Identification and SAR of glycine benzamides as potent agonists for the GPR139 receptor

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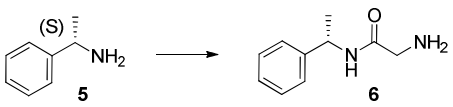
SUPPORTING INFORMATION

Experimental Section.

General Procedures. Reagents were purchased from commercial suppliers and were used without purification. Anhydrous solvents were obtained from a GlassContour Solvent Dispensing System. Reactions were performed at room temperature (20-23 °C) under an atmosphere of N_2 unless otherwise noted. Chromatography was performed using prepacked ISCO RediSep^{TM} silica cartridges utilizing gradient elution. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 or 500 MHz spectrometers. Chemical shifts are reported in parts per million downfield from an internal standard (Me_4Si). All spectra were taken in $CDCl_3$ unless otherwise noted. Mass spectra were obtained on an Agilent series 1100 MSD using electrospray ionization (ESI) in either positive or negative modes as indicated. Thin-layer chromatography was performed using Merck silica gel 60 F254 2.5 cm x 7.5 cm 250 µm or 5.0 cm x 10.0 cm 250 µm pre-coated silica gel plates. Preparative thin-layer chromatography was performed using EM Science silica gel 60 F254 20 cm x 20 cm 0.5 mm pre-coated plates with a 20 cm x 4 cm concentrating zone. Melting points are uncorrected and were obtained on a MelTemp apparatus. Analytical reverse phase HPLC was performed on a Hewlett Packard Series 1100 instrument with an Agilent ZORBAX® Bonus RP, column utilizing an acetonitrile/water (0.05%TFA) gradient. Rotations were obtained on a Perkin Elmer model 341 polarimeter.

Experimental Details:

General procedure A:

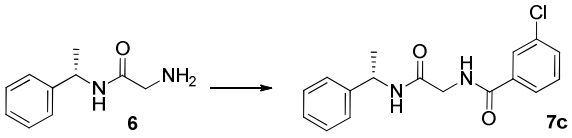


(S)-2-amino-N-(1-phenylethyl)acetamide (**6**)

To a solution of 2-((tert-butoxycarbonyl)amino)acetic acid (11.9 g, 67.6 mmol) and (S)-1-phenylethanamine (6.83 g, 56.4 mmol) in DCM (250 mL) was added EDCI (19.5 g, 101.5 mmol), HOBt (13.7 g, 101.5 mmol) and DIEA (24 mL, 141 mmol). The mixture was stirred at room temperature overnight. The mixture was diluted with DCM (100 mL), washed with water (2 X 100 mL) and brine (100 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0-100% EtOAc/ Hex) to afford (S)-tert-butyl (2-oxo-2-((1-phenylethyl)amino)ethyl)carbamate (14.1 g, 90% yield). LC/MS (ES+) *m/z* found 302 $[M+1+Na]^+$; 223 $[M+1-tBu]^+$.

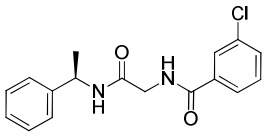
To a solution of (S)-tert-butyl (2-oxo-2-((1-phenylethyl)amino)ethyl)carbamate (14.1 g, 60.8 mmol) in DCM (200 mL) was charged with 4 N HCl in Dioxane (20 mL). The resulting solution was allowed to stir at room temperature overnight. The reaction was then concentrated to afford (S)-2-amino-N-(1-phenylethyl)acetamide Hydrochloride (**6**) as a white solid (8.5 g, 94% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.41 – 7.28 (m, 4H), 7.28 – 7.18 (m, 1H), 5.04 (q, *J* = 7.0 Hz, 1H), 3.80 – 3.59 (m, 2H), 1.47 (d, *J* = 7.0 Hz, 3H). LC/MS (ES+) *m/z* found 179 $[M+1]^+$.; $[\alpha]_D^{20}$ -87° (c = 1; ethanol); MP = 171-173 °C.

General procedure B:



(S)-3-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (7c)

To (S)-2-amino-N-(1-phenylethyl)acetamide Hydrochloride (5.2 g, 23.1 mmol) in DCM (200 mL) was added TEA (4.8 mL, 34.7 mmol). To the resulting was added 3-Chlorybenzoyl chloride (4.5 g, 25.4 mmol) slowly. The mixture was stirred at room temperature overnight. The reaction was then diluted with DCM (100 mL), washed with water (2 X 100 mL) and brine (100 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by crystallization from hot EtOAc to afford (S)-3-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (7c) as a white solid (6.04 g, 83% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (t, *J* = 1.9 Hz, 1H), 7.69 – 7.64 (m, 1H), 7.52 - 7.46 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.34 – 7.29 (m, 5H), 7.12 (s, 1H), 6.53 (d, *J* = 7.6 Hz, 1H), 5.13 (t, *J* = 7.2 Hz, 1H), 4.20 - 4.03 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.91, 166.51, 142.98, 135.19, 134.80, 131.86, 129.86, 128.68, 127.63, 127.41, 126.06, 125.19, 49.34, 43.93, 22.12. LC/MS (ES+) *m/z* found 317.0 and 318.9 $[M+1]^+$; $[\alpha]_D^{20}$ -91° (c = 1.04; ethanol); MP = 158-160 °C. SFC: 99%; rt = 9.6min (stationary phase (S,S) Whelk O1 5um 4.6 x 250mm, mobile phase 30% EtOH, 70% CO2, 2mL/min, 150Bar).



LC/MS (ES+) *m/z* found 316.9 and 318.9 $[M+1]^+$; $[\alpha]_D^{20}$ +91° (c = 1; ethanol); MP = 158-160 °C) SFC:99%, rt = 4.2min (stationary phase (S,S) Whelk O1 5um 4.6 x 250mm, mobile phase 30% EtOH, 70% CO2, 2mL/min, 150Bar)

The following were prepared by general procedure B.

(S)-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7a**)

^{1}H NMR (400 MHz, MeOD) δ 7.91 - 7.85 (m, 2H), 7.59 - 7.53 (m, 1H), 7.51 - 7.45 (m, 2H), 7.38 - 7.29 (m, 4H), 7.27 - 7.20 (m, 1H), 5.07 (q, J = 7.0 Hz, 1H), 4.14 - 4.01 (m, 2H), 1.49 (d, J = 7.1 Hz, 3H). LC/MS (ES+) *m/z* found 282.9 $[M+1]^+$.

(S)-2-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7b**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.5 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.34 – 7.28 (m, 4H), 7.28 – 7.22 (m, 2H), 7.06 (s, 1H), 6.81 – 6.75 (m, 1H), 5.12 (t, *J* = 6.7 Hz, 1H), 4.17 (d, *J* = 5.1 Hz, 2H), 1.50 (d, *J* = 7.0, 1.4 Hz, 3H). LC/MS (ES+) *m/z* found 316.9 and 319.0 $[M+1]^+$.

(S)-4-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7d**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.36 – 7.28 (m, 5H), 7.05 (s, 1H), 6.55 – 6.40 (m, 1H), 5.13 (t, *J* = 7.2 Hz, 1H), 4.12 (t, *J* = 5.0 Hz, 2H), 1.56 (s, 3H). LC/MS (ES+) *m/z* found 317.1 and 318.1 $[M+1]^+$.

(S)-3-methyl-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7e**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.61 – 7.56 (m, 1H), 7.35 – 7.28 (m, 6H), 7.22 -- 7.27 (m, 2H), 7.17 (d, *J* = 7.6 Hz, 1H), 5.11 (t, *J* = 7.2 Hz, 1H), 4.26 – 4.09 (m, 2H), 2.37 (s, 3H), 1.51 (d, *J* = 7.0 Hz, 3H). LC/MS (ES+) *m/z* found 297.1 $[M+1]^+$.

(S)-3-cyano-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7f**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (t, *J* = 1.6 Hz, 1H), 8.06 – 7.97 (m, 1H), 7.82 – 7.73 (m, 1H), 7.61 – 7.49 (m, 2H), 7.35 – 7.28 (m, 4H), 7.28 – 7.24 (m, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 5.16 – 5.07 (m, 1H), 4.22 – 4.04 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 308.0 $[M+1]^+$.

(S)-3-fluoro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7g**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 – 7.51 (m, 2H), 7.43 – 7.37 (m, 1H), 7.34 – 7.28 (m, 5H), 7.27 – 7.25 (m, 1H), 7.24 – 7.18 (m, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 5.12 (t, *J* = 7.2 Hz, 1H), 4.14 (qd, *J* = 16.4, 4.9 Hz, 2H), 1.52 (d, *J* = 7.0 Hz, 3H). LC/MS (ES+) *m/z* found 301.0 $[M+1]^+$.

(S)-3-methoxy-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7h**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 - 7.15 (m, 10H), 7.08 - 7.01 (m, 1H), 5.17 - 5.03 (m, 1H), 4.24 - 4.07 (m, 2H), 3.80 (d, J = 2.4 Hz, 3H), 1.50 (d, J = 7.1, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.04, 167.81, 129.78, 128.84, 127.55, 126.18, 119.19, 118.30, 112.44, 55.57, 49.41, 44.08, 22.31. LC/MS (ES+) *m/z* found 313.0 $[M+1]^+$; $[\alpha_D]^{20}$ -66° (c = 1.06; ethanol).

(S)-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)-3-(trifluoromethyl)benzamide (**7i**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.03 (m, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.64 – 7.52 (m, 1H), 7.39 – 7.27 (m, 5H), 7.22 – 7.14 (m, 1H), 6.50 – 6.39 (m, 1H), 5.20 – 5.06 (m, 1H), 4.24 – 4.05 (m, 2H), 1.61 – 1.50 (m, 3H). LC/MS (ES+) *m/z* found 351.1 $[M+1]^+$.

(S)-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)-3-(trifluoromethoxy)benzamide (**7j**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.66 (m, 2H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.39 – 7.35 (m, 1H), 7.35 – 7.29 (m, 4H), 7.28 – 7.23 (m, 2H), 6.69 (d, *J* = 7.6 Hz, 1H), 5.12 (t, *J* = 7.2 Hz, 1H), 4.22 – 4.06 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 367.1 $[M+1]^+$.

(S)-2,3-dichloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7k**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.43 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.38 – 7.29 (m, 4H), 7.29 – 7.22 (m, 2H), 6.84 (s, 1H), 6.47 (d, *J* = 7.8 Hz, 1H), 5.13 (t, *J* = 7.2 Hz, 1H), 4.14 (dd, *J* = 5.3, 1.1 Hz, 2H), 1.52 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 351.1 and 353.0 $[M+1]^+$.

(S)-3,4-dichloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7l**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 2.1 Hz, 1H), 7.66 – 7.57 (m, 1H), 7.56 – 7.50 (m, 1H), 7.50 – 7.45 (m, 1H), 7.34 – 7.27 (m, 4H), 7.27 – 7.23 (m, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 5.11 (t, *J* = 7.2 Hz, 1H), 4.20 – 4.02 (m, 2H), 1.52 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 351.0 and 353.0 $[M+1]^+$.

(S)-2,5-dichloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7m**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 – 7.57 (m, 1H), 7.38 – 7.29 (m, 6H), 7.29 – 7.23 (m, 1H), 7.19 – 7.07 (m, 1H), 6.60 (d, *J* = 7.7 Hz, 1H), 5.12 (t, *J* = 7.2 Hz, 1H), 4.21 – 4.09 (m, 2H), 1.54 – 1.50 (m, 3H). LC/MS (ES+) *m/z* found 351.0 and 353.0 $[M+1]^+$.

(S)-3,5-dichloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7n**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 1.9 Hz, 2H), 7.49 (t, *J* = 1.9 Hz, 1H), 7.37 – 7.26 (m, 6H), 6.55 (d, *J* = 7.8 Hz, 1H), 5.12 (t, *J* = 7.2 Hz, 1H), 4.19 – 4.01 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 351.0 and 353.0 $[M+1]^+$.

(S)-3-chloro-5-fluoro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7o**)

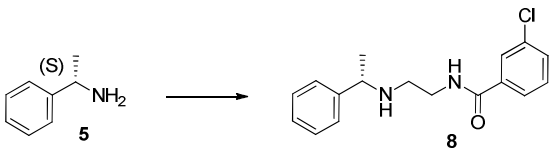
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.58 (m, 1H), 7.45 – 7.40 (m, 1H), 7.36 – 7.27 (m, 5H), 7.28 – 7.20 (m, 2H), 6.57 (d, *J* = 7.7 Hz, 1H), 5.12 (t, *J* = 7.1 Hz, 1H), 4.19 – 4.00 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 335.1 and 337.1 $[M+1]^+$.

(S)-3-bromo-5-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7p**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 1H), 7.77 – 7.71 (m, 1H), 7.67 – 7.62 (m, 1H), 7.40 – 7.20 (m, 6H), 6.51 (d, *J* = 7.7 Hz, 1H), 5.12 (t, *J* = 7.2 Hz, 1H), 4.19 – 3.98 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 395.0 and 397.0 $[M+1]^+$.

(S)-3,5-dimethoxy-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (**7q**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (t, J = 4.9 Hz, 1H), 7.33 - 7.18 (m, 6H), 6.94 (d, J = 2.3 Hz, 2H), 6.57 (t, J = 2.3 Hz, 1H), 5.16 - 5.03 (m, 1H), 4.22 - 4.04 (m, 2H), 3.77 (s, 6H), 1.50 (d, J = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 343.0 $[M+1]^+$.

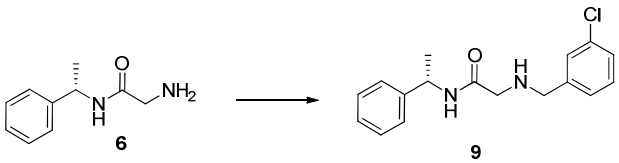


(S)-3-chloro-N-(2-((1-phenylethyl)amino)ethyl)benzamide (**8**)

A solution of tert-butyl (2-oxoethyl)carbamate (0.7 g, 4.4 mmol) and (S)-1-phenylethanamine (0.8 g, 6.6 mmol) in DCM (50 mL) was charged with sodium triacetoxyborohydride (2.8 g, 13.2 mmol) and stirred at room temperature overnight. The mixture diluted with DCM (30 mL), washed with water (2 X 20 mL) and brine (20 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated *in vacuo* giving the crude residue (S)-tert-butyl (2-((1-phenylethyl)amino)ethyl)carbamate that was carried forward without further purification (1.05 g, 90% yield). LC/MS (ES+) *m/z* found 265.1 $[M+1]^+$.

To a solution of (S)-tert-butyl (2-((1-phenylethyl)amino)ethyl)carbamate (1.05 g, 3.8 mmol) in DCM (25 mL) was charged with 4 N HCl in Dioxane (4 mL). The resulting solution was allowed to stir at room temperature overnight. The reaction was then concentrated to afford (S)-N1-(1-phenylethyl)ethane-1,2-diamine Hydrochloride (0.48 g, 77% yield). LC/MS (ES+) *m/z* found 165.1 $[M+1]^+$.

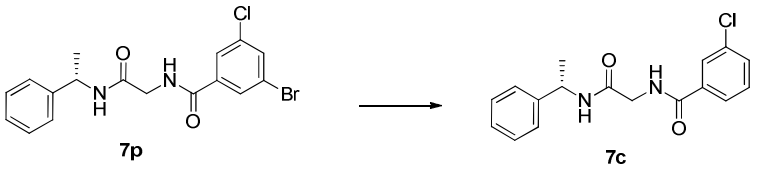
To (S)-N1-(1-phenylethyl)ethane-1,2-diamine hydrochloride (75 mg, 0.3 mmol) in DCM (3 mL) was added TEA (70 μL, 0.5 mmol). This solution was then treated with 3-Chlorybenzoyl chloride (46 mg, 0.3 mmol). The mixture was stirred at room temperature overnight. The mixture was diluted with DCM (10 mL), washed with water (2 X 10 mL) and brine (10 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0-5% MeOH/DCM) to afford (S)-3-chloro-N-(2-((1-phenylethyl)amino)ethyl)benzamide (**8**) (35 mg, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (t, *J* = 1.9 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.51 – 7.45 (m, 1H), 7.42 – 7.29 (m, 5H), 7.27 – 7.22 (m, 2H), 6.70 (s, 1H), 3.79 (q, *J* = 6.6 Hz, 1H), 3.51 – 3.37 (m, 2H), 2.90 – 2.78 (m, 1H), 2.71 – 2.58 (m, 1H), 1.39 (d, *J* = 6.6 Hz, 3H). LC/MS (ES+) *m/z* found 303.1 and 305.1 $[M+1]^+$.



(S)-2-((3-chlorobenzyl)amino)-N-(1-phenylethyl)acetamide (**9**)

To a solution of (S)-2-amino-N-(1-phenylethyl)acetamide Hydrochloride (50 mg, 0.23 mmol) and 3-Chlorobenzaldehyde (33 mg, 0.23 mmol) in DCM (4 mL) was charged with Sodium

triacetoxyborohydride (150 mg, 0.7 mmol) and stirred at room temperature overnight. The mixture diluted with DCM (10 mL), washed with water (2 X 10 mL) and brine (10 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography (0-5% MeOH/DCM) to afford (S)-2-((3-chlorobenzyl)amino)-N-(1-phenylethyl)acetamide (**9**) (22 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.19 (m, 10H), 7.17 – 7.06 (m, 1H), 5.25 – 5.06 (m, 1H), 3.73 (s, 2H), 3.30 (d, *J* = 1.5 Hz, 2H), 1.49 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 303.1 and 305.1 $[M+1]^+$.



To a flask was added (S)-3-bromo-5-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide (30 mg, 0.0758 mmol) and Pd/C (10%) (8.0 mg, 0.00758 mmol) suspended in EtOH (5 mL, 0.789 g/mL) and the solution was evacuated and backfilled with N_2. A hydrogen balloon was affixed to the flask and was then evacuated and backfilled with H_2 from the balloon (3X) then allowed to stir under an atmosphere of H_2 at rt for 1 hour. The reaction mixture was filtered through celite, washed with EtOAc and concentrated. Sample was purified on SiO2 eluting with 0-100% EtOAc to afford the desired product (S)-3-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl)benzamide as a white solid 44% yield.

The following were prepared by general procedures A and B.

3-methoxy-N-((S)-1-oxo-1-(((S)-1-phenylethyl)amino)propan-2-yl)benzamide (**10**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 1H), 7.31 – 7.22 (m, 5H), 7.22 – 7.14 (m, 3H), 7.07 (s, 1H), 7.04 – 7.00 (m, 1H), 5.16 – 5.03 (m, 1H), 4.90 – 4.75 (m, 1H), 3.77 (s, 3H), 1.54 (d, *J* = 6.9 Hz, 3H), 1.51 (d, *J* = 7.0 Hz, 3H). LC/MS (ES+) *m/z* found 327.2 $[M+1]^+$

3-methoxy-N-((R)-1-oxo-1-(((S)-1-phenylethyl)amino)propan-2-yl)benzamide (**11**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 8H), 7.29 – 7.22 (m, 1H), 7.19 (d, *J* = 7.4 Hz, 1H), 7.06 – 7.01 (m, 1H), 5.18 – 4.99 (m, 1H), 4.97 – 4.78 (m, 1H), 3.78 (s, 3H), 1.49 (d, *J* = 6.9 Hz, 3H), 1.46 (d, *J* = 7.0 Hz, 3H). LC/MS (ES+) *m/z* found 327.2 $[M+1]^+$.

N-((S)-3-hydroxy-1-oxo-1-(((S)-1-phenylethyl)amino)propan-2-yl)-3-methoxybenzamide (**12**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 1H), 7.37 – 7.34 (m, 1H), 7.34 – 7.32 (m, 6H), 7.29 – 7.21 (m, 1H), 7.08 – 7.03 (m, 1H), 5.13 – 5.01 (m, 1H), 4.79 – 4.67 (m, 1H), 4.18 – 4.06 (m, 1H), 3.98 – 3.90 (m, 1H), 3.81 (s, 3H), 3.77 – 3.65 (m, 1H), 1.46 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 343.2 $[M+1]^+$.

3-methoxy-N-((S)-2-oxo-1-phenyl-2-(((S)-1-phenylethyl)amino)ethyl)benzamide (**13**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 7H), 7.25 – 7.17 (m, 4H), 7.15 – 7.11 (m, 2H), 7.07 – 6.98 (m, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 6.01 (d, *J* = 7.9 Hz, 1H), 4.97 (t, *J* = 7.2 Hz, 1H), 4.88 – 4.78 (m, 1H), 3.81 (s, 3H), 3.39 – 3.26 (m, 1H), 3.14 – 3.01 (m, 1H), 1.27 (d, *J* = 6.9 Hz, 3H). LC/MS (ES+) *m/z* found 403.2 $[M+1]^+$.

In vitro functional assays (calcium mobilization assays):

HEK293 cells stably expressing human GPR139 were grown to confluency in F12K (Cellgro), 10% FBS, 1X penicillin/streptomycin, 1X sodium pyruvate, 20mM HEPES, 600ug/ml G418. Cells were detached with trypsin and resuspended in plating media (F12K, 10% charcoal treated FBS, 1X penicillin/streptomycin, 1X sodium pyruvate, 20mM HEPES, 600ug/ml G418). Cells were seeded at 50,000 cells/well in a volume of 50ul in Poly-D-Lysine coated, black walled, clear bottom tissue culture 96-well plates and incubated overnight at 37°C and 5% CO2. On the day of the assay, a 2X loading dye solution (BD Calcium Assay Kit) was added to the cells and incubated for 45 minutes at 37°C in 5% CO. Dilutions of test compounds were prepared in Hanks Balanced Salt Solution (HBSS) from 10 mM DMSO stocks. Addition of test compounds were done on the Fluorometric Imaging Plate Reader (FLIPR) Tetra instrument (Molecular Devices) and changes in fluorescence were monitored. Raw data from the FLIPR Tetra were exported as the difference between maximum and minimum fluorescence observed for each well. Results were calculated using GraphPad Prism (San Diego, CA) software. A non-linear regression was used to determine the agonist EC50.

Total Binding:

Cell membranes from CHO-TRex cells stably expressing the human GPR139 receptor was incubated with 10 nM of [^{3}H]-**7c** (specific activity 24.7 Ci/mmol) for 60 min at room temperature. Reaction was terminated by filtration through GF/C filterplates pre-treated with PEI followed by washes with cold TE buffer (binding buffer is 50 mM Tris-HCl, pH 7.4, 5 mM EDTA). Filterplates were dried in a 60°C oven followed by addition of scintillation fluid. Bound radioactivity was read on a Topcount scintillation counter. Non-specific binding was determined with 10 uM of **1** (TC-O9311; Tocris). Total and non-specific binding was plotted on Graphpad Prism software (San Diego, CA).

Pharmacokinetic Studies. The pharmacokinetic study with JNJ-63533054 was conducted in non-fasted male Sprague Dawley rats with an indwelling jugular vein catheter. JNJ-63533054 was formulated at 0.5mg/ml in 20% HP-β-Cyclodextrin and was delivered in a volume of 2 ml/kg for the IV dose and 10 ml/kg for the PO dose. Three animals received a bolus oral dose of the compound via a 16-gauge intragastric gavage needle at a dose of 5mg/kg. Three animals received a bolus dose of the compound in the lateral tail vein at a dose of 1 mg/kg. Blood sampling of the jugular vein followed dosing over a time course. Blood samples consisted of 300 μL samples taken from the indwelling jugular vein catheter and expelled into 0.5 mL microtainer tubes containing K2 EDTA.. Animals were given 300 μL of 50 units/mL of heparinized saline to keep the catheters patent and for fluid replacement after sampling. These blood samples were then centrifuged at 14,000 rpm in a micro-centrifuge for 5 minutes. The plasma was retained and kept frozen in a -80 °C freezer until processed for analysis. The time course for sampling was as follows: 0.25, 0.5, 1, 2, 4, 8, and 24 hours for oral administration and 0.03, 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 hours for intravenous administration. A non-compartmental model was used to determine the PK parameters using Phoenix (Pharsight, Sunnyvale, CA).

DMPK. Detailed descriptions of plasma protein binding, brain tissue binding, and solubility assays can be found in Letavic, et al. *J. Med. Chem*. **2015**. DOI:10.1021/acs.jmedchem.5b00742

General Methods. A 10 mM compound stock solution in DMSO further diluted in acetonitrile/water to yield a secondary 1 mM solution was used in all in vitro assays. Pooled human liver microsomes of mixed gender, mouse, rat, beagle dog, and cynomolgus monkey of male gender were purchased from BD Gentest (Woburn, MA). Plasma from various species was purchased from Bioreclamation, Inc. (Westbury, NY). All other commercially available reagents and solvents were either analytical or HPLC grade. In vitro and PK samples were analyzed by LC−MS/MS in the multiple reaction monitoring (MRM) scan mode with electrospray ionization (ESI).

Stability in Liver Microsomes. Stability in liver microsomes from human, mouse, rat, dog, or monkey was assessed over a time course of 1h. The incubation mixture consisted of 0.5 mg/mL microsomal protein, 1 μM compound, 1.8 mM MgCl2, and 2 mM NADPH in 0.1 M potassium phosphate buffer, pH 7.4. After incubation for 5, 10, 20, 40, and 60 min at 37 °C, the reaction was terminated by the addition of 5 volumes of acetonitrile containing internal standard (5,5-diphenylhydantoin). The samples were analyzed by LC−MS/MS, and the percent remaining at various time points was calculated by dividing the observed analyte/internal standard peak ratio by the ratio at the 0 time point. The half-life (t1/2) was calculated as $t1/2 = \ln(2)/(-k)$, where k is the slope of the linear regression from natural log percent remaining versus time. In vitro hepatic clearance (CL) was calculated as $CL = (Q)(CLint)/(Q+CLint)$, where Q is the hepatic blood flow in mL min−1 kg−1 (mouse, 90; rat, 55; dog, 31; monkey, 44, human, 21) and CLint is the intrinsic microsomal clearance determined as $CLint = (-k)(\text{microsomal protein conc})-1(A)(N)$, where A is the microsomal protein yield (45 mg) per 1 g of liver and N is 25.7, 30, 32, 40, and 87.5 g of

liver per kg of body weight for human, monkey, dog, rat, and mouse, respectively. Extraction ratio (ER) was calculated as ER = CL/Q.

Plasma Protein Binding. The stock solution of compound was spiked into blank plasma to yield 0.1, 1, or 10 μM final concentration. Equilibrium dialysis was performed using the RED Device (Thermo Scientific, Rockford, IL) consisting of Teflon base plate, and RED Device inserts comprised two (sample and buffer) side-by-side chambers separated by dialysis membrane (MWCO ≈ 8000). A 300 μL aliquot of plasma spiked with a compound in triplicate was placed into the sample chamber, and 500 μL of dialysis buffer was loaded into the buffer chamber. The dialysis unit was covered with sealing tape and incubated in a 37 °C orbital shaking incubator at 100 rpm for 6 h. After a 6 h dialysis, 10 μL of plasma sample was diluted with 90 μL of PBS buffer, and 90 μL of each buffer sample was mixed with 10 μL of blank plasma in a 96-well plate and extracted with 150 μL of acetonitrile to precipitate proteins. The supernatant was analyzed by LC−MS/MS. The preliminary (tier 1) assay was conducted at a compound concentration of 1 μM using a two-point (0 and 1 μM) calibration curve. The definitive plasma protein binding for compound 34 was conducted in five species at three different concentrations (0.1, 1, or 10 μM) using the full calibration curve and three sets of quality control samples. Calibration curve samples were prepared by spiking appropriate stock solutions into a mixture of blank plasma and PBS buffer (1:9, v/v).

CYP Inhibition. The potential to inhibit various isoforms of human cytochrome P450 (CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4) was investigated by incubating human liver microsomes (0.2 mg/mL) with a "cocktail" of CYP probe substrates (phenacetin for CYP1A2, paclitaxel for CYP2C8, diclofenac for CYP2C9, S-mephenytoin for CYP2C19, dextromethorphan for 2D6, and midazolam for CYP3A4) in the presence of compound at concentrations ranging from 0 (vehicle) to 10 μM. Metabolite formation by probe substrates was quantified by LC−MS/MS. The percent inhibition was measured as the ratio of metabolite peak in the presence and absence of compound × 100. The IC50 values were calculated from the percent inhibition data relative to vehicle.

Solubility in Aqueous Systems. Solubility in 30 mM phosphate buffers (pH 2 and pH 7), simulated gastric (SGF, 0.2% NaCl in 0.1 N HCl, pH 1.2) and intestinal fluids (FasSIF, 0.029 M phosphate buffer, 5mM sodium taurocholate, and 1.5 mM lecithin, pH 6.8) was investigated. Compound was dissolved in DMSO solutions at a concentration of 10 mM and was used for the solubility experiment. DMSO solutions (20 μL) are dispensed in 96-well plates, and the solvent is removed by evaporation using a Caliper TurboVap 96 set at 30 °C and a flow rate of 40 Fh. Buffers (400 μL) of interest are added to the residual solids, and the resulting mixtures are stirred at room temperature for 3 days using magnetic stir bars. The samples are then filtered using an AcroPrep 1 mL 96 filter plate, and the supernatant is analyzed for compound concentration, against external standards.