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Synthesis and σ_1 Receptor Binding of Halogenated N,N´-Diphenethylethylenediamines

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Abstract

Eight halogenated *N,N´*-diphenethylethylenediamines were synthesized, characterized and evaluated for σ_1 receptor binding affinity in vitro. Measurements of lipophilicity also were obtained. The substitution pattern on one of the aromatic rings remained constant as 3,4-dichloro, while the substituents on the other aromatic ring were varied to include fluorine, bromine or iodine in either the 2-, 3- or 4- positions. Two main structure activity relationships were observed. First, halogen substitution on the 3- or 4-positions of the aromatic ring conferred higher binding affinities (K_i values 6.35 - 15.82 nM) than the corresponding substitutions at the 2-position (K_i values 12.08 - 43.15 nM). Second, derivatives containing either a bromo or fluoro substituent at a given position showed higher σ_1 receptor binding affinities than derivatives with a corresponding iodo substituent. The data indicate that σ_1 receptor affinity for this structural series is sensitive to steric bulk at the 2-position. Log k'_w measurements for the halogenated *N,N´*-diphenethylethylenediamines were determined by high performance liquid chromatography, and varied from 2.54 - 3.71. In particular, the 3-fluoro analog exhibited a log k'_w = 2.54 accompanied by a σ_1 receptor K_i = 7.8 nM. These novel N,N´-diphenethylethylenediamines warrant further investigation in behavioral assays, and radiolabeled versions may prove suitable for in vivo studies of σ_1 receptors.

Keywords: Sigma receptor; Binding studies; Structure activity relationships; lipophilicity

Abbreviations: SAR: Structure-activity relationship; SPECT: Single photon emission computed tomography; PET: Positron emission tomography; HPLC-MS: High performance liquid chromatography-mass spectrometry; ESI-MS: Electrospray ionization mass spectrometry; TFA: Trifluoroacetic acid; DCC: N,N´-dicyclohexylcarbodiimide; DCU: N,N´-dicyclohexylurea; THF: Tetrahydrofuran; NHP: N-hydroxyphthalimide; MOPS: 3-(N-morpholino)propanesulfonic acid; Tris-HCl: **T**ris(hydroxymethyl)aminomethane hydrochloride; BSA: Bovine serum albumin; SEM: Standard error of the mean; BD1047: N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine; BD1063: 1-[2-(3,4-dichlorophenyl) ethyl]-4-methylpiperazine; YZ069: N-phenylpropyl-N´-(3,4-dichlorophenethyl)piperazine.

Introduction

Sigma (σ) receptors can be classified into two distinct subtypes, σ_1 and σ_2, based upon their relative protein sizes, tissue and cellular distributions, and pharmacological / biochemical profiles [1-5]. There is considerable current interest in σ_1 receptors as therapeutic targets for multiple central nervous system disorders, including schizophrenia, depression, anxiety, Alzheimer's disease and stroke [5-9]. Further, there is a growing body of evidence that σ_1 receptor ligands, particularly selective antagonists, reduce the reinforcing effects of alcohol [10] and attenuate the behavioral effects of psychostimulant drugs of abuse [1,5,11-15]. The σ_2 receptors also may play a modest role in mitigating the actions of abused drugs [1,15], but truly selective σ_2 receptor ligands are just now being identified that might allow definitive discrimination of individual σ receptor subtype contributions [15-17].

Over the years, a number of *N,N´*-disubstituted ethylenediamines and piperazines have been investigated as σ_1 receptor ligands [1,15,18]. Such compounds typically exhibit K_i values of 1 - 10 nM for σ_1 receptors, accompanied by 2- to 50-fold selectivities against σ_2 sites. Prototypical ligands include BD1047 and BD1063 (Figure 1) that mitigate cocaine-induced lethality, locomotor activity and conditioned place preference in mice [1]. A series of *N*-benzyl-*N´*-benzylpiperazines (Figure 1,1) showed higher affinities for σ_1 receptors, K_i values of 0.39 - 7.6 nM, accompanied by greater selectivities, 13- to 340-fold selectivity against σ_2 sites [19]. This active series potently attenuates cocaine-induced convulsions in mice, except for the 3,4-dichloro derivative that unexpectedly behaves as an agonist.

Structural modifications of the ethylenediamine and piperazine scaffolds generally are well tolerated. For instance, *N*-phenylpropyl-*N´*-phenethylpiperazines, such as YZ069 (Figure 1,2), display σ_1 receptor K_i values of 0.7 - 3.9 nM, 2- to 22-fold selectivities against σ_2 sites, and protect mice against cocaine-induced convulsions [20]. Recently, a series of ten *N*-(3-phenylpropyl)-*N´*-benzylpiperazines (Figure 1,3) showed σ_1 receptor K_i values of 0.37 - 2.8 nM, with 1.4 - to 52-fold selectivities against σ_2 sites [21]. Robust quantitative SAR were established for their σ_1 and σ_2 receptor binding, and these compounds profiled as probable σ_1 receptor antagonists based upon an in vitro test using phenytoin as an allosteric modulator of competition against [^{3}H]-(+)-pentazocine.

In order to gain further insight into σ receptor SAR, and to expand the armamentarium of ligands available for biological testing, we have synthesized and characterized a series of eight halogenated *N,N´*-diphenethylethylenediamines (Figure 1,4-11), determined σ_1 receptor

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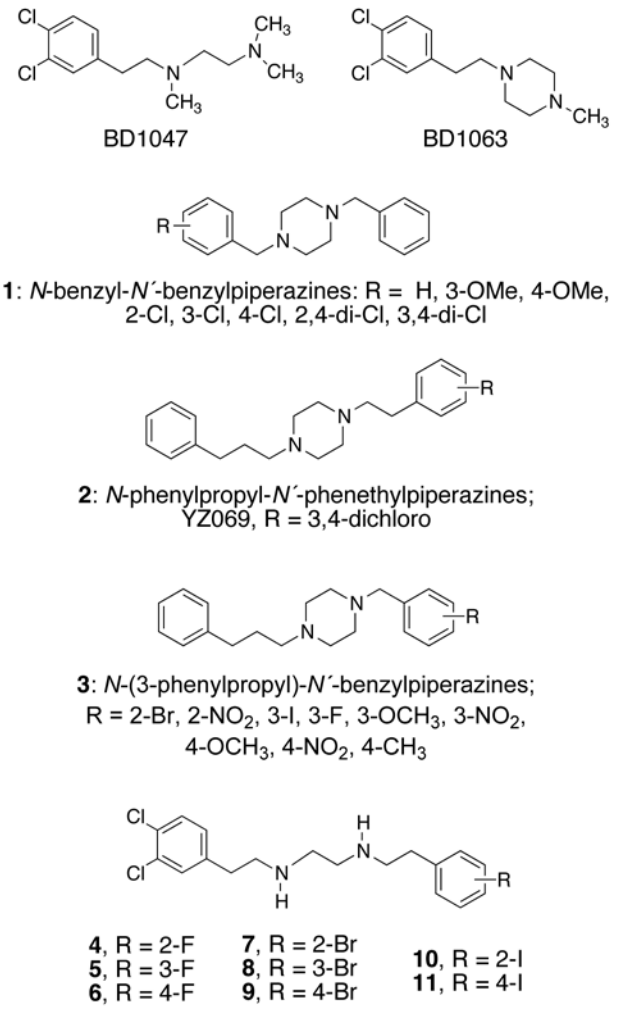

Figure 1: Structures of representative σ_1 receptor ligands, and the novel series of halogenated *N,N´*-diphenethylethylenediamines **4 - 11**.

binding parameters in vitro, and measured lipophilicity by HPLC (log k'_w) and computational (ClogP) methods. One of the aromatic rings was held constant with a 3,4-dichloro pattern, while the other ring was varied to include fluoro, bromo and iodo substituents at the 2-, 3- or 4- positions. We focused on halogen substitution with a longer-term view toward radiolabeling with fluorine-18 for PET imaging, or radiolabeling with iodine-123 for SPECT imaging [22,23].

Materials and Methods

General information

Chemical reagents and HPLC solvents were the best grade available from Aldrich Chemical Co. (Milwaukee, WI), and were used as received unless further noted. Reaction solvents (CH_2Cl_2, CH_3CN, THF and benzene) were dried, and freshly distilled under nitrogen before use. Ethylenediamine was distilled from freshly activated, 5 Å molecular sieves, and the heart cut was then distilled from sodium metal (bp 117.0-117.5 °C). ^1H and ^{13}C NMR were performed using ARX-250, DRX-300 or DRX-500 MHz spectrometers (Bruker BioSpin Corp., Westmont, IL). Chemical shifts are reported in ppm (δ) relative to internal Me_4Si in $CDCl_3$ unless otherwise stated. Elemental analyses were determined by Atlantic Microlab, Inc. (Norcross, GA). The C, H, N analyses were performed by combustion using automated analyzers, and the accuracy and precision are ± 0.3%. ESI-MS analyses were performed on a Finnigan TSQ7000 mass spectrometer (Thermo Finnigan, San Jose, CA). The HPLC-MS analyses utilized a Waters (Milford, MA) C18 Nova Pak® column (3.9 x 300 mm) with a solvent system comprised of an aqueous phase including 0.1 % TFA and an organic phase including 0.1 % TFA in acetonitrile with the following gradient program: Time (t) = 0 min 5 % B, t = 2 min 40 % B, t = 7 min 40 % B, t = 37 min 80 % B, t = 39 min 95 % B, t = 45 min 95 % B. [^{3}H]-(+)-Pentazocine (36 Ci / mmol) was purchased from Perkin Elmer Life Sciences (Waltham, MA), and fresh-frozen English Hartley guinea pig brains were obtained from Rockland Immunochemicals, Inc. (Gilbertsville, PA). A Brandel R48 manifold (Brandel Instruments, Gaithersburg, MD) was used for receptor binding filtrations. Radioactivity was measured using a Wallac 1409 (Turku, Finland) liquid scintillation counter and OptiPhase® HiSafe 2 cocktail (Perkin Elmer) at a tritium efficiency of 45%.

Chemistry

N-(2-aminoethyl)-2-(3,4-dichlorophenyl)-acetamide (12): 3,4-Dichlorophenylacetic acid (5.36 g, 0.0261 mol) and *N*-hydroxyphthalimide (4.36 g, 0.0267 mol) were added to a flask containing CH_2Cl_2 (50 mL), treated with a solution of DCC (7.78 g, 0.0377 mol) in CH_2Cl_2 (50 mL), and stirred for 30 min. DCU was removed by filtration, and the filtrate added dropwise to neat ethylenediamine (13.5 g, 0.224 mol) over 30 min. The solution was stirred overnight, filtered and treated with 10% citric acid (75 mL). The pH was adjusted to 3 by drop-wise addition of concentrated HCl. The aqueous layer was separated, the organic layer was extracted with water, and the aqueous layers were combined. The aqueous solution was brought to pH 12 with concentrated NH_4OH, and extracted with $CHCl_3$. The extracts were pooled, dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The yellow oil was dissolved in absolute ethanol (10 mL), and treated with 49% HBr (2 mL) to give **12** as a white salt (0.34 g, 52%). ^1H NMR (300 MHz, D_2O): δ 3.09 (t, 2H, CH_2NH_2); 3.45 (t, 2H, (C=O)NHCH$_2$); 3.58 (s, 2H, Ar CH_2); 7.1 (d, 1H, ArH); 7.461 (t, 2H, ArH). ^{13}C NMR (75 MHz, D_2O^i): δ 36.89, 38.98, 40.96, 129.04, 130.43, 130.46, 130.98, 131.61, 134.86, 174.4. MS-ESI direct infusion: Theory (M^+ m/z, %): 246.8, 100; 248.7, 69. Found (M^+ m/z, %): 246.0, 100; 248.0, 64.

General method for synthesis of amides 13 – 20: The hydrobromide salt of 12 was dissolved in water, treated with NH_4OH, and the free amine extracted with CH_2Cl_2 (3 x 100 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered and volatiles removed under reduced pressure. The residual oil was dissolved in THF (50 mL) and 1 equivalent of Et_3N was added. Then 1.5 to 2 equivalents of the appropriate acid chloride were prepared by refluxing the halogenated phenylacetic acid with thionyl chloride (2.5 - 10 volume equivalents per gram acid). THF (2 mL) was added to the acid chloride solution, and volatiles were evaporated under reduced pressure. The residual oil was dissolved in THF, and added drop-wise to the amine solution. The reaction was stirred overnight, and then slowly added to 2 M HCl (10 mL). The mixture was concentrated under reduced pressure to approximately 10 mL, and extracted with $CHCl_3$ (3 x 20 mL). The organic fractions were pooled, and evaporated under reduced pressure. The remaining oil was dissolved in absolute ethanol, and added drop-wise to water to yield a white precipitate that was isolated by filtration, dried and characterized.

N-{2-[2-(3-Fluorophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (14): The acid chloride generated from 3-fluorophenylacetic acid (0.56 g, 0.0036 moles) was added to a

solution of **12** (0.61 g, 0.0019 moles) and Et_3N (0.54 mL, 0.0039 moles) to generate the diamide (0.34 g, 47%). ^1H NMR (300 MHz, D_6-DMSO): δ 3.10 (s, 4H, (C=O)NHCH$_2$); 3.41 (s, 4H, Ar CH$_2$); 7.05 (d, 2H, ArH); 7.22 (d, 1H, ArH); 7.31 (q, 1H, ArH); 7.51 (d, 1H, ArH); 7.55 (s, 1H, ArH); 8.10 (br s, 2H, NH$_2$). ^{13}C NMR (75 MHz D_6-DMSO): δ 40.98, 41.84, 112.97, 113.25, 115.62, 115.90, 125.15, 129.03, 129.52, 129.90, 130.01, 130.22, 130.59, 131.09, 137.39, 139.07, 169.42, 169.72. MS-ESI: Theory (M+H m/z, %): 383.07, 100; 385.1, 64. Found (M+H m/z, %): 383.09, 100; 385.0, 60.

N-{2-[2-(4-Fluorophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (15): The acid chloride generated from 4-fluorophenylacetic (1.01 g, 0.0065 moles) acid was added to a solution of **12** (0.64 g, 0.0019 moles) and Et_3N (0.87 mL, 0.0062 moles) to generate the diamide (0.69 g, 93%). ^1H NMR (300 MHz, D_6-DMSO): δ 2.18 (m, 4H, (C=O)NHCH$_2$); 3.38 (d, 4H, Ar CH$_2$); 7.09 (t, 2H, ArH); 7.23 (m, 3H, ArH); 7.52 (t, 2H, ArH); 8.07 (br d, 2H, NH). ^{13}C NMR (75 MHz, D_6-DMSO): δ 38.72, 38.84, 41.37, 41.70, 115.07, 115.35, 129.42, 129.91, 130.61, 130.97, 131.12, 131.22, 131.48, 132.80, 169.80, 170.54. HPLC-MS, retention time = 21.7 min. Theory (M+H m/z, %): 383.0, 100; 384.0, 19; 385.0, 64. Found (M+H m/z, %): 383.0, 100; 384.0, 13; 385.0, 69.

N-{2-[2-(2-Bromophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (16): The acid chloride generated from 2-bromophenylacetic acid (1.57 g, 0.0073 moles) was added to a solution of **12** (1.5 g, 0.0046 moles) and Et_3N (1.03 mL, 0.0074 moles) to generate the diamide (1.48 g, 72%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.5-3.6 (s and m, 8H, CH$_2$(C=O)NHCH$_2$); 6.5 (br s, 1H, NH); 7.1-7.2 (d, 1H NH); 7.3-7.4 (m, 1H, ArH); 7.37-7.40 (m, 3H, ArH); 7.5-7.6 (dd, 1H, ArH); 7.69-7.73 (m, 1H, ArH); 7.95-7.99 (m, 1H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 37.29, 39.78, 42.52, 43.93, 49.77, 124.98, 127.60, 128.15, 128.76, 128.89, 129.25, 130.66, 131.26, 131.86, 132.83, 133.20, 134.40, 170.48, 171.08. HPLC-MS, retention time = 8.5 min. Theory (M+H m/z, %): 443.9, 100; 441.9, 62; 445.9, 45. Found (M+H m/z, %): 443.0, 100; 441.0, 65; 445.1, 43.

N-{2-[2-(3-Bromophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (17): The acid chloride generated from 3-bromophenylacetic acid (1.3 g, 0.0062 moles) was added to a solution of **12** (1.6 g, 0.0048 moles) and Et_3N (0.86 mL, 0.0062 moles) to generate the diamide (0.98 g, 46%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.35 (t, 4H, (C=O)NHCH$_2$); 3.46 (d, 4H, Ar CH$_2$); 6.10 (br s, 1H, NH); 6.26 (br s, 1H NH); 7.07 (dd, 1H, ArH); 7.17 (d, 1H, ArH); 7.22 (d, 1H, ArH); 7.37 (d, 1H, ArH); 7.40-7.46 (m, 3H, ArH).^{13}C NMR (75 MHz, $CDCl_3$) δ 40.07, 40.50, 42.46, 43.06, 122.90, 128.0, 128.7, 130.5, 130.6, 130.7, 131.2, 132.3, 134.7, 136.7, 170.9, 171.63. MS-ESI: Theory (M+H m/z, %): 444.9, 100; 442.9, 62. Found (M+H m/z, %): 444.7, 81; 442.7, 43. Theory (M+NH$_4$ m/z, %): 462.0, 100; 464.0, 44. Found (m/z, %): 462.9, 100; 464.9, 74.

N-{2-[2-(4-Bromophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (18): The acid chloride generated from 4-bromophenylacetic acid (2.23 g, 0.0104 moles) was added to a solution of **12** (2.9 g, 0.0088 moles) and Et_3N (1.59 mL, 0.0114 moles) to generate the diamide (2.5 g, 65%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.3 (s, 4H, (C=O)NHCH$_2$); 3.4 (d, 4H, Ar CH$_2$); 5.9 (br s, 1H, NH); 6.2 (br s, 1H, NH); 7.0 (m, 3H, ArH); 7.36 (d, 1H, ArH); 7.41 (d, 1H, ArH); 7.48 (d, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.88, 40.01, 40.51, 40.63, 42.48, 42.52, 42.90, 42.94, 121.52, 128.75, 130.75, 131.06, 131.22, 131.57, 132.10, 132.81, 133.48, 134.80, 170.78, 171.77. MS-ESI(+): Theory (M+Na m/z, %): 466.9, 100; 464.9, 62; 468.9, 45. Found (m/z, %): 465.1, 100; 463.2, 84, 466.6, 21.

N-{2-[2-(2-Iodophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (19): The acid chloride generated from 2-iodophenylacetic acid (1.65 g, 0.0063 moles) was added to a solution of **12** (2.03 g, 0.0062 moles) and Et_3N (0.87 mL, 0.0063 moles) to generate the diamide (0.6311g, 21%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.39 (d, 6H, CH$_2$(C=O)NHCH$_2$)), 3.82 (s, 2H, Ar CH$_2$), 5.87 (br s, 1H, NH), 6.42 (br s, 1H, NH), 7.02 (t, 1H, ArH), 7.10 (d, 1H, ArH) 7.28-7.41 (m, 4H, ArH), 7.87 (d, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.61, 39.58, 40.64, 42.42, 48.29, 128.71, 128.95, 129.34, 130.59, 130.99, 131.20, 131.37, 132.63, 134.82, 137.71, 139.83, 170.47, 171.01. HPLC-MS, retention time = 7.7 min. Theory (M+H m/z, %): 490.9, 100; 492.9, 64. Found (M+H m/z, %): 490.9, 100; 493.0; 60.

N-{2-[2-(4-Iodophenyl)-acetylamino]-ethyl}-2-(3,4-dichlorophenyl)-acetamide (20): The acid chloride generated from 4-iodophenylacetic acid (1.33 g, 0.0051 moles) was added to a solution of **12** (1.00 g, 0.0031 moles) and Et_3N (0.90 mL, 0.0065 moles) to generate the diamide (0.50 g, 33%). ^1H NMR (300 MHz, CDCl3): δ 3.33 (s, 4H, (C=O)NHCH$_2$)), 3.42 (s, 4H, Ar CH$_2$), 6.10 (br s, 1H, NH), 6.34 (br s, 1H, NH), 6.97 (d, 2H, ArH), 7.08 (d, 1H, ArH), 7.39 (m, 2H, ArH), 7.67 (d, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.97, 40.52, 42.45, 42.99, 128.75, 130.72, 131.21, 131.21, 132.76, 134.16, 138.0, 170.88, 171.82. HPLC-MS, retention time = 11.1 min. Theory (M+H m/z, %): 490.9, 100; 492.9, 64. Found (M+H m/z, %): 490.9, 100; 492.9; 65.

General method for reduction of amides 13 – 20: AlH_3-Et_3N was prepared as previously described [24]. Briefly, to a 100 mL, 2-necked flask under nitrogen was added 1M $LiAlH_4$ (35 mL, 0.035 mol) in THF. The solution was stirred, cooled in an ice bath and then treated drop-wise with concentrated H_2SO_4 (1.22 mL, 0.0439 mol). Neat Et_3N (5.4 mL, 0.039 mol) was added to form the reducing agent in situ, and the appropriate amide (**13** - **20**) dissolved in freshly distilled THF (20 mL) was added drop-wise. A molar ratio of 2.5 to 1 of AlH_3-Et_3N to amide was used, and reactions were kept under nitrogen at ambient temperature for 72 h. Mixtures were then poured into ice-cold 2 M HCl (15 mL), and concentrated under reduced pressure to 15 mL. CH_2Cl_2 (30mL) was added, and the pH adjusted to > 11 with aqueous NaOH (15%). Mixtures were extracted with CH_2Cl_2 (3 x100 mL), dried (Na_2SO_4) and filtered. Evaporation under reduced pressure provided oils that were dissolved in absolute ethanol, and converted to salts by addition of 49% HBr. The free bases were obtained for NMR spectroscopy by treatment of the salts with 15% NaOH (1 mL) and extraction with CH_2Cl_2 (3 x 2 mL). After drying (Na_2SO_4), the CH_2Cl_2 was removed under reduced pressure, and the oil was dissolved in the appropriate NMR solvent.

N-[2-(3,4-Dichlorophenyl)-ethyl]-*N'*-[2-(2-fluorophenyl)-ethyl]-ethane-1,2-diamine (4): The acid chloride generated from 2-fluorophenylacetic acid (0.55 g, 0.0035 moles) was added to a solution of **12** (0.60 g, 0.0018 moles) and Et_3N (0.54 mL, 0.0039 moles). The reaction was stirred overnight, purified an oil and the amide **13** (0.694 g, 0.0018 moles) was directly reduced with AlH_3-Et_3N (9 mL, 0.009 moles) and isolated as the dihydrobromide salt (0.02 g, 2%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.73-3.8 (m, 12H, CH$_2$); 7.05 (m, 2H, ArH); 7.21 (p, 3H, ArH); 7.30 (d, 1H, ArH); 7.35 (d, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.81, 35.63, 48.92, 49.14, 49.66, 50.59, 115.12, 115.41, 123.95, 127.78, 127.88, 128.15, 130.04, 130.28, 130.60, 130.92, 130.99, 140.51. HPLC-MS, retention time = 6.1 min. Theory (M+H m/z, %): 355.11, 100; 356.12, 19; 357.11, 64. Found (M+H m/z, %): 354.98, 100; 356.01, 19; 356.98, 68. Elemental analysis for $C_{18}H_{21}Cl_2FN_2$ • 2 HBr: Theory: C, 41.81; H, 4.48; N, 5.42; Found: C, 41.69; H, 4.43; N, 5.27.

N-[2-(3,4-Dichlorophenyl)-ethyl]-*N'*-[2-(3-fluorophenyl)-

ethyl]-ethane-1,2-diamine (5): Compound **14** (1.30 g, 0.0034 moles) was reduced with AlH_3-Et_3N (17 mL, 0.017 moles) and isolated as the dihydrobromide salt (0.13 g, 7%). ^{1}H NMR (300 MHz, D_6-DMSO): δ 2.07 (s, 2H, NH); 2.97 (t, 4H Ar CH_2); 3.28 (CH_2NHCH_2); 7.15 (t, 3H, ArH); 7.32 (d, 1H, ArH); 7.39 (q, 1H, ArH); 7.61 (d, 2H, ArH). ^{13}C NMR (75 MHz, DMSO): δ 30.51, 60.67, 31.16, 42.52, 47.25, 47.42, 113.64, 113.91, 115.39, 115.67, 124.94, 129.37, 130.55, 130.72, 130.87, 131.11, 137.96, 139.50. HPLC-MS, retention time = 21.8 min. Theory (M+H m/z, %): 355.1, 100; 357.1, 64; 359.1, 10. Found (M+H m/z, %): 354.9, 100; 356.9, 73; 359.0, 10. Elemental analysis for $C_{18}H_{21}Cl_2FN_2$ • 2 HBr: Theory: C, 41.81; H, 4.48; N, 5.42; Found: C, 42.07; H, 4.28; N, 5.36.

N-[2-(3,4-Dichlorophenyl)-ethyl]-*N'*-[2-(4-fluorophenyl)-ethyl]-ethane-1,2-diamine (6): Compound **15** (0.57 g, 0.0015 moles) was reduced with AlH_3-Et_3N (7.4 mL, 0.0074 moles) and isolated as the hydrobromide salt (0.065 g, 9%). ^{13}C NMR (75 MHz, DMSO): δ 30.55, 30.76, 42.57, 47.31, 47.88, 115.23, 115.51, 129.37, 129.55, 130.58, 130.69, 130.87, 131.08, 138.08. HPLC-MS, retention time = 6.5 min. Theory (M+H m/z, %): 355.1, 100; 357.1, 64; 359.1, 10. Found (M+H m/z, %): 355.0, 100; 357.0, 73; 359.0, 11.

N-[2-(2-Bromophenyl)-ethyl]-*N'*-[2-(3,4-dichlorophenyl)-ethyl]-ethane-1,2-diamine (7): Compound **16** (0.44 g, 0.0010 moles) was reduced with AlH_3-Et_3N (10 mL, 0.010 moles) and isolated as the dihydrobromide salt (0.32 g, 56%). ^{1}H NMR (250 MHz, D_2O): δ 2.96 (t, 2H, Ar CH_2); 3.12 (t, 2H, Ar CH_2); 3.31-3.25 (s and m, 8H, CH_2NHCH_2); 7.25-7.18 (m, 2H, ArH); 7.35-7.34 (m and s, 2H, ArH); 7.51-7.46 (s and d, 2H, ArH); 7.64 (d, 1H, ArH). ^{13}C NMR (62 MHz, $CDCl_3$): δ 35.61, 36.73, 48.99, 49.14, 49.36, 50.66, 124.57, 127.42, 127.88, 128.16, 130.06, 130.29, 130.61, 130.74, 132.27, 132.87, 139.36, 140.44. HPLC-MS, retention time = 20.9 min. Theory (M+H m/z, %): 417.0, 100; 415, 62; 419.0 45. Found (M+H m/z, %): 416.9, 100; 414.9, 62; 418.9, 44. Elemental analysis for $C_{18}H_{21}BrCl_2N_2$ • 2 HBr • 1.5 H_2O: Theory: C, 35.73; H, 4.33; N, 4.63; Found: C, 35.72; H, 4.10; N, 4.69.

N-[2-(3-Bromophenyl)-ethyl]-*N'*-[2-(3,4-dichlorophenyl)-ethyl]-ethane-1,2-diamine (8): Compound **17** (0.66 g, 0.0015 moles) was reduced with AlH_3-Et_3N (7.5 mL, 0.0075 moles) and isolated as the dihydrobromide salt (0.38 g, 44%). ^{1}H NMR (300 MHz, D_2O): δ 3.02 (s, 4H, Ar CH_2); 3.42 (s, 8H CH_2NHCH_2); 7.52-7.30 (m, 7H, ArH). ^{13}C NMR (75MHz, $CDCl_3$): δ 35.41, 36.51, 48.77, 48.90, 49.17, 124.39, 127.29, 127.73, 128.53, 129.85, 130.14, 130.47, 130.60, 130.73, 132.06, 132.69, 139.21, 140.4. HPLC-MS, retention time = 22.1 min. Theory (M+H m/z, %): 417.0, 100; 415.0, 62; 419.0, 45. Found (M+H m/z, %): 416.9, 100; 414.9, 60; 418.9, 45. Elemental analysis for $C_{18}H_{21}BrCl_2N_2$ • 2 HBr: Theory: C, 37.40; H, 4.01; N, 4.85; Found: C, 37.62; H, 3.96; N, 4.78.

***N*-[2-(4-Bromophenyl)-ethyl]-*N'*-[2-(3,4-dichlorophenyl)-ethyl]-ethane-1,2-diamine (9):** Compound **18** (0.4 g, 0.0009 moles) was reduced with AlH_3-Et_3N (4.52 mL, 0.0045 moles) and isolated as the salt (0.13 g, 25%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.72-2.76 (s and m, 8H, CH_2NHCH_2); 2.85 (t, 4H Ar CH_2); 7.04-7.06 (d of d, 1H, ArH); 7.09 (d, 2H, ArH); 7.32 (d, 1H, ArH); 7.37 (d, 1H, ArH); 7.42 (d, 2H, ArH). ^{13}CNMR (75 MHz, $CDCl_3$): δ 29.69, 32.42, 35.63, 35.9, 49.18, 50.89, 51.13, 119.88, 128.17, 128.43, 128.69, 129.73, 130.05, 130.44, 130.60, 131.47, 132.26, 139.10. HPLC-MS, retention time = 22.4 min. Theory (M+H m/z, %): 417.0, 100; 415.0, 62; 419.0, 45. Found (M+H m/z, %): 416.9, 100; 414.9, 60; 418.9, 43. Elemental analysis for $C_{18}H_{21}BrCl_2N_2$ • 1.75 HBr • 0.25 H_2O: Theory: C, 38.45; H, 4.17; N, 4.98; Found: C, 38.44; H, 4.09; N, 4.65.

N-[2-(3,4-Dichlorophenyl)-ethyl]-*N'*-[2-(2-iodophenyl)-ethyl]-ethane-1,2-diamine (10): Compound **19** (0.31 g, 0.0006 moles) was reduced with AlH_3-Et_3N (3 mL, 0.003 moles) at 0 °C and isolated as the salt (0.12 g, 32%). ^{1}H NMR (300 MHz, D_6-DMSO): δ 2.9-3.3 (m, 8H CH_2) 7.30 (s, 1H, ArH), 7.38 (d, 2H, ArH) 7.63 (s, 1H, ArH) 7.89 (d, 1H, ArH) 8.98 (d, 3H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.47, 36.36, 42.53, 46.59, 47.27, 101.74, 128.69, 128.83, 129.21, 129.39, 129.59, 129.90, 130.71, 130.89, 131.11, 137.99, 139.37. HPLC-MS, retention time = 24.9 min. Theory (M+H m/z, %): 463.0, 100; 464.0, 20; 465.0, 64. Found (M+H m/z, %): 462.9, 100; 464.0, 21; 464.9, 63. Elemental analysis for $C_{18}H_{21}ICl_2N_2$ • 1.75 HBr • 0.75 H_2O: Theory: C, 35.48; H, 4.22; N, 4.47; Found: C, 35.48; H, 4.16; N, 4.35.

***N*-[2-(3,4-Dichlorophenyl)-ethyl]-*N'*-[2-(4-iodophenyl)-ethyl]-ethane-1,2-diamine(11):** Compound **20** (0.25 g, 0.0005 moles), was reduced with AlH_3-Et_3N (3 mL, 0.003 moles) at 0 °C and isolated as the dihydrobromide salt (0.052g, 17%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.75-2.88 (m, 12H, CH_2), 7.00 (dd, 1H, ArH), 7.2 (d, 2H, ArH), 7.33 (t, 2H, ArH), 7.36-7.38 (m, 1H, ArH), 7.63 (d, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.45, 35.78, 50.47, 50.67, 53.32, 58.31, 128.09, 128.69, 130.00, 130.22, 130.50, 130.71, 132.19, 137.38, 139.57, 140.27. HPLC-MS, retention time = 24.9 min. Theory (M+H m/z, %): 463.0, 100; 464.0, 20; 465.0, 64. Found (M+H m/z, %): 462.9, 100; 464.0, 21; 464.9, 67. Elemental analysis for $C_{18}H_{21}ICl_2N_2$ • 2 HBr: Theory: C, 34.59; H, 3.71; N, 4.48; Found: C, 34.36; H, 3.78; N, 4.44.

Lipophilicity Measurements

Computational method: Specific algorithms for calculating ClogP utilized fragment-based methods developed by the Medicinal Chemistry Project and BioByte [25] contained as a subroutine in ChemDraw 9.0 (CambridgeSoft Corporation, Cambridge, MA).

Reverse-phase HPLC method: These procedures were performed as described by Minick et al. [26]. The HPLC equipment consisted of Waters M6000A pumps and a Waters 490E programmable multiwavelength detector. The guard column (C18) and main column (Econosil C8, 4.6 mm X 10 cm) were from Alltech Applied Science (State College, PA). The organic phase was methanol containing 0.25% (v/v) *n*-octanol and the aqueous phase was 0.02 M MOPS buffer containing 0.12% (v/v) n-decylamine (pH 7.5). Multiple different organic / aqueous compositions were utilized at a flow rate of 2 mL / min. Ligand samples were dissolved in 1 mL of the organic phase, and 3 injections each of samples and standards at 3 different concentrations of organic phase were performed. The void volume was determined with urea, detected at UV = 214 nm. All other compounds were detected at 280 and 254 nm. The $\kappa\times_{\sqrt{}}$ value for all injected samples and standards was calculated with the following formula: $\kappa\times_{\sqrt{}}$ = (retention time of compound / dead time) - 1. Then a graph of λογ $\kappa\times_{\sqrt{}}$ versus fraction methanol was generated. The data for standards and ligand samples were fit to a linear equation, and the intercept was determined which is the λογ $\kappa\times_{\sqrt{}}$. Next, a curve was generated for the standards by plotting the known log PC values versus the experimental λογ $\kappa\times_{\sqrt{}}$ and a linear equation ($y = 0.92 x + 0.90$; $r^2 = 0.94$) was generated where y = k'_w calculated and x = log $k'_{\sqrt{}}$. Then, k'_w was determined for the samples from the equation for the standard curve and the intercept log $k'_{\sqrt{}}$ of the samples.

σ_1 Receptor Binding Assays

Binding assays were performed using 1.0 nM [^{3}H](+)-pentazocine and membranes prepared from fresh-frozen guinea pig brains as previously described [27,28] with minor modifications. Non-specific binding was defined by haloperidol (10.0 μM), each assay tube contained 0.24 mg protein, and assays were performed in Tris-HCl buffer (50 mM,

pH 7.4) at 37 ºC for 150 min. Assays were terminated by the addition of ice-cold buffer, and rapid filtration through Whatman GF/B glass fiber filters that had been presoaked in 0.5 % polyethylenimine. Filter papers were then washed with ice-cold buffer (3 x 5 mL), soaked in cocktail, dark-adapted overnight and then counted for tritium. Test compounds were dissolved in the minimum amount of ethanol, and assay buffer was added to make a concentrated stock (1×10^{-3} M) that was used to prepare serial dilutions in buffer. Ligand concentrations in the assays ranged from 1000 to 0.1 nM. The final concentration of ethanol in any assay tube never exceeded 0.5%, an amount that did not affect [^{3}H](+)-pentazocine binding in control studies. The IC_{50} and K_i values were determined in two to four assays, each performed in duplicate, by non-linear regression of binding data using curve-fitting programs Prism 4.0b (Graph-Pad Software, San Diego, CA) and Radlig 6.0 (Biosoft, Inc., Ferguson, MO). K_i values were derived from IC_{50} data by the Cheng-Prusoff relationship [29] using an input K_d of 2.3 nM for [^{3}H](+)-pentazocine [28].

Results and Discussion

The construction of the eight halogenated *N,N´*-diphenethylethylenediamines (**4** - **11**) involved linear synthesis from amide **12** as a common precursor (Figure 2). The synthesis of **12** was accomplished by a two-step, activation - amidation process. Several methods for activation of 3,4-dichlorophenylacetic acid were explored, including conversion of the acid to either an active ester or to an anhydride with DCC, followed by amide formation by coupling with anhydrous ethylenediamine. Based upon the initial yields and ease of performance, activation using *N*-hydroxyphthalimide and DCC proved to be the method of choice. This reaction was conducted six times on a 5 - 6 gram scale of the carboxylic acid, and yielded **12** as the white hydrobromide salt in a reproducible 31 ± 6% yield.

The formation of diamides **13** - **20** also was investigated using two different routes. The first involved DCC-mediated coupling of **12** with the carboxylic acid, and the second involved coupling of **12** with the carboxylic acid chloride. Synthesis via the carboxylic acid chloride route was advantageous because the reaction side products are soluble in water while the diamides readily precipitate. Thus, the final diamide products were obtained in high purity by simple filtration, and procedural issues involving removal of DCU during the alternative route were avoided. Isolated yields for diamides **13** - **20** ranged between 21 - 93%.

The final target compounds **4** - **11** were prepared by reduction of the corresponding diamides. Exploratory attempts to accomplish the transformations using $LiAlH_4$ at 0 ºC resulted in complex product

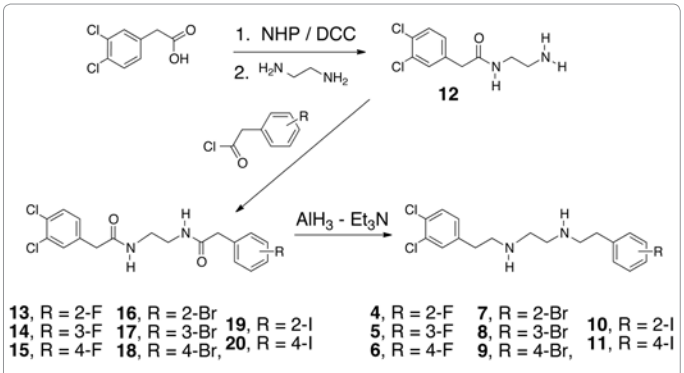

Figure 2: Synthetic route to halogenated *N,N´*-diphenethylethylenediamines **4 - 11**.

mixtures as a consequence of loss of aromatic halogens. Subsequently, the more selective aluminum hydride-triethylamine (AlH_3-Et_3N) reducing agent developed by Cha and Brown [24] was investigated. The AlH_3-Et_3N was prepared in situ, and a molar ratio of 2.5 to 1 of AlH_3-Et_3N to amide was employed. Reactions were allowed to proceed at room temperature under nitrogen for 2.5 days. Isolated yields of the fluoro analogs **4** - **6** as the dihydrobromide salts were low, < 10%, but the procedure was straightforward. Improved isolated yields, 25 - 56%, were obtained for the bromo congeners **7** - **9**. For the iodinated diamides **19** and **20**, AlH_3-Et_3N reductions at room temperature gave mixtures of the iodinated and the deiodinated derivatives. However, deiodination was not observed when the reactions were kept at 0 ºC. Thus, the 2- and 4-iodophenethyl derivatives, **10** and **11**, were at hand in yields of 32% and 17%, respectively. Attempts to prepare **10** and **11** from bromo analogs **7** and **9** by copper-assisted iodine for bromine exchange, or through stannylated intermediates, were unsuccessful.

The log k'_w values for representative isomeric *N,N´*-diphenethylethylenediamines were determined experimentally by a reverse-phase HPLC method [26], as well as calculated using ClogP [25] (Table 1). When comparing the experimental k'_w values for compounds with similar structures, the expected order of lipophilicity is fluoro- < bromo- < iodo- based on known lipophilicity constants. This relationship held true for analogs **6**, **9** and **11** having the three different halogens in the 4-position (Table 1). There is no significant difference in k'_w between the 2-bromophenyl and 3-bromophenyl derivatives **7** and **8**; however, the value for the 4-bromophenyl derivative **9** was 0.22 - 0.25 units higher. Considering that this is an HPLC method, it appears that the interaction of these isomers with the stationary phase promotes subtle differences. The experimentally determined k'_w values ranged from 2.54 – 3.71 while the calculated Clog P values ranged from 5.03 – 6.01. This difference in magnitude can be explained by the fact that the pH of the aqueous component of the HPLC solvent system is 7.5, and it is known that the computer program does not adjust for the protonation status of the amines.

To determine the ability of compounds **4** - **11** to bind to σ_1 receptors, competition assays against [^{3}H](+)-pentazocine were performed using established methods in guinea pig brain membranes [27,28]. Binding parameters are summarized in (Table 1). Within each individual halogen series, the 3- and 4- substituted derivatives displayed higher binding affinities than the 2-substituted derivative. This difference is pronounced in relation to the size of the halogen. The 2-fluoro substituted ligand **4** shows only a 1.5-fold lower σ_1 receptor affinity than positional isomers **5** and **6**. By contrast, the 2-bromo substituted ligand **7** exhibits 2.7 fold lower affinity than isomers **8** and **9**. Similarly, 2-iodo analog **10** suffers a 2.7-fold loss of affinity compared to the 4-iodo isomer **11**. These data indicate that σ_1 receptor binding in this series is sensitive to steric bulk at the 2-position. The apparent affinity (K_i) values for the 3- and 4-substituted bromo derivatives **8** and **9** are quite similar to those observed for the 3- and 4-substituted fluoro derivatives **5** and **6**. Conversely, the 4-substituted iodo derivative **11** has a 2-fold poorer K_i value than either one of compounds **6** or **9**. In keeping with this trend, **10** displays lower affinity than either **4** or **7**. Thus, fluoro and bromo substituents impart enhanced σ_1 receptor binding affinity as compared to the corresponding iodinated derivatives for this series of ligands.

Conclusions

The synthesis and evaluation of σ_1 receptor binding diphenethylethylenediamines with a 3,4 dichlorophenyl moiety and a halogenated phenyl ring was explored. The structural modifications

	Lipophilicity		σ_1 Receptor Binding Parameters		
Compound	k'_w	**ClogP**	IC_{50} (nM)	K_i (nM)	**Hill Slope**
4, 2-F; n = 4	ND	5.03	17.48 ± 0.31	12.08 ± 0.22	-1.07 ± 0.01
5, 3-F; n = 4	ND	5.03	11.21 ± 1.29	7.74 ± 0.89	-1.13 ± 0.09
6, 4-F; n = 4	2.54 ± 0.05	5.03	11.34 ± 2.02	7.83 ± 1.39	-1.21 ± 0.05
7, 2-Br; n = 3	3.09 ± 0.05	5.75	26.62 ± 3.95	18.39 ± 2.73	-0.78 ± 0.03
8, 3-Br; n = 3	3.06 ± 0.07	5.75	9.14 ± 0.85	6.35 ± 0.59	-1.02 ± 0.15
9, 4-Br; n = 3	3.31 ± 0.08	5.75	9.85 ± 3.92	6.80 ± 2.71	-1.00 ± 0.02
10, 2-I; n = 2	ND	6.01	62.47 ± 0.96	43.15 ± 0.66	-1.12 ± 0.03
11, 4-I; n = 4	3.71 ± 0.05	6.01	22.89 ± 0.94	15.82 ± 0.65	-1.59 ± 0.08*

ND = not determined. Binding parameters are means ± SEM for two to four assays, each performed in duplicate, n = number of trials. * Significantly different from unity

Table 1: Lipophilicity measurements and σ_1 receptor binding parameters.

provided differences in lipophilicity, and influenced σ_1 receptor binding. Affinity was moderately sensitive to steric bulk at the 2- position. Bromine or fluorine substituents at a given position gave higher σ_1 receptor binding affinity than an iodine substituent. The 8-atom spacer between the hydrophobic (aromatic) groups in compounds **4** - **11** is consistent models described by Glennon, Ablordeppey and colleagues [18,30], which indicate that 7 - 10 atoms are needed between the primary and secondary hydrophobic regions of the postulated pharmacophore to confer high σ_1 receptor binding affinity. Lipophilicity measurements, coupled with σ_1 receptor binding affinities, suggest that ligands from this active series, such as 3-fluoro analog **5**, may be good candidates for in vivo studies.

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