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Supporting Information

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Design, Synthesis and In Vivo Efficacy of Novel Glycine Transporter-1 (GlyT1) Inhibitors Derived from a Series of [4-Phenyl-1-(propylsulfonyl)piperidin-4-yl] methyl benzamides

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General. All NMR spectra were recorded on either a Varian Inova 400 (400 MHz) or Varian Inova 500 (500 MHz) spectrophotometer. ^{1}H chemical shifts are reported in δ values in ppm downfield from Me_4Si as the internal standard in $CDCl_3$. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constant (Hz). ^{13}C chemical shifts are reported in δ values in ppm with the $CDCl_3$ carbon peak set to 77.23 ppm. Low resolution mass spectra were obtained on an HP1100 MSD with electrospray ionization. High resolution mass spectra were recorded on a Bruker Daltonics 3T Fourier transform ion cyclotron resonance mass spectrometer (FT/ICR) with electrospray ionization. Analytical thin layer chromatography was performed on EM Reagent 0.25 mm silica gel 60-F plates. Analytical HPLC was performed on an HP1100 with UV detection at 214 and 254 nm along with ELSD detection, LC/MS (J-Sphere80-C18, 3.0 x 50 mm, 4.1 min gradient, 5%[0.05%TFA/CH_3CN]:95%[0.05%TFA/H_2O] to 100%[0.05%TFA/CH_3CN]. Preparative purification was performed on a custom HP1100 purification system (reference 16) with collection triggered by mass detection. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from either Avocado Research or Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Argonaut Technologies.

General Procedures for Library Synthesis (Schemes 1 and 2):

Amide Coupling. A Bohdan Mini-Block (24-position rack) was charged with PS-DCC (145 mg, 0.2 mmol, 1.38 mmol/g, 2.0 equivalents), HOBt (13.5 mg, 0.1 mmol, 1.0 equivalent) and 3 mL of dry CH_2Cl_2. Then, one of 24 RCOOH (0.1 mmol) was added to each reaction tube, followed by 1,3-diphenyl-1*H*-pyrazol-5-amine (21 mg, 0.09 mmol, 0.9 equivalents). The Mini-Block was then then sealed and vortexed overnight on the Mini-Block workstation. The next morning, MP-carbonate (77 mg, 0.25 mmol, 3.38 mmol/g, 2.5 equivalents) was added, and vortexed for another 3 hours. After filtration, washing with DCM (3 x 3 mL) and drying, the desired analogs were purified by mass-guided preparative HPLC.

Acylation. A Bohdan Mini-Block (24-position rack) was charged with PS-DIEA (94 mg, 0.3 mmol, 3.2 mmol/g, 3.0 equivalents) and 3 mL of dry CH_2Cl_2. Then, 1,3-diphenyl-1*H*-pyrazol-5-amine (21 mg, 0.09 mmol, 0.9 equivalents) was added to each reaction tube, followed by one of 24 RCOCl (0.12 mmol). The Mini-Block was then then sealed and vortexed overnight on the Mini-Block workstation. The next morning, PS-trisamine (77 mg, 0.25 mmol, 3.38 mmol/g, 2.5

equivalents) was added, and vortexed for another 3 hours. After filtration, washing with DCM (3 x 3 mL) and drying, the desired analogs were purified by mass-guided preparative HPLC.

Standard Experimental Procedures for Key Compounds:

4-Phenyl-1-(propylsulfonyl)piperidine-4-carbonitrile (**6**)

1-Propanesulfonyl chloride (23.6g, 165 mmole) was added to a solution of 4-phenylpiperidine-4-carbonitrile hydrochloride (**5**) (33.3 g, 150 mmol) and DIEA (51.6 g, 400 mmol) in DCM (300 mL) with stirring at 0 °C. The resultant reaction mixture was stirred at 0 °C for 2 hours. After this time, LCMS indicated that the reaction was complete. 1N NaOH (200 mL) was added. The reaction mixture was stirred another hour from 0 °C to room temperature. The DCM phase was then separated and the aqueous phase was extracted with DCM (2 × 200 mL). The combined DCM solution was washed with brine (200 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the pure desired product **(6)** (43.8 g, 100%) Analytical LCMS: single peak (214 nm), 3.092 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33-7.52 (m, 5H), 4.00 (d, *J*=13.8 Hz, 2H), 3.19-3.31 (m, 2H), 2.92-3.10 (m, 4H), 1.83-1.96 (m, 2H), 3.47 (t, *J*=7.4 Hz, 3H); HRMS, calc'd for $C_{15}H_{21}N_2O_2S$ (M+1), 292.1245; found 292.1301.

1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl amine (7)

A mixture of 4-phenyl-1-(propylsulfonyl)piperidine-4-carbonitrile (**6**) (8.76 G, 30 mmole) and Raney-Ni (2.5 g) in ammonia-MeOH (2M, 100 mL) was hydrogenated under H_2 (55 psi) at room temperature for 48 hours. After this time LCMS indicated that the reaction was complete. The catalyst was filtered and washed with MeOH. The MeOH solution was concentrated on a rotary evaporator to afford pure amine (**7**) (8.88 g, 100%) as a wax solid. Analytical LCMS: single peak (214 nm), 1.959 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.44 (m, 5H), 3.60 (d, *J*=12.2 Hz, 2H), 2.95 (t, *J*=11.5 Hz, 2H), 2.78 (t, *J*=7.6 Hz, 2H), 2.38 (d, *J*=13.9 Hz, 2H), 1.88 (t, *J*=12.0 Hz, 2H), 1.74-1.83 (m, 2H), 1.00 (t, *J*=7.5 Hz, 3H); HRMS, calc'd for $C_{15}H_{25}N_2O_2S$ (M+1), 297.1558; found 297.1676.

2-Chloro-*N*-{[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl}benzamide (8c)

A mixture of 1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methanamine (**7**) (30 mg, 0.10 mmol) and 2-chlorobenzoyl chloride (21 mg, 0.12 mmol) in DCM (1 mL) containing DIEA (26 mg, 0.2 mmol) was shaken for 2 hours at room temperature. After this time, the solvent was evaporated and the residue was purified by LCMS to afford the desire pure product (**8c**) as a white solid (41 mg, 95%). Analytical LCMS: single peak (214 nm), 3.136 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, *J*=7.5 Hz, 1H), 7.26-7.43 (m, 8H), 5.85 (t, *J*=5.6 Hz, 1H), 3.73 (d, *J*=6.2 Hz, 2H), 3.48-3.55 (m, 2H), 3.23-3.31 (m, 2H), 2.83-2.88 (m, 2H), 2.24-2.32 (m, 2H), 2.03-2.11 (m, 2H), 1.79-1.87 (m, 2H), 1.04 (t, *J*=7.5 Hz, 3H); HRMS, calc'd for $C_{22}H_{28}ClN_2O_3S$ (M+1), 435.1504; found 435.1504.

(1*E*)-1-[4-Phenyl-1-(propylsulfonyl)piperidin-4-yl]ethanone oxime (**10**)

1-Propanesulfonyl chloride (6.42 g, 45.9 mmole) was added to solution of 4-acetyl-4-phenyl piperidine hydrochloride (**9**) (10.0 g, 41.7 mmol) and DIEA (12.9 g, 100 mmole) in DCM (200 mL) with stirring on an ice-cooled bath. The resultant reaction mixture was stirred at 0 °C for 2 hours. After this time, LCMS indicated that the reaction was complete. 1N NaOH (120 mL) was added. The reaction mixture was stirred another hour from 0 °C to room temperature. The DCM phase was then separated and the aqueous phase was extracted with DCM (2 × 200 mL). The combined DCM solution was washed with brine (200 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the pure desired product (12.3 g, 95%) Analytical LCMS: single peak (214 nm), 3.062 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (t, *J*=7.8 Hz, 2H), 7.25-7.33 (m, 3H), 3.56-3.66 (m, 2H), 3.08 (t, *J*=11.5 Hz, 2H), 2.81-2.87 (m, 2H), 2.50 (d, *J*=15.0 Hz, 2H), 2.07-2.16 (m, 2H), 1.92 (s, 3H), 1.78-1.87 (m, 2H), 1.04 (t, *J*=7.4 Hz, 3H). A mixture of hydroxylamine hydrochloride (11.3 g, 162.5 mmole) and 4-acetyl-4-phenyl-1-(propylsulfonyl)piperidine (10.1 g, 32.5. mmole) in pyridine was heated at 90 °C overnight. Next morning, LCMS indicated that the reaction was complete. The pyridine was evaporated. The residue was stirred with water-EtOAc (1:2, 300 mL). The organic phase was separated and the aqueous solution was extracted with EtOAc (2 × 100 mL). The combined organic solution was washed with saturated $NaHCO_3$ (2 × 150 mL), brine (2 × 100 mL), dried over $MgSO_4$, filtered and concentrated to afford the pure desired product (**10**) as a white solid (10.2 g, 97%). Analytical LCMS: single peak (214 nm), 2.929 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.23-7.38 (m, 5H), 3.51-3.60 (m, 2H), 3.22 (t, *J*=11.6 Hz, 2H), 2.84-2.89 (m, 2H), 2.38 (d, *J*=15.0 Hz, 2H), 2.07-2.15 (m, 2H), 1.80-1.89 (m, 2H), 1.58 (s, 3H), 1.06 (t, *J*=7.5 Hz, 3H); HRMS, calc'd for $C_{16}H_{25}N_2O_3S$ (M+1), 325.1508; found 325.1515.

{(1*S*)-1-[4-Phenyl-1-(propylsulfonyl)piperidin-4-yl]ethyl}amine (**(S)-13**)

A mixture of (1*E*)-1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]ethanone oxime (10.0 g, 30.8 mmole) and Raney-Ni (3.0 g) in ammonia-MeOH (2M, 100 mL) was hydrogenated under H_2 (55 psi) at room temperature for 48 hours. After this time LCMS indicated that the reaction was complete. The catalyst was filtered and washed with MeOH. The MeOH solution was concentrated on a rotary evaporator to afford the desired product as a 1:1 (R) and (S) mixture (9.36, 98%). The mixture was separated by chiral HPLC to afford the pure (R) (4.57 g) and (S) (4.62 g) enantiomers. The absolute stereo-configuration was determined by X-ray after the amine was coupled with (R)-(-)-MTPA-Cl (Mosher's acid chloride) to form the amide. Analytical data of the (S) amine (**(S)-13**) was reported here. Analytical LCMS: single peak (214 nm), 2.012 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40 (t, *J*=7.8 Hz, 2H) 7.25-7.30 (m, 3H), 3.67 (d, *J*=6.6 Hz, 2H), 2.66-2.86 (m, 5H), 2.53 (d, *J*=14.2 Hz, 1H), 2.41 (d, *J*=3.9 Hz, 1H), 1.20-1.40 (s, broad, 2H), 0.98 (t, *J*=7.3 Hz, 3H), 0.88 (t, *J*=6.2 Hz, 3H); HRMS, calc'd for $C_{16}H_{27}N_2O_2S$ (M+1), 311.1715; found 311.1819.

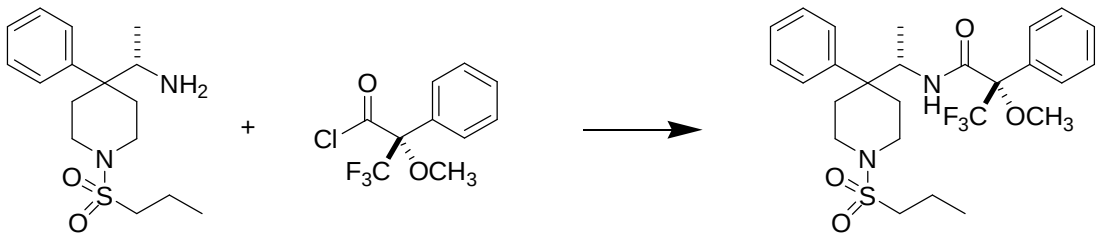
2-Amino-6-chloro-*N*-{(1*S*)-1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]ethyl}benzamide (**(S)-13h**)

A mixture of 2-amino-6-chloro benzoic acid (34 mg, 0.20 mmole), HOBt (27 mg, 0.20), PS-Carbodiimide (200 mg, 0.24 mmol), DIEA (52 mg, 0.4 mmol), and {(1*S*)-1-[4-phenyl-1-(propylsulfonyl)piperidin-4-yl]ethyl}amine (**(S)-13**) (31 mg, 0.10 mmol) in DCM was shaken over night at room temperature. Next morning, LCMS indicated that the amine was consumed. The resin was filtered and washed with DCM (4 × 5 mL). The combined DCM solution was concentrated and the residue was purified by LCMS. The LCMS purified collection was concentrated. The concentrated residue was dissolved in DCM (20 mL) and washed with 1N NaOH (10 mL), brine (2 × 10 mL), dried over $MgSO_4$, filtered and concentrated to afford the pure product (**(S)-13h**) as a white solid (51 mg, 94%). Analytical LCMS: single peak (214 nm), 2.248 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40 (t, *J*=7.7, 1H), 7.28-7.34 (m, 3H), 7.05 (t *J*=8.1 Hz, 1H), 6.71 (d *J*=7.9 Hz, 1H), 6.59 (td *J*=7.9 Hz, 1H), 5.58 (d, *J*=9.9 Hz, 1H), 4.41-4.50 (m, 1H), 3.57-3.70 (m, 2H), 2.81-2.93 (m, 2H), 2.74-2.80 (m, 2H), 2.57 (d, *J*=14.0 Hz, 1H), 2.57 (d, *J*=14.0 Hz, 1H), 1.96-2.07 (m, 2H), 1.73-1.83 (m, 2H), 0.98-1.02 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 166.4, 148, 147.4, 138.4, 131.5, 130.9, 129.2, 128.3, 127.2, 120.7, 119.1, 115, 104.5,

52.9, 51.5, 44.4, 42.4, 42.2, 33.3, 32.2, 17, 15.9, 13.3; HRMS, calc'd for $C_{23}H_{31}ClN_3O_3S$ (M+1), 464.1696; found 464.1766.

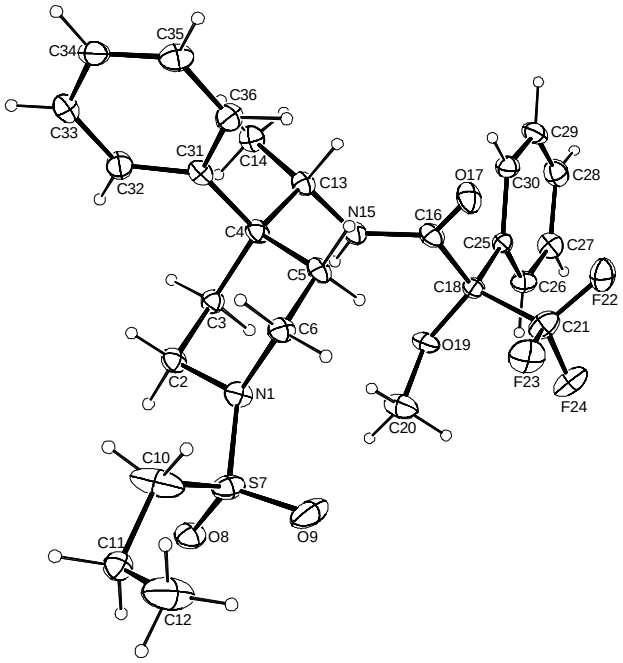
X-Ray Crystal Structure Data

Attempts to grow X-ray quality crystals of either **(S)-13** or **(R)-13** to assign absolute steerochemistry were unsuccessful. **(S)-12** was converted into the Mosher's amide using (R)-Mosher's acid chloride **(R)-14** to provide **15**. Compound **15** readily afforded crystalline material of sufficient quality for X-ray analysis. The ORTEP drawing of **15** unambiguously assigns the *(S)*-sterochemsitry to **(S)-12**. All of the **(S)-13** analogs reported in this manuscript were prepared from enantiopure **(S)-12**.



(S)-12 **(R)-14** **15**

Crystal data for **15**: $C_{26}H_{33}F_3N_2O_4S$, $M_r = 526.610$, orthorhombic, space group $P2_12_12_1$, $a = 10.409(5)$, $b = 12.989(6)$, $c = 19.231(9)$ Å, $V = 2600(2)$ Å^3, $Z = 4$, $D_x = 1.345$ g cm^{-3}, monochromatized radiation l(Mo K_a) = 0.71073 Å, $m = 0.18$ mm^{-1}, $F(000) = 1112$, $T = 100$ K. Data were collected on a Bruker CCD diffractometer to a q limit of 28.35°. There are 6284 unique reflections out of 30738 measured with 3155 observed at the $I \geq 2\sigma(I)$ level. The structure was solved by direct methods and refined using full-matrix least-squares on F^2 using 322 parameters and all unique reflections. The refinement converged with agreement statistics of $R = 0.057$, $wR = 0.104$, $S = 0.96$, $(\Delta/\sigma)_{max} = 0.01$. The maximum peak height in a final difference Fourier map is 0.410 eÅ^{-3}. Perspective view (ORTEP) of **15** shows the crystallographic numbering scheme. Non-hydrogen atoms are represented by ellipsoids corresponding to 30% probability envelopes. Hydrogen atoms have been drawn at an arbitrary size. CCDC 603055 (**15**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.



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Microdialysis. Rats were handled in strict accordance with regulations of the Merck Research Laboratories, West Point-Institutional Animal Care and Use Committee (WP-IACUC). A guide cannula for a microdialysis probe with an obturator (occupies the lumen of the cannula) was stereotaxically placed in anesthetized Male Sprague-Dawley rats (250-300 grams). On the day of the experiment (2-3 days later) the rats were again anesthetized, and the obturator was then removed and replaced by the microdialysis probe. Using a microinfusion pump, the probe was continuously perfused with an artificial cerebrospinal fluid (CSF) solution at a rate of 1-2microliters/minute. Approximately 2 hours was allowed for stabilization of injury-mediated release of neurotransmitter (from probe insertion) before collecting dialysate samples. The basal level of neurotransmitter was determined by collecting dialysate samples at 20 minute intervals until 3 consecutive samples do not vary by more than 10% (determined by HPLC). Test compounds were administered by subcutaneous, i.p., or i.v. (tail vein) routes. Note: in the conscious model, the rat was not anesthetized during test article administration. Microdialysate samples were collected every 20 minutes until the concentration of neurotransmitter reached a plateau before the next higher dose was administered. Neurotransmitter concentration was measured as a percent change from basal level. In some experiments the rats received a single dose of compound. In other experiments, the rat received multiple doses in a time interval

dictated by when the neurotransmitter level plateaus (i.e. a cumulative dose-response curve). When sample collection was complete, the probe was removed and in the case of urethane anesthetized rats a 1 ml blood sample was obtained by cardiac puncture to determine the plasma concentration of the test compound. In the conscious model, the rat was first anesthetized with isoflurane under a Flow Sciences hood and then a 1 ml blood sample is procured by cardiac puncture. After the blood sample is obtained, the anesthetized rat is immediately euthanized by placing it in a CO_2 chamber. After euthanasia is complete, the brain was removed and frozen. The brain was then placed in an acrylic matrix and sectioned to verify position of the probe. Glycine levels were determined by HPLC (Agilent 1100 series) of microdialysis samples derivatized by o-phthalaldehyde (OPA) using a fluorescence detector, and analyzed by ChemStation software (Agilent). Samples were OPA-derivatized by standard methods and injected onto a Hypersil C18 column (Thermo Hypersil-Keystone) in 0.03M sodium acetate, 0.25 % tetrahydrofuran pH 7.2 (mobile phase component A), and eluted with a gradient of 80% acetonitrile, 20% 0.1 M sodium acetate pH 7.2 (mobile phase component B) at a flow rate of 0.5ml/min at room temperature. Detector fluorescence excitation and emission wavelengths were 340 nm and 450nm, respectively.

Prepulse Inhibition. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used in the present studies. Prepulse inhibition was evaluated using SR-LAB (San Diego Instruments, San Diego, (CA) acoustic startle chambers as described previously (reference 18). **(S)-13h** (3-30 mg/kg) or vehicle (60:40 DMSO:PEG400) was administered 10 minutes pre-test. All injections were given subcutaneously at an injection volume of 1 cc/kg. In each test session, rats were exposed to a 5 minute acclimation period where background noise was continuously present (65 dB) followed by a series of five 40 ms 118-120 dB bursts of white noise. Following these five presentations, the test session began. Each test session consisted of 10 repetitions of trials composed of a 120 ms pulse at 70, 75, 80 or 85 dB prepulse followed by 100 ms later by the 118 or 120 bD 40 ms pulse, the startle pulse alone (pulse alone) and a period where no stimulus was presented (nostim). Prepulse inhibition (PPI) was determined by the formula (100-((prepulse pulse/pulse alone)x100)) and expressed as a %PPI +/- standard error of the mean (SEM). %PPI was evaluated using a repeated analysis of variance (ANOVA) with treatment as the between factor and prepulse level as the within factor followed by post hoc testing using the Dunnett procedure, where appropriate.