + 2]^{2+}/2$); HPLC 100 area % (254 nm). Anal. Calcd for for $C_{26}H_{21}N_5 \cdot 2HCl \cdot 1.7H_2O$: C, 61.59; H, 5.25; N, 13.81; Cl, 13.98. Found: C, 61.81; H, 5.17; N, 13.51; Cl, 13.77



16SAB079 (dihydrochloride salt)

The compound was similarly prepared from *m*-xylylenediamine (0.306 g, 2.25 mmol) and *S*-(2-naphthyl)methylbenothioimidate hydrobromide (1.70 g, 4.74 mmoL) in EtOH (20 mL). Yield, 0.611 g (66%): mp > 165 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.51 (t, J = 6.3 Hz, 2H), 9.71 (s, 2H), 9.48 (s, 2H), 7.90 – 7.79 (m, 4H), 7.79 – 7.67 (m, 2H), 7.63 – 7.56 (m, 4H), 7.54 (s, 1H), 7.45 (d, J = 1.3 Hz, 3H), 4.78 (d, J = 6.1 Hz, 4H); ESI-MS *m/z* 343.3 ([M + 1]$^+$); HPLC 100 area % (230 nm). Anal. Calcd for for $C_{22}H_{22}N_4 \cdot 2.3HCl \cdot 1.2H_2O$: C, 58.99; H, 6.01; N, 12.51; Cl, 18.20. Found: C, 58.96; H, 5.95; N, 12.39; Cl, 18.07.

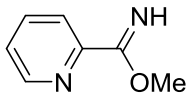


18SAB075 (dihydrochloride salt)

The title compound was prepared by the method of Lange *et al.*[3] but using lithium bis(trimethylsilyl)amide in place of sodium bis(trimethylsilyl)amide. A solution of 4,4′-diaminodiphenylacetylene[4, 5] (0.416 g, 2.00 mmol) in dry THF (10 mL) was treated with lithium

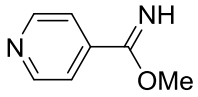
bis(trimethylsilyly)amide (1 M solution in THF, 4 mL). After 20 minutes, a solution of 2-cyanopyridine (0.416 g, 4.00 mmol) in THF (2 mL) was added dropwise, and the mixture was stirred for 2 days before being poured over ice-water and extracted into CH_2Cl_2. The crude product was purified by preparative HPLC and converted to the HCl salt using 1 M HCl. Yield 0.155 g (16%): mp > 350 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 2H), 10.25 – 10.13 (m, 2H), 9.43 (s, 2H), 8.93 – 8.87 (m, 2H), 8.54 (d, J = 8.0 Hz, 2H), 8.23 (td, J = 7.8, 1.8 Hz, 2H), 7.86 (dd, J = 7.8, 4.7 Hz, 2H), 7.77 (d, J = 8.0 Hz, 4H), 7.57 (d, J = 7.9 Hz, 4H); ESI-MS *m/z* 417.6 ($[M + 1]^+$);HPLC 100 area % (254 nm). Anal. Calcd for $C_{26}H_{20}N_6 \cdot 2HCl$: C, 63.81; H, 4.53; N, 17.17; Cl, 14.49. Found: C, 63.90; H, 4.36; N, 17.02; Cl, 14.29.

Imidate derivatives of 2- and 4-cyanopyridine



Methyl picolinimidate

A suspension of 2-cyanopyridine (20.8 g, 200 mmol) in dry MeOH (250 mL) was treated with sodium methoxide (0.5 M solution in MeOH, 50 mL, 25 mmol). The mixture was stirred for 1 week, and aliquots of this solution (0.667 M) were used directly in the next step.

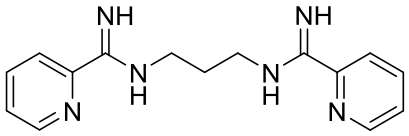


Methyl isonicotinimidate

By similar methodology, a 0.75 M solution of the title compound was prepared using 4-cyanopyridine (20.3 gm, 195 mmol), MeOH (210 mL), and NaOMe (0.5 M solution in MeOH, 50 mL, 25 mmol).

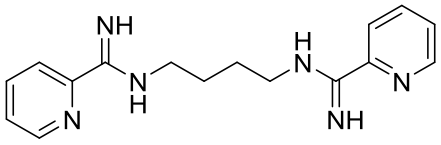
General procedure for 23SMB022, 23SMB026, and 23SMB054

To a solution of the appropriate diamine (6.65 mmol) in MeOH (10 mL) was added HCl/dioxane (4 M solution, 3.3 mL, 13.3 mmol) followed by the methyl picolinimidate solution (20 mL, 13.3 mmol). The solution was stirred at room temperature and the progress of the reaction was monitored by HPLC. The product was purified by preparative HPLC and converted to the hydrochloride salt using aqueous HCl unless stated otherwise.



23SMB022 (tetrahydrochloride salt)

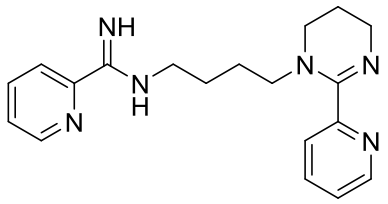
The title compound was prepared from methyl picolinimidate and 1,3-diaminopropane by the general procedure. The product was converted to the HCl salt using 4 M HCl/dioxane and EtOH. Yield, 1.3 g (46%): mp 198 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (s, 2H), 9.99 (s, 2H), 9.84 (s, 2H), 8.81 (d, J = 3.8 Hz, 2H), 8.50 (d, J = 7.9 Hz, 2H), 8.16 (t, J = 7.5 Hz, 2H), 7.79 (dd, J_1 = 5.10 Hz, J_2 = 4.8 Hz), 3.71-3.69 (m, 4H), 2.06-2.02 (m, 2H); ESI-MS *m/z* 283.2 ([M + 1]$^+$); HPLC 99.1 area % (254 nm). Anal. Calcd for $C_{15}H_{18}N_6$·4HCl: C, 42.08; H, 5.18; N, 19.63; Cl, 33.12. Found: C, 42.33; H, 5.34; N, 19.37; Cl, 32.88.



23SMB026 (trihydrochloride salt)

The title compound was prepared from methyl picolinimidate and 1,4-diaminobutane by the general procedure. Yield, 2.27 g (92%): mp 305-307°C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.28-10.25 (m, 2H), 9.88 (s, 2H), 9.62 (s, 2H), 8.82 (d, J = 4.6 Hz, 2H), 8.43 (d, J = 7.9 Hz, 2H), 8.16 (t, J = 7.9 Hz, 2H), 7.79 (dd, J_1 = 4.8 Hz, J_2 = 4.5 Hz, 2H), 3.60-3.59 (m, 4H), 1.76 (s, 4H); ESI-MS *m/z* 297.2 ([M + 1]$^+$); HPLC 100 area % (230 nm). Anal. Calcd for

$C_{16}H_{20}N_6 \cdot 3.1HCl \cdot 0.2H_20$: C, 46.53; H, 5.74; N, 20.35; Cl, 26.61. Found: C, 46.77; H, 5.69; N, 20.25; Cl, 26.37.

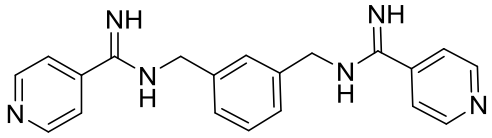


23SMB054 (trihydrochloride salt)

The title compound was prepared from methyl picolinimidate and spermidine by the general procedure. An unexpected cyclization occured, resulting in the next loss of NH_3. The product was converted to the HCl salt using 4 M HCl/dioxane, EtOH, and ether. Yield, 0.912 g (5%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.48 (s, 1H), 10.31 (m, 1H), 9.92 (s, 1H), 9.80 (s, 1H), 8.82 (d, J = 4.0 Hz, 1H), 8.74 (d, J = 4.8 Hz, 1H), 8.54 (d, J = 8.0 Hz, 1H), 8.19-8.07 (m, 2H), 7.85 (d, J = 7.7 Hz, 1H), 7.79 (dd, J_1 = 7.6 Hz, J_2 = 4.8 Hz, 1H), 7.69 (dd, J_1 = 7.7 Hz, J_2 = 4.8 Hz, 1H), 3.73-3.69 (m, 2H), 3.49-3.45 (m, 4H), 3.33-3.28 (m, 2H), 2.11-2.08 (m, 2H), 1.71-1.67 (m, 2H), 1.53-1.48 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ159.5, 159.2, 150.4, 150.2, 147.8, 145.0, 138.9, 138.7, 127.3, 125.5, 124.1, 52.4, 46.0, 42.4, 39.1, 24.8, 24.5, 19.0; ESI-MS *m/z* 337.3 ([M + 1]$^+$); HPLC 99.4 area % (254 nm). Anal. Calcd for $C_{19}H_{24}N_6 \cdot 3.2HCl \cdot 3H_20 \cdot 0.5C_2H_5OH$: C, 45.31; H, 6.88; N, 15.85; Cl, 21.40. Found: C, 45.16; H, 6.77; N, 15.91; Cl, 21.32.

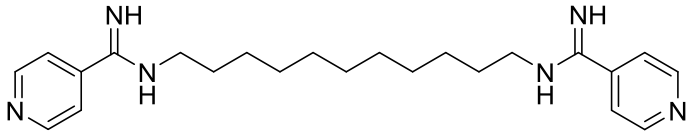
General procedure for 27SMB009 and 26SMB070

The procedures were similar to those above for 23SMB022, 23SMB026, and 23SMB054, using solutions of the appropriate diamine (7.5 mmol) in MeOH (10 mL), HCl/dioxane (4 M solution, 3.75 mL, 15 mmol) and the methyl isonicotinimidate solution (20 mL, 15 mmol).



26SMB070 (tetrahydrochloride salt)

The title compound was prepared from methyl isonicotinimidate and *m*-xylylenediamine by the general procedure. Yield, 0.92 g (22%): mp 280-285 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.12 (s, 2H), 10.25 (s, 2H), 10.09 (d, J = 9.1 Hz, 2H), 9.06 – 8.97 (m, 4H), 8.15 – 8.04 (m, 4H), 7.75 (s, 1H), 7.58 (qq, J_1 = 7.7, J_2 =3.1 Hz, 3H), 5.03 – 4.90 (m, 4H); ESI-MS *m/z* 345.2 ($[M + 1]^+$); HPLC 100 area % (230 nm). Anal. Calcd for $C_{20}H_{20}N_6 \cdot 4.2HCl \cdot 3H_20$: C, 43.55; H, 5.52; N, 15.24; Cl, 26.99. Found: C, 43.67; H, 5.35; N, 15.16; Cl, 26.99.



27SMB009 (tetrahydrochloride salt)

The title compound was prepared from methyl isonicotinimidate and 1,11-diaminoundecane by the general procedure. Yield, 0.63 g (15%); mp 233-235 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (d, J = 6.3 Hz, 2H), 9.95 (s, 2H), 9.68 (s, 2H), 8.95 (d, J = 5.3 Hz, 4H), 8.01 – 7.91 (m, 4H), 3.53 – 3.39 (m, 4H), 1.63 (q, J = 7.2, 6.6 Hz, 4H), 1.39 – 1.29 (m, 14H); ESI-MS *m/z* 395.3

([M + 1]$^{+}$); HPLC 98.0 area % (230 nm). Anal. Calcd for $C_{23}H_{34}N_6 \cdot 4HCl \cdot 1.5H_20$: C, 48.68; H, 7.28; N, 14.81; Cl, 24.99. Found: C, 48.65; H, 7.33; N, 14.58; Cl, 25.23.