Supporting Information

Design, synthesis and progress towards optimization of potent small molecule antagonists of CC-Chemokine Receptor 8 (CCR8)

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CCR8 Binding Assay Protocol

L1.2-CCR8 cells are stable recombinant L1.2 cells overexpressing the human CCR8 receptor. The cells were routinely cultured and passaged in RPMI based medium. The incubators were set at 37°C, 6% CO_2 and 90% relative humidity. The density of the cell suspension was maintained around 0.7 to 1.0 million cells per ml. Cells were removed from the culture after about 2 months and replaced with freshly thawed cells of lower passage number. On Day 1, the cells were split to be approximately 0.5 millions/ml for next day assay by dilution into fresh RPMI medium in the morning. N-butyric acid (500 mM) to a final concentration of 5 mM was added into the cell suspension (1:100 dilution) in late afternoon. On Day 2, the cells were harvested by spinning down the cells for 5 minutes (1350 rpm) in a table top centrifuge, and the cells were washed with 35 ml of assay binding buffer once, and then re-suspend the cells into the binding buffer at 2 millions cells per ml of the buffer.

A 10-point dose-response curve (final concentrations are 100µM, 33.3 µM, 11.1 µM, 3.70 µM, 0.411 µM, 0.137 µM, 0.0457 µM, 0.0152 µM, 0.00508 µM) was prepared by diluting a 20 mM solution of the compounds 1:2 (6 µL into 6 µL DMSO) and then serially diluting the sample 1:3 (4 µL into 8 µL DMSO). To prepare a screen for the compounds (at 10 µM and 1 µM), a 20 mM solution of the compounds was diluted 1:20 (1 µL into 19 µL DMSO). The sample was then subsequently diluted 1:10 dilution (2 µL into 18 µL of DMSO).

To prepare the compound plate, 1 µL from each of the above DMSO solutions was transferred into each well of a polypropylene 96-well plate for the following binding experiment. 1 µL of DMSO was stamped into each well of the blank control. 50 µL of the L1.2-CCR8 cell suspension (2 million cells/mL) was added into each well of the compound plate (100,000 cells/well), and pipette up and down three times to mix. Then 1 µL of the 10 µM cold I-309 solution was added into control wells, A11, B11, C11 and D11 as non-specific control. The cells were incubated with the compounds for 40 min. at room temperature. Then 50 µL of 0.2 nM^{125} I-I-309 solution was added into each well of the above plate. The radioligand was added to the mixture of cells and compounds and incubated at room temperature for one hour. 100 µL of 0.33% PEI solution was added into each well of the filter plate (GF/B), and incubated for about half an hour at room temperature. The samples were harvested using Packard cell harvester, the plates were washed with 4 wells of cold assay wash buffer, the harvester was opened, and the plate was dried under vacuum for about 30 seconds. The filter plate was then air-dried overnight, the plates were bottom-sealed, 500 µL MicroScint-20 fluid was added to each well, and the top of the plate sealed using Topseal. The plate was read on the Topcount.

CCR8 Chemotaxis Assay Protocol

The chemotaxis buffer was 1(x) Hanks Balanced salt solution supplemented with 10 mM HEPES and 0.5% fatty acid free BSA. The cells were prepared by culturing L1.2 transfectants @ 37 °C, 5.0% CO_2, and humid air overnight at 0.7-1.0 x 10^6 cells/ml in fresh media.

A dilution series with a sufficient volume per dilution to give 30 or 300 µL of sample/well of a 30 or 300 µL 96-well chemotaxis plate, respectively, was prepared. All dilutions were prepared in chemotaxis assay buffer. Samples were assayed in triplicate or quadruplicate.

The cells were counted and centrifuged at low speed (~1200 RPM in swing bucket rotor) to pellet out cells. The cells were re-suspended in an equal volume of warm chemotaxis buffer then centrifuged again, aspirated and re-suspended at 1.0 x 10^7 cells/ml in warm chemotaxis buffer.

The bottom wells were filled with +/- chemokine or inhibitors, (Add 30 or 300 µL to your 30 or 300 µL 96-well chemotaxis plate respectively) then membrane was snapped down onto the plate and checked that all wells made good contact. 20-25 µL droplets of cells were added to the open membrane

area. The plates were incubated at 37°, 5.0% CO_2, and humid air for 2.0 hrs. After the incubation step, the cells were scraped from the top of the membrane filter and rinsed well with PBS. Once plates were washed carefully, the membrane was removed from the plate. The migrated cells were lysed by freezing @ -80°C, 30 min, and then thawed @ at 37 °C. 6 µl 5X Lysis/CytoQuant buffer was added for 30µl chemotaxis plate, or 15 µl 20X Lysis/CytoQuant buffer for 300µl chemotaxis plate and fluorescence was read in Fluorometer at 485ex/535em.

Selectivity data for Compound 12c and 17c

Assay	12c % Inhibtion @ 1 uM	17c % Inhibtion @ 1 uM
Serotonin, Non-Selective (rat)	-9.78	ND
Serotonin, 5HT1A (Human Recombinant)	ND	-13.2
Serotonin, 5HT2A (human)	14.0	3.2
Adrenergic, Alpha 1, Non-selective (rat)	4.4	38.4
Adrenergic, Beta 1 (Human Recombinant)	9.5	9.0
Adrenergic, Beta 2 (Human Recombinant)	15.1	-7.5
Calcium Channel, Type L, Benzothiazepine Site (rat)	15.5	12.7
Calcium Channel, Type L, Dihydropyridine Site (rat)	-8.3	17.0
Cholecystokinin, CCKA (rat)	-7.1	-0.2
Dopamine, non selective (rat)	3.5	ND
Dopamine, D1 (Human Recombinant)	-10.1	8.3
Dopamine, D2 (Human Recombinant)	1.5	-6.4
Histamine, H2 (rat)	34.4	8.0
Muscarinic, central (rat)	-1.3	-13.1
Sodium, Site 2 (rat)	14.6	-11.1
Opiate, Non-selective (rat)	2.2	19.9

Experimental Section:

Materials and Methods: All reactions involving air-sensitive reagents were performed under a nitrogen atmosphere. Reagents were used as received from commercial suppliers unless otherwise noted. ^{1}H NMR data were recorded using the Bruker UltraShield 300 MHz/54mm instrument equipped with Bruker B-ACS60 Auto Sampler or the Varian 300 MHz instrument. Intermediates and final compounds were purified by flash chromatography using one of the following instruments: 1. Biotage 4-channel Quad UV Flash Collector equipped with a Quad 1 Pump Module and the Quad 12/25 Cartridge module. 2. Biotage 12-channel Quad UV Flash Collector equipped with a Quad 3 Pump Module and a Quad 3 Cartridge module. 3. ISCO combi-flash chromatography instrument. Mass Spectrometry: LC/MS spectra were obtained using a MicroMass Platform LC (Phenomenx C18 column, 5 micron, 50x4.6 mm) equipped with a Gilson 215 Liquid Handler. Purity determined by running two diverse purity methods in an ammonium acetate and a formic acid method on LCMS as described below: Mass Spectrometry was performed on an LCMS consisting of an Agilent 1100 series HPLC with binary pumps, a LEAP autosampler and a ZQ single quad mass spectrometer. Samples were diluted in 90:10 MeOH:DMSO and 10 uL injected onto a Waters Symmetry C18 column (3.5 um, 4.6mm x 100 mm). Compounds were identified by MS and quantified by diode array detection in an ammonium acetate and a formic acid method

3-(2-Methoxy-phenoxy)-benzaldehyde (2b). 3-Formyl phenyl boronic acid (10g, 66.7 mmol) and 2-methoxy-phenol (5.78g, 46.7 mmol) were mixed with copper acetate (8.47g, 46.7 mmol), 4Å molecular sieves and triethylamine (32.2 mL, 233.5 mmol) in dichloroethane (0.1 M solution). The resulting mixture was stirred vigorously for 18 h at ambient atmosphere and room temperature. The reaction mixture was filtered and concentrated. Column chromatography of the residue using 95% hexane/ 5% ethyl acetate provided the title compound (5.91g, 55%). ^{1}H-NMR ($CDCl_3$) δ: 3.78 (s, 3H), 6.95 (m, 1H), 7.0-7.05 (m , 2H), 7.14-7.24 (m , 2H), 7.32 (br s, 1H), 7.43 (m, 1H), 7.50 (m, 1H), 9.91 (s, 1H).

3-(2-Chloro-phenoxy)-benzaldehyde (2c). The title compound was prepared using the same procedure as **2b** with 2-chloro-phenol in place of 2-methoxy-phenol (65g, 68 %). ^{1}H-NMR ($CDCl_3$) δ: 7.06 (dt, 2H, J=1.22, 7.94 Hz), 7.21 (m, 3H), 7.60 (m, 2H), 7.81 (d, 1H, J = 7.94 Hz), 9.94 (s, 1H).

1-(3-Phenoxy-benzyl)-piperidine-4-carboxylic acid ethyl ester (1). 3-Phenoxybenzaldehyde (1.0g, 5.0 mmol), **2a**, was mixed with the piperidine-4-carboxylic acid ethyl ester (1.59g, 10.1 mmol) and sodium triacetoxyborohydride (3.21g, 15.1 mmol) in dichloroethane (50 mL) containing acetic acid (1%) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Column chromatography with 2% methanol/ 98% dichloromethane provides the corresponding biaryl ether piperdine **1** (1.2g, 70%). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (dt, 3H, J = 6.7, 7.3 Hz), 2.15-2.37 (m, 4H), 2.59 (m, 1H,), 2.85 (m, 2H), 3.40 (d, 1H, J = 11.5 Hz), 3.61 (d, 1H, J = 11.5 Hz), 4.14 (m, 4H), 6.97 (d, 2H, J = 8.50 Hz), 6.99 (m, 2H), 7.14-7.20 (m, 2H), 7.32 -7.36 (m, 3H). UV Retention time = 3.18, MS m/z: 340 (M+1), purity >95%.

1-(3-Phenoxy-benzyl)-piperidine-4-carboxylic acid ethylamide (4) Boc-isonipecotic acid (2.28g, 9.94 mmol) was mixed with ethyl amine (7.5 mL, 14.9 mmol), HOBt (1.61g, 11.93 mmol) and EDCI (2.28g, 11.93 mmol) in dichloromethane. The reaction was allowed to stir at room temperature for 5h. The mixture was diluted with water and washed 3 x water, 1x 1N HCl, 1x brine and dried over sodium sulfate, filtered and concentrated to a residue (2.28g, 84%). The residue was dissolved in 20 mL of

methylene chloride/ trifluoroacetic acid (1:1) and stirred for 2h at room temperature. The reaction mixture was quenched with sodium bicarbonate and extracted with butanol to give piperidine-4-carboxylic acid ethylamide (0.98g, 68%). Piperidine-4-carboxylic acid ethylamide (0.96g, 6.15 mmol) and 1-bromomethyl-3-phenoxy-benzene (1.94g, 7.4 mmol) were dissolved in acetonitrile, diisopropylethylamine (1.6 mL, 9.22 mmol) was added and stirred at room temperature for 16h. The reaction mixture was concentrated down and partitioned between dichloromethane and water. The organics were washed 2x water, 1x brine and dried over sodium sulfate, filtered and concentrated. Column chromatography with 5% methanol/ 94% dichloromethane/ 1% ammonium hydroxide provided the title compound (0.235g, 11%). ^{1}H-NMR ($CDCl_3$) δ: 1.12 (t, 3H, J = 7.5 Hz), 1.76 (m, 5H), 2.01 (m, 3H), 2.90 (d, 2H, J = 12.0 Hz), 3.28 (q, 2H, J = 7.2 Hz), 3.46 (s, 2H), 6.88 (d, 1H, J = 5.2 Hz), 7.06 (m, 4H), 7.30 (m, 3H). UV Retention time = 1.91 min, MS m/z: 339 (M+1), purity >95%.

1-(3-Phenoxy-benzyl)-piperidin-4-ylamine (5a). 3-phenoxy benzaldehyde **2a** (3.28g, 16.6 mmol) was mixed with 4-*N*-Boc-amino-piperidine (4 g, 19.9 mmol) and sodium triacetoxyborohydride (4.2 g, 19.9 mmol) in dichloroethane (60 mL) containing acetic acid (1%) and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Column chromatography with 25 % ethyl acetate/hexane provided 4-*N*-Boc-amino-1-(3-phenoxy-benzyl) piperidine (5g, 81%). Treatment of 4-*N*-Boc-amino-1-(3-phenoxy-benzyl) piperidine with 4M HCl/ Dioxane solution provided 1-(3-Phenoxy-benzyl)-piperidin-4-ylamine (**5a**) as the dihydrochloride salt in quantitative yield. UV Ret time = 0.97 min, MS m/z: 283 (M+1)

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1-[3-(2-Methoxy-phenoxy)-benzyl]-piperidin-4-ylamine (5b). 3-(2-Methoxy-phenoxy)-benzaldehyde, **2b**, (1.69g, 7.5 mmol) was mixed with 4-*N*-Boc-amino-piperidine (1.66 g, 8.3 mmol) and sodium triacetoxyborohydride (4.79g, 22.6 mmol) in dichloroethane (100 mL) containing acetic acid (1%) and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Column chromatography with 25 % ethyl acetate/hexane provided 1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-yl}-carbamic acid tert-butyl ester. Treatment of 1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-yl}-carbamic acid tert-butyl ester with 4M HCl/ Dioxane solution provided 1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-ylamine (**5b**) as the dihydrochloride salt (2.6 g, 91%). UV Retention time = 1.04 min, MS m/z: 313 (M+1)

1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylamine (5c). 3-(2-Chloro-phenoxy)-benzaldehyde , **2c**, (2.59g, 11.3mmol) was mixed with 4N-Boc-amino-piperidine (2.5 g, 12.5 mmol) and sodium triacetoxyborohydride (7.21 g, 34.0 mmol) in dichloroethane (113 mL) containing acetic acid (1%) and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Column chromatography with 25 % ethyl acetate/hexane provided 1-[3-(2-chloro-phenoxy)-benzyl]-piperidin-4-yl}-carbamic acid tert-butyl ester. Treatment of 1-[3-(2-chloro-phenoxy)-benzyl]-piperidin-4-yl}-carbamic acid tert-butyl ester with 4M HCl/ Dioxane solution provided 1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylamine (**5c**) as the dihydrochloride salt (4 g, 91%). UV Retention time = 1.02 min, MS m/z: 317 (M+1).

N-[1-(3-Phenoxy-benzyl)-piperidin-4-yl]-propionamide (6a). The dihydrochloride salt, **5a** (0.32 g, 1.13 mmol), was treated with propionyl chloride (0.088g, 0.94 mmol) in the presence of DIEA (0.49 mL, 2.8 mmol) in CH_2Cl_2 for 18h at room temperature. The solvent was evaporated and the residue was taken up in CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Column chromatography with 3% methanol/CH_2Cl_2 provided the title compound (0.3 g, 79%). ^{1}H-NMR ($CDCl_3$) δ: 1.11 (3H, m), 1.42 (2H, m), 1.88 (2H, m), 2.09 (4H, m),

2.75 (2H, m), 3.43 (2H, s), 3.76 (1H, m), 5.26 (1H, m), 6.83 (1H, m), 7.06 (5H, m), 7.28 (3H, m). UV Retention time = 2.41 min, MS m/z: 339 (M+1), purity >95%

N-[1-(3-Phenoxy-benzyl)-piperidin-4-yl]-2-phenyl-acetamide (6b). The title compound was prepared using the same procedure as for **6a** with phenyl acetyl chloride in place of propionyl chloride and purification with 2% methanol/ 98% methylene chloride (0.25 g, 54%). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (q, 2H, J = 12.9 Hz), 1.79 (m, 2H), 2.05 (t, 2H, J = 9.9 Hz), 2.66 (m, 2H), 3.39 (s, 2H), 3.52 (s, 2H), 3.77 (m, 1H), 3.83 (s, 3H), 5.18 (d,1H, J = 7.6 Hz), 6.83 (dd, 1H, J = 1.7, 8.2 Hz), 6.99-7.04 (m, 4H), 7.09-7.43 (m, 8H). UV Retention time = 2.68 min, MS m/z: 401 (M+1), Purity >95%

N-{1-[3-(2-Methoxy-phenoxy)-benzyl]-piperidin-4-yl}-2-phenyl-acetamide (6c) The title compound was prepared using the same procedure as for **6a** with **5b** replacing **5a** in the first step and phenyl acetyl chloride in place of propionyl chloride in the last step and purification with 5% methanol/ 95% methylene chloride (75 mg, 16%). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (2H, m), 1.79 (2H, m), 2.07 (2H, m), 2.68 (2H, m), 3.40 (2H, s), 3.36 (2H, s), 3.77 (1H, m), 3.84 (3H, s), 5.25 (1H, m), 6.78 (1H, m), 6.91 (5H, m), 7.13 (1H, m), 7.30 (6H, m). UV Retention time = 1.65 min, MS m/z: 431 (M+1), purity >95%

1-(4-Chloro-phenyl)-cyclohexanecarboxylic acid {1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-yl}-amide (6d). 1-(4-Chloro-phenyl)-cyclohexanecarboxylic acid (0.155 g, 0.65 mmol) was mixed with **5b** (0.300g, 0.76 mmol), HOBt (0.263g, 1.95 mmol), EDCI (0.560 g, 2.92 mmol and N-methylmorpholine (1.06 mL, 9.75 mmol) in dichloromethane. The reaction was stirred at room temperature for 18h. The mixture was diluted with water and washed water, 1x 2N NaOH, 1x brine and dried over sodium sulfate. Chromatography with 50% ethyl acetate/ 50% hexane provided the title compound (155 mg, 63%). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (2H, m), 1.35 (1H, m), 1.53 (4H, m), 1.75 (2H, m), 1.87 (2H, m), 2.02 (2H, m), 2.22 (2H, m), 2.61 (2H, m), 3.38 (2H, s), 3.72 (1H, m), 3.79 (3H, s), 5.12 (2H, m), 6.77 (1H, m), 6.91 (5H, m), 7.15 (2H, m), 7.29 (4H, m). UV Retention time = 2.13 min, MS m/z: 534 (M+1), purity >95%.

4-Phenyl-piperidine-4-carboxylic acid {1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-yl}-amide (9b) Commercially available 4-phenyl-piperidine-1,4-dicarboxylic acid mono-tert-butyl ester (1.94g, 6.35 mmol) was mixed with **5b** (2.69g, 7 mmol), HOBt (0.858g, 6.35 mmol) and EDCI (1.82g, 9.52 mmol) in dichloromethane. The reaction was stirred at room temperature for 5h. The mixture was diluted with water and washed 3x water, 1x 1N HCl, 1x brine and dried over sodium sulfate. Chromatography with 40% ethyl acetate/ 60% hexane provided *tert*-Butyl-4-((1-(3-(2-methoxyphenoxy)benzyl)piperidin-4-yl)carbamoyl)-4-phenylpiperidine-1-carboxylate (0.314g, 85%). *tert*-Butyl-4-((1-(3-(2-methoxyphenoxy)benzyl)piperidin-4-yl)carbamoyl)-4-phenylpiperidine-1-carboxylate was treated with 4M HCl/Dioxane for 2h at room temperature. The solvent was evaporated and the residue was triturated with ether and filtered to give the dihydrochloride salt **9b** (0.245 g, 82%). ^{1}H-NMR (CD_3OD) δ: 1.73 (2H, m), 1.95 (2H, m), 2.17 (2H, m), 2.68 (2H, m), 3.11 (2H, m), 3.17 (2H, m), 3.41 (2H, m), 3.62 (2H, s), 3.70 (3H, s), 3.92 (1H, m), 4.20 (2H, m), 6.88 (1H, m), 7.01 (5H, m), 7.15 (3H, m), 7.37 (5H, m). UV Retention time = 1.36 min, MS m/z: 500 (M+1) and M-15.

4-Phenyl-piperidine-4-carboxylic acid {1-[3-(2-chloro-phenoxy)-benzyl]-piperidin-4-yl}-amide (9c). 4-{1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylcarbamoyl}-4-phenyl-piperidine-1-carboxylic acid tert-butyl ester was prepared using the same procedure as for **9b** with **5c** replacing **5b**. 4-{1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylcarbamoyl}-4-phenyl-piperidine-1-carboxylic acid tert-butyl ester was treated with 4M HCl/Dioxane for 2h at room temperature. The solvent was evaporated and the residue was triturated with ether and filtered to give the dihydrochloride salt **9c** (0.250mg, 82%). UV Retention time =1.42 min, MS m/z: M+1 = 505.

1-Ethyl-4-phenyl-piperidine-4-carboxylic acid {1-[3-(2-methoxy-phenoxy)-benzyl]-piperidin-4-yl}-amide (11b). Compound **9b** (0.300g, 0.53 mmol) was mixed with triethylamine (0.25 mL, 0.18 mmol) and ethyl bromide (0.089 mL, 0.6 mmol) in CH_2Cl_2 and the resulting solution was stirred at room temperature for 18h. Standard work-up (as above) and column chromatography with 50% ethyl acetate/ 50% hexane provided the corresponding *N*-ethyl analog **11b** (0.237g, 85%) ^{1}H-NMR ($CDCl_3$) δ: 1.25 (6H, m), 1.73 (2H, m), 2.04 (2H, m), 2.45 (4H, m), 2.64 (2H, m), 2.73 (2H, m), 2.86 (2H, m), 3.06 (2H, m), 3.38 (2H, s), 3.70 (1H, m), 3.81 (3H, s), 6.72 (1H, m), 6.91 (6H, m), 7.08 (1H, m), 7.16 (1H, m), 7.38 (4H, m). UV Retention time = 1.28 min, MS m/z: M+1 = 528, purity >95%.

1-Ethyl-4-phenyl-piperidine-4-carboxylic acid {1-[3-(2-chloro-phenoxy)-benzyl]-piperidin-4-yl}-amide (11c). The title compound was prepared using the same procedure as for **11b**. Column chromatography with 50% ethyl acetate/50% hexane provided the corresponding *N*-ethyl analog **11c** (0.168g, 73%). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (t, 3H, J = 5.87), 1.22 (m, 2H), 1.84 (m, 2H), 2.06 (m, 2H), 2.19 (m, 2H), 2.49-2.90 (m, 10H), 3.36 (s, 2H), 3.70 (m, 1H), 5.06 (m, 1H), 6.77 (dd, 1H, J=1.76, 8.22 Hz), 6.95-7.10 (m, 5H), 7.15-7.39 (m, 6H), 7.41 (dd, 1H, J=1.2, 8.8 Hz). UV Retention time = 1.33 min, MS m/z: 532 (M+1), purity >95%.

2-(4-{1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylcarbamoyl}-4-phenyl-piperidin-1-yl)-2-methyl-propionic acid (12 c). Compound **9c** (2.16g, 3.7 mmol) was mixed with potassium carbonate (1.62 g, 11.5 mmol) and ethyl-2-bromoisobutyrate (0.548 mL, 3.7 mmol) in DMF and the resulting solution was heated to 50 ^{o}C for 18h. The reaction mixture was allowed to cool to room temperature and diluted with ethyl acetate and water. The organics were washed 2x with water, 1 x brine and dried over magnesium sulfate. The solution was filtered, concentrated to a residue. Column chromatography with 50% ethyl acetate/ 50 % hexane to 100% ethyl acetate provided the corresponding *N*-isobutyric ester (0.900mg, 39%). The ester (0.600g, 0.96 mmol) was dissolved in ethanol (5 mL) and hydrolyzed in a solution of 6N HCl (aqueous, 10 mL). The reaction mixture was heated to reflux for 20 h, and then concentrated down to a residue. The acid was purified by HPLC to yield the title compound as a formate salt (0.267 mg, 57%). ^{1}H NMR ($CDCl_3$) d 1,43 (s, 6H), 1.67-1.81 (m, 3H), 2.41 (t, 2H, J = 10.9 Hz), 2.50 (bs, 1H), 2.71 (s, 1H), 2.74 (d, 2H, J = 13.8 Hz), 3.12 (m, 4H), 3.46 (s, 3H), 3.81 (s, 2H), 6.88-7.14 (m, 4H), 7.21-7.34 (m, 8H), 7.44 (d, 1H, J = 7.7 Hz), 8.2 (s, 1H). UV Retention time = 1.43 min, purity >95%; HRMS calcd for $C_{34}H_{40}ClN_3O_4$ $(M + H)^+$ 590.2786, found 590.2791.

(+/-) 3-Phenyl-pyrrolidine-3-carboxylic acid {1-[3-(2-chloro-phenoxy)-benzyl]-piperidin-4-yl}-amide (10c). 3-{1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylcarbamoyl}-3-phenyl-pyrrolidine-1-carboxylic acid tert-butyl ester (1.1g, 1.86 mmol) was prepared using 3-phenyl-pyrrolidine-1,3-dicarboxylic acid 1-tert-butyl ester (Padwa, A.; Chen, Y.; Dent, W.; Nimmesgem, H. *J. Org. Chem.* **1985**, *50*, 4006 and Hagen, S.E.; Domagala, J. M.; Heifetz, C. L.; Sanchez, J. P.; Solomon, M. *J. Med. Chem*. ***1990***, 33, *849.)* as the amine source. 3-{1-[3-(2-Chloro-phenoxy)-benzyl]-piperidin-4-ylcarbamoyl}-3-phenyl-pyrrolidine-1-carboxylic acid tert-butyl ester was treated with 4M HCl/Dioxane for 2h at room temperature. The solvent was evaporated and the residue was triturated with ether and filtered to give the dihydrochloride salt **10c** (0.9g, 86%). ^{1}H-NMR (CD_3OD) δ: 1.78 (4H, m), 2.65 (1H, m), 2.78 (1H, m), 3.03 (2H, m), 3.44 (3H, m), 3.65 (2H, s), 3.92 (1H, m), 4.27 (3H, m), 6.99 (1H, m), 7.12 (2H, m), 7.23 (2H, m), 7.38 (8H, m), 7.64 (1H, m). UV Retention time =1.19 min, MS m/z: 490 (M+1), purity >95%.

(+/-) 2-Methyl-2-{3-[1-(3-phenoxy-benzyl)-piperidin-4-ylcarbamoyl]-3-phenyl-pyrrolidin-1-yl}-propionic acid (13c). Compound **10c** (0.23g, 0.41 mmol) was mixed with potassium carbonate (0.331g, 2.4 mmol) and ethyl-2-bromoisobutyrate (0.095g, 0.49 mmol) in DMF and the resulting solution was heated to 50 ^{o}C for 18h. The reaction mixture was allowed to cool to room temperature and diluted with ethyl acetate and water. The organics were washed 2 x with water, 1 x brine and dried over magnesium sulfate. The solution was filtered, concentrated to a residue. Column chromatography

with 50% ethyl acetate/50 % hexane to 100% ethyl acetate provided the corresponding *N*-isobutyric ester. The ester was dissolved in ethanol and hydrolyzed in a solution of 6N HCl (aqueous). The reaction mixture was heated to reflux for 20 h, and then concentrated down to a residue. The acid was purified by HPLC to yield the title compound as a formate salt (0.035g, 15%). ^{1}H-NMR (CD_3OD) δ: 1.45 (6H,s), 1.59 (2H, m), 1.86 (2H,m), 2.67 (3H, m), 2.87 (1H, m), 3.17 (2H, m), 3.38 (1H, m), 3.56 (2H, m), 3.80 (1H, m), 3.96 (2H, s), 4.41 (1H, m), 6.92 (1H, m), 6.99-7.22 (4H, m), 7.34 (7H, m), 7.50 (1H, m), 8.34 (1H, s). UV Retention time = 1.58 min, MS m/z: 576 (M+1), purity >95%. HRMS calcd for $C_{33}H_{38}ClN_3O_4$ $(M + H)^+$ 576.2629, found 576.2656.

N-Fmoc-amino-piperidinyl-1, 1 carboxylic acid methyl ester hydrochloride (15). Commercially available N-Boc-4-(Fmoc-amino)-piperidine-4-carboxylic acid (10.71g, 22.9 mmol) was treated with (trimethylsilyl)diazomethane (13.0 mL) in toluene: methanol (9:1) solvent mixture and stirred for 2 h at room temperature. Solvent was evaporated and crude product was purified on silica gel by column chromatography using 95% dichloromethane/ 5% methanol to yield the desired corresponding methyl ester (11 g, 90%). UV Retention time = 3.30 min, MS m/z: 481.3 (M+1).
Removal of the Boc protecting group (11.01g, 22.9 mmol) with 4.0 M HCl in dioxane (20 mL) provides **15** in a 91% yield**.** UV Retention time = 1.61 min MS m/z: 381.24 (M+1) as the hydrochloride salt.

4-Amino-1-[3-(2-methoxy-phenoxy)-benzyl]-piperidine-4-carboxylic acid methyl ester (16). 3-(2-Methoxy-phenoxy)-benzaldehyde **2b** (2.56g, 11.22 mol) was added to a solution of 4-(9H-fluoren-9-ylmethoxycarbonylamino)-piperidine-4-carboxylic acid methyl ester (4.67g, 11.22 mol) and sodium triacetoxyborohydride (7.14g, 33.66 mol) in dichloroethane (100 mL) containing acetic acid (1%) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Product was purified on silica gel using 100% ethyl acetate by column chromatography to yield 1-[3-(2-methoxy-phenoxy)-benzyl]-4-(9H-fluoren-9-ylmethoxycarbonylamino)-piperidine-4-carboxylic acid methyl ester (4.00g, 60%) UV Retention Time = 2.11 min, MS m/z: 593.44 (M+1) . 1-[3-(2-methoxy-phenoxy)-benzyl]-4-(9H-fluoren-9-ylmethoxycarbonylamino)-piperidine-4-carboxylic acid methyl ester (4.00g, 6.75 mmol) was followed by removal of the Fmoc protecting group with diethyl amine (20%) (1.0 mL) in DMF (25 mL) gave the corresponding 4-amino-1-[3-(2-methoxy-phenoxy)-benzyl]-piperidine-4-carboxylic acid methyl ester**,** which was purified with 2% methanol/ 98% dichloromethane to 10% methanol/ 90% methanol to yield **16** (1.15g, 46%). UV Retention Time = 1.17 min, MS m/z: 371.35 (M+1).

1-[3-(2-methoxy-phenoxy)-benzyl]-4-[(4-phenyl-piperidine-4-carbonyl)-amino]-piperidine-4-carboxylic acid (17b). 4-Amino-1-[3-(2-methoxy-phenoxy)-benzyl]-piperidine-4-carboxylic acid methyl ester **16** (0.750g, 2.02 mmol) was treated with the acid chloride (prepared from 4-phenyl-piperidine-1, 4-dicarboxylic acid mono-*tert*-butyl ester) (0.618g, 2.02 mmol) in the presence of TEA (0.350 mL) in CH_2Cl_2 (10 mL) for 18h at room temperature. The solvent was evaporated from the reaction mixture and the residue was re-dissolved in ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Crude product was purified on silica by column chromatography using 100% ethyl acetate (0.657g, 49%) UV Retention time = 2.14 min, MS m/z: 658.35 (M+1)**.** 1-[3-(2-Methoxy-phenoxy)-benzyl]-4-[(4-phenyl-*N*-Boc-piperidine-4-carbonyl)-amino]-piperidine-4-carboxylic acid methyl ester (0.657g, 0.99mol) was hydrolyzed by treatment with 1.0 M NaOH (aqueous, 5.0 mL) in methanol (5.0 mL) and refluxed for 2 hrs. The reaction mixture was concentrated to a minimal volume and the aqueous layer was acidified to pH 5.0 using 1.0 N HCl. The desired acid was filtered off and purified by RP HPLC using acetonitrile:methanol (0.1% formic acid) to give 1-[3-(2-methoxy-phenoxy)-benzyl]-4-[(4-phenyl-N-Boc-piperidine-4-carbonyl)-amino]-piperidine-4-carboxylic acid, (0.400g, 62%) UV Retention Time = 2.13 min, MS m/z: 644.48 (M+1)**.**

1-[3-(2-Methoxy-phenoxy)-benzyl]-4-[(4-phenyl-*N*-Boc-piperidine-4-carbonyl)-amino]-piperidine-4-carboxylic acid (0.400g, 0.621mmol) was treated with 4.0 N HCl in dioxane (5.0 mL) to yield **17b** as dihydrochloride salt (0.374g, 97%) ^{1}H NMR (CD_3OD) δ: 2.0-2.40 (m, 6H), 2.8 (m, 2H), 3.1(m, 2H), 3.25 (m, 2H), 3.40(m, 4H), 4.10 (s, 2H), 4.00(s, 3H), 7.00-7.60 (m, 13H), 8.40 (s, 1H). UV Retention Time = 1.39 min, MS m/z: 544 (M+1), purity >95%. HRMS calcd for $C_{32}H_{37}N_3O_5$ $(M + H)^+$ 544.2811, found 544.2824**.**

1-[3-(2-chlorophenoxy)benzyl]-4-{[(1-ethyl-4-phenylpiperidin-4-yl)carbonyl]amino} piperidine-4-carboxylic acid (17c). 1-[3-(2-chlorophenoxy)benzyl]-4-{[(1-ethyl-4-phenylpiperidin-4-yl)carbonyl] amino}piperidine-4-carboxylic acid was synthesized in a similar fashion to 1-[3-(2-methoxy-phenoxy)-benzyl]-4-[(4-phenyl-piperidine-4-carbonyl)-amino]-piperidine-4-carboxylic acid**,** except 1-ethyl-4-phenylpiperidine-4-carbonyl chloride was substituted for *tert*-butyl 4-(chlorocarbonyl)-4-phenylpiperidine-1-carboxylate. UV Retention Time = 1.49 min, MS m/z: 576 (M+1). ^{1}H-NMR (CD_3OD) δ: 1.29 (t, 3H), 1.96-2.19 (m, 9H), 2.80-2.88 (m, 4H), 3.11 (q, 2H), 3.31-3.40 (m, 3H), 3.50 (s, 2H), 6.86-6.91 (m, 2H), 6.99-7.04 (m, 2H), 7.16 (t, 1H), 7.27-7.32 (m, 3H), 7.38-7.42 (m, 4H), 7.49 (d, 1H). HRMS calcd for $C_{33}H_{38}ClN_3O_4$ exact Mass for $(M+H)^+$ 576.2629, found 576.2630**.**