Supporting Information

From Selective α4β2 Nicotinic Acetylcholine Receptor Ligands to the Discovery of Selective Sigma-1 Receptor Ligands: Pharmacophore Analysis and Rational Design

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Abbreviations: CC, column chromatography; rt, room temperature; TFA, trifluoroacetic acid.

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Synthetic Methods and Procedures

General Methods. Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Anhydrous THF and CH_2Cl_2 were obtained by distillation over sodium wire or CaH_2, respectively. All non-aqueous reactions were run under an argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. The progress of reactions was monitored by TLC on SiO_2. Spots were visualized by their quenching of the fluorescence of an indicator admixed to the SiO_2 layer, or by dipping into $KMnO_4$ solution followed by heating. SiO_2 for column chromatography (CC) was of 230–400 mesh particle size, and an EtOAc/hexane mixture or gradient was used unless stated otherwise. ^{1}H NMR spectra were recorded at a spectrometer frequency of 300 or 400 MHz, ^{13}C NMR spectra at 75 MHz or 100 MHz. ^{1}H chemical shifts are reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ or the δ 4.80 signal of D_2O as internal standard. ^{13}C chemical shifts are reported in δ (ppm) using the δ 77.23 signal of $CDCl_3$ as internal standard. ^{13}C NMR spectra in D_2O were not adjusted. Purities of final compounds (>98%) were established by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Synergi 4 µ Hydro-RP 80A column, with detection at 220 or 254 nm on a variable wavelength detector G1314A; flow 1.4 mL/min; gradient of 10% to 100% methanol in water (both containing 0.05 vol% of TFA) in 18 min.

3-[[3-(2(*S*)-Azetidinylmethoxy)isoxazol-5-yl]methoxy]-5-chloropyridine (14): To a solution of the *N*-Boc protected precursor **23** (200 mg, 0.51 mmol) in CH_2Cl_2 (10 mL) was added TFA (0.5 mL) under argon with ice cooling. The mixture was stirred overnight at rt. After the solvent was evaporated, the residue was dissolved in distilled water (5 mL). The solution was filtered over a syringe filter (polytetrafluoroethylene, 17 mm diameter, 0.45 µm pore size), then concentrated to 2–3 mL under reduced pressure at 30 °C bath temperature. The crude product was purified by preparative HPLC under the following conditions: column, ACE AQ, 150 x 21.2 mm; flow, 17 mL/min; all solvents containing 0.05 vol% TFA; UV detection at 254 nm and 220 nm; Gradient: 25–100% MeOH in water in 30 min, 100% for 5 min, return to 25% in 4 min, and equilibration at 25% for 1 min. After the solvent was

evaporated, the residue was dissolved in distilled water (about 2–3 mL). The solution was lyophilized to obtain the TFA salt (150 mg, 66%) as a white solid. Purity ~100%. $[\alpha]_D^{20}$ 0.7 (*c* = 0.84, MeOH). ^{1}H NMR (400 MHz, D_2O) δ 8.34 (m, 2H), 7.90 (d, 1H, *J* = 2.0 Hz), 6.37 (s, 1H), 5.28 (s, 2H), 4.94–4.92 (m, 1H), 4.55–4.53 (m, 2H), 4.15–4.10 (m, 1H), 4.06–4.02 (m, 1H), 2.69–2.62 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 170.8, 167.1, 162.4 (TFA), 154.9, 137.1, 133.6, 131.8, 126.9, 115.7 (TFA), 95.7, 67.7, 61.6, 58.4, 43.1, 19.9. Anal Calcd for $C_{13}H_{14}ClN_3O_3 \cdot 1.25\ C_2HF_3O_2 \cdot 0.7\ H_2O$ (MW 453.4): C, 41.29, H, 3.72, F, 15.80; N, 9.32. Found: C, 41.08; H, 3.43; F, 15.62; N, 9.06.

5-(Phenoxymethyl)-3-(2(*S*)-pyrrolidinylmethoxy)isoxazole (15). To a solution of the *N*-Boc protected precursor **24** (180 mg, 0.48 mmol) in CH_2Cl_2 (10 mL) was added TFA (0.5 mL) under argon with ice cooling. The mixture was stirred overnight at rt. After the solvent was evaporated, the residue was dissolved in distilled water (5 mL). The solution was filtered over a syringe filter (polytetrafluoroethylene, 17 mm diameter, 0.45 μm pore size), then concentrated to 2–3 mL under reduced pressure at 30 °C bath temperature. The crude product was purified by preparative HPLC under the following conditions: column, ACE AQ, 150 x 21.2 mm; flow, 17 mL/min; all solvents containing 0.05 vol% TFA; UV detection at 254 nm and 220 nm; gradient: 8–100% MeOH in water in 30 min, 100% for 5 min, return to 8% in 4 min, and equilibration at 8% for 1 min. After the solvent was evaporated, the residue was dissolved in distilled water (2–3 mL). The solution was lyophilized to obtain the TFA salt (71 mg, 37%) as a white solid. Purity 99.3%. $[\alpha]_D^{20}$ 11.7 (*c* = 0.50, MeOH). ^{1}H NMR (300 MHz, D_2O) δ 7.16–7.10 (m, 2H), 6.84–6.81 (m, 3H), 6.05 (s, 1H), 4.94 (s, 2H), 4.39 (dd, 1H, *J* = 11.4, 3.3 Hz), 4.22 (dd, 1H, *J* = 11.4, 8.4 Hz), 3.22–3.16 (m, 2H), 2.09–1.65 (m, 4H). ^{13}C NMR (75 MHz, D_2O) δ 172.4, 171.2, 162.4 (TFA), 159.2, 130.6, 122.8, 115.8, 115.7 (TFA), 95.8, 69.3, 62.1, 59.9, 46.8, 27.2, 24.7. Anal Calcd for $C_{15}H_{18}N_2O_3 \cdot 1.1\ C_2HF_3O_2 \cdot 0.05\ H_2O$ (MW 400.6): C, 51.56; H, 4.83; F, 15.65; N, 6.99. Found: C, 51.39; H, 4.81; F, 15.86; N, 7.11.

3-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]methoxy]isoxazol-5-ylmethanol (18). To a stirred solution of 1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylmethanol (240 mg, 1.7 mmol), 3-hydroxyisoxazole-

5-carboxylic acid methyl ester (**16**; 406 mg, 2.0 mmol), and PPh_3 (660 mg, 2.5 mmol) in anhydrous THF (30 mL) was added dropwise diethyl azodicarboxylate (1.5 mmol). After stirring overnight at rt, the solvent was evaporated, and the residue was dissolved in EtOAc. The solution was washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by CC on SiO_2 to give the isoxazolecarboxylic acid ester. To a solution of this ester in anhydrous THF (20 mL) was added $LiBH_4$ (150 mg, 4 mmol) with ice cooling under Ar. After stirring overnight at rt, saturated aqueous NH_4Cl solution was added with ice cooling. Extraction with EtOAc, drying over Na_2SO_4, and CC on SiO_2 gave the alcohol **18** (385 mg, 78%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (s, 1H), 4.58 (s, 2H), 4.26 (m, 1H), 4.16–4.10 (m, 2H), 3.66 (br, 1H), 3.35 (m, 2H), 1.94–1.79 (m, 4H), 1.42 (s, 9H).

2(*S*)-[[5-(Iodomethyl)isoxazol-3-yloxy]methyl]azetidine-1-carboxylic Acid *tert*-Butyl Ester (19). To a stirred solution of **17** (270 mg, 0.95 mmol), imidazole (142 mg, 2.1 mmol), and PPh_3 (500 mg, 1.9 mmol) in anhydrous CH_2Cl_2 (20 mL) was added I_2 (482 mg, 1.9 mmol) with ice cooling under Ar. After stirring overnight at rt, the solvent was evaporated. The residue was purified by CC on SiO_2 to give the iodide (290 mg, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (s, 1H), 4.47–4.42 (m, 2H), 4.27–4.22 (m, 3H), 3.79 (t, 2H, J = 7.6 Hz), 2.30–2.22 (m, 1H), 2.19–2.10 (m, 1H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 169.5, 156.1, 94.1, 79.7, 70.1, 60.0, 46.7, 28.4, 18.8.

2(*S*)-[[5-(Iodomethyl)isoxazol-3-yloxy]methyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (20). To a stirred solution of **18** (240 mg, 0.8 mmol), imidazole (83 mg, 1.2 mmol), and PPh_3 (320 mg, 1.2 mmol) in anhydrous PhMe (8 mL) was added I_2 (308 mg, 1.2 mmol) with ice cooling under Ar. After stirring overnight at rt, the solvent was evaporated. The residue was purified by CC on SiO_2 to give the iodide (240 mg, 73%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (s, 1H), 4.30–4.25 (m, 3H), 4.16–4.11 (m, 2H), 3.36 (s, 2H), 1.96–1.80 (m, 4H), 1.45 (s, 9H).

2(*S*)-[[5-[(5-Chloropyridin-3-yloxy)methyl]isoxazol-3-yloxy]methyl]azetidine-1-carboxylic Acid *tert*-Butyl Ester (21). To a stirred solution of **19** (250 mg, 0.6 mmol) and 5-chloropyridin-3-ol (120 mg, 0.8

mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (522 mg, 3.8 mmol) under Ar. After stirring overnight at rt, saturated aqueous NH_4Cl solution was added. The mixture was extracted with EtOAc (2 × 15 mL), and the combined organic phases were washed with water (3 x 10 mL), dried over Na_2SO_4, and evaporated. The residue was purified by CC on SiO_2 to give the product (200 mg, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.15 (s, 1H), 7.22 (s, 1H), 5.99 (s, 1H), 5.02 (s, 2H), 4.46–4.42 (m, 2H), 4.25 (d, 1H, *J* = 10.0 Hz), 3.77 (t, 2H, *J* = 7.6 Hz), 2.28–2.20 (m, 1H), 2.17–2.10 (m, 1H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 167.2, 156.1, 154.1, 141.9, 136.2, 132.0, 121.7, 95.6, 79.7, 70.2, 61.9, 59.9, 46.8, 29.6, 28.3, 18.8.

2(*S*)-[[(5-(Phenoxymethyl)isoxazol-3-yloxy]methyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (22). To a stirred solution of **20** (220 mg, 0.54 mmol) and phenol (101 mg, 1.1 mmol) in anhydrous DMF (4 mL) was added K_2CO_3 (450 mg, 3.3 mmol) under Ar. After stirring overnight at rt, saturated aqueous NH_4Cl solution was added. The mixture was extracted with EtOAc (2 × 15 mL), and the combined organic phases were washed with water (3 × 10 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by CC on SiO_2 to give the product (180 mg, 89%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (t, 2H, *J* = 7.5 Hz), 7.01 (t, 2H, *J* = 7.5 Hz), 6.94 (d, 2H, *J* = 9.0 Hz), 5.97 (s, 1H), 5.04 (s, 2H), 4.33 (m, 1H), 4.16 (m, 2H), 3.37 (m, 2H), 1.97–1.86 (m, 4H), 1.46 (s, 9H).

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