Investigation of Cardiovascular Effects of Tetrahydro-β-carboline sstr3 antagonists

Supporting Information

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Contents of Supporting Information

General

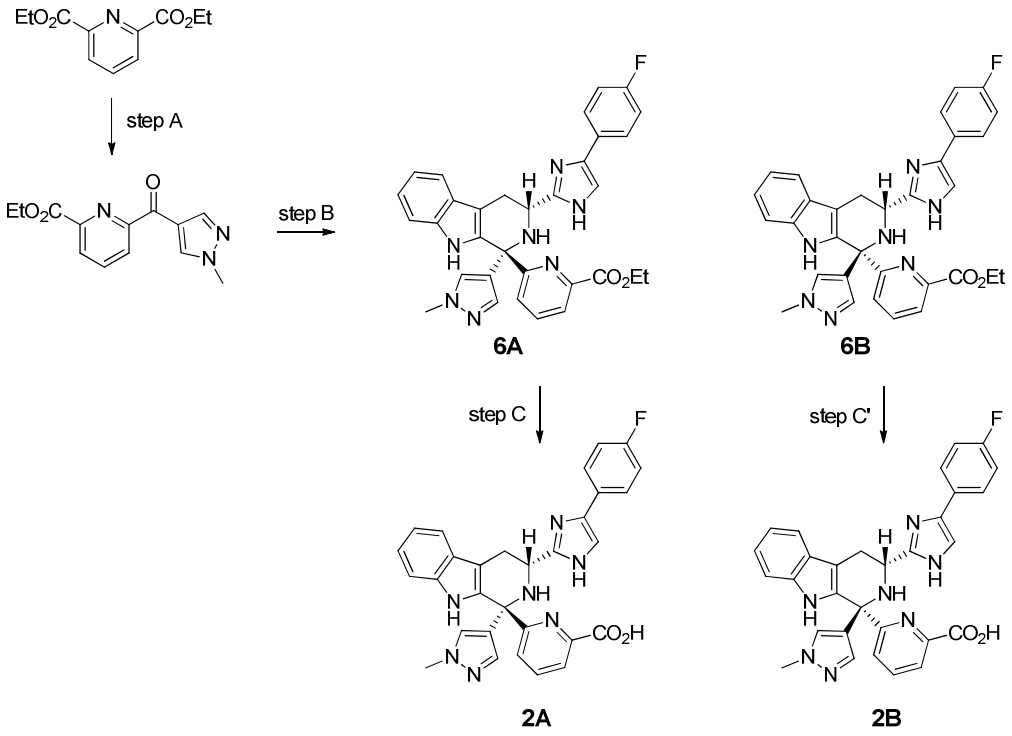
Normal phase column chromatography was carried out in the indicated solvent system (in the percentage of volume) using pre-packed silica gel cartridges for use on the Isco CombiFlash® or Biotage SP1®. Analytical thin layer chromatography (TLC) visualization was performed using 254 nm wavelength ultraviolet light. The LC/MS analyses were performed using a MICROMASS ZMD mass spectrometer coupled to an AGILENT 1100 Series HPLC utilizing a YMC ODS-A 4.6 x 50 mm column eluting at 4.5 mL/min with a solvent gradient of 10 to 95% B over 2.5 min, followed by 0.5 min at 95% B: solvent A = 0.06% TFA in water; solvent B =0.05% TFA in acetonitrile. Nuclear Magnetic Resonance spectra were recorded on Varian spectrometers. Spectra were taken in the indicated solvent at ambient temperature, and the chemical shifts are reported in parts per million (ppm (δ)) relative to the lock of the solvent used. Resonance patterns are recorded with the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). High resolution mass spectra (HRMS) were acquired by use of a Bruker Daltonics 7T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Samples were dissolved in acetonitrile:water:acetic acid (50:50:0.1%v/v), and ionized by use of electrospray ionization (ESI) yielding [M+H]+. External calibration was accomplished with oligomers of polypropylene glycol (PPG, average molecular weight 1000 Da.

Ethyl 6-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)picolinate (**6A**)

Ethyl 6-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)picolinate (**6B**)

6-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)picolinic acid (**2A**)

6-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)picolinic acid (**2B**)



Step A

To a solution of 4-iodo-1-methyl-1H-pyrazole (3.2 g, 0.015 mol) in dry THF (20 mL) was added 2 M solution of *i*-PrMgCl in THF (8.4 mL) at -20 °C, and the solution was stirred for 1 h at this temperature. To the mixture was added a solution of diethyl pyridine-2, 6-dicarboxylate (10 g, 0.045 mol) in THF (50 mL) at -78 °C. The resulting solution was stirred for further 2 hours at -78 °C, allowed to warm to room temperature slowly, and stirred overnight. 1N HCl aqueous solution was added slowly to the reaction mixture at -10 °C. THF was removed and the residue was diluted with ethyl acetate. The organic layer was separated and washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography to afford ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)picolinate (2.1 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (s, 1 H), 8.46 (s, 1 H), 8.37-8.35 (m, 1 H), 8.30 (m, 1 H), 8.05-8.02 (m, 1 H), 4.51 (q, J = 5.4 Hz, 2 H), 1.52 (t, J = 5.4 Hz, 3 H). LC-MS m/z = 260(M+1)$^+$.

Step B

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine mono to-

sylate salt (1.0 g, 1.50 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with p-toluenesulfonic acid (1 eq)] in DMSO (2 mL) was added ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)picolinate (0.55 g, 2.11 mmol, 1.4 eq.) followed by NaOAc (123 mg, 1.50 mmol) and tetraethoxysilane (0.337 mL, 0.31 g, 1.50 mml). The mixture was heated in oil bath (92 °C) for two days. The mixture was cooled to room temperature and quenched by ice. The mixture was then basified by NaOH (1.0 N, aq. 50 mL). The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give a residue, which was purified by silica gel chromatography eluted with a gradient of 20-100% EtOAc in hexanes to give a mixture of isomers 670 mg. The mixture was separated by chiral SFC (Chiral IA column, 40% MeOH/CO_2) to give two individual isomers.

The fast eluting peak was **6A** (259 mg, 31%). LCMS *m/z* = 562 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 7.93 (d, 1H), 7.79 (m, 1H), 7.73 (d, 1H), 7.65 (m, 2H), 7.45 (d, 1H), 7.37 (d, 1H), 7.30 (d, 2H), 7.25 (s, 1H), 7.09 (t, 1H), 7.01 (m, 3H), 4.36 (q, 2H), 4.15 (m, 1H), 3.72 (m, 2H), 1.34 (t, 3H).

The slow eluting peak was **6B** (285 mg, 34%). LCMS *m/z* = 562 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (m, 1H), 7.89 (m, 2H), 7.66 (m, 2H), 7.44 (d, 1H), 7.31 (m, 4H), 7.04 (m, 3H), 6.97 (t, 1H), 4.43 (m, 1H), 4.37 (m, 2H), 3.72 (s, 3H), 3.22 (m, 1H), 3.14 (m, 1H), 1.36 (t, 3H).

The configurations of **6A** and **6B** at the C1 position of β-carboline were determined by nOe experiments.

Step C

Ethyl ester **6A** (60 mg, 0.11 mmol) was treated with LiOH monohydrate (22.4 mg, 0.534 mmol) in THF (3 mL) and water (1 mL). The mixture was stirred overnight at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water to afford **2A** (62 mg, 66%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (d, 1H), 8.11 (t, 1H), 7.92 (d, 1H), 7.88 (m, 2H), 7.83 (s, 1H), 7.57 (d, 1H), 7.46 (m, 2H), 7.23 (m, 4H), 7.12 (m, 1H), 5.25 (m, 1H), 3.83 (s, 3H), 3.79 (m, 1H), 3.43 (m, 1H).

Step C'

Ethyl ester **6B** (60 mg, 0.11 mmol) was treated with LiOH monohydrate (22.4 mg, 0.534 mmol) in THF (3 mL) and water (1 mL). The mixture was stirred overnight at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a

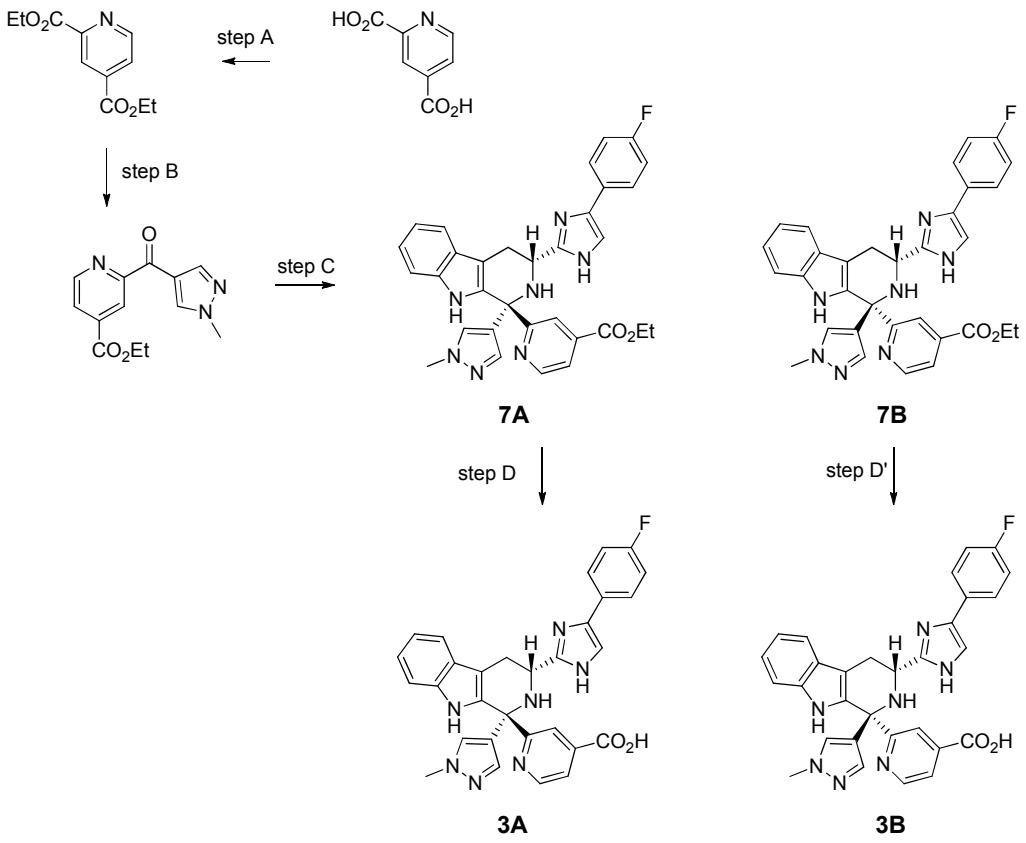
gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **2B** (60 mg, 64%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 8.13 (d, 1H), 8.06 (t, 1H), 7.87 (d, 1H), 7.77 (m, 2H), 7.75 (s, 1H), 7.54 (d, 1H), 7.50 (s, 1H), 7.40 (s, 1H), 7.33 (d, 1H), 7.23 (m, 2H), 7.13 (t, 1H), 7.06 (t, 1H), 4.81 (m, 1H), 3.84 (s, 3H), 3.53 (m, 1H), 3.41 (m, 1H).

Ethyl 2-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)isonicotinate (**7A**)

Ethyl 2-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)isonicotinate (**7B**)

2-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)isonicotinic acid (**3A**)

2-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)isonicotinic acid (**3B**)



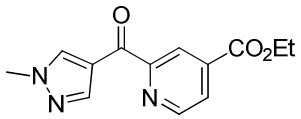
Step A

Thionyl chloride (34.8 mL, 57.0 g, 479 mmol) was added dropwise to EtOH (200 mL) at 0 °C under N_2. The mixture was stirred for 30 min. This mixture was transferred to a suspension of pyridine-2,4-dicarboxylic acid (40 g) suspended in EtOH (300 mL). The resulting mixture was then heated to reflux for 3 h. The solvent was removed in vacuo. The residue was dissolved in EtOAc. The mixture was washed with $NaHCO_3$ (sat. aq.), dried (Na_2SO_4), and concentrated to afford diethyl pyridine-2,4-dicarboxylate (44 g, 82%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.89 (m, 1H), 8.61 (s, 1H), 8.01 (m, 1H), 4.49 (m, 2H), 4.42 (m, 2H), 1.42 (m, 6H).

Step B

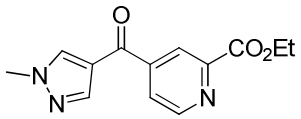
To a solution of 1-methyl-4-iodo-1H-pyrazole (29.8 g, 143 mmol) in THF (200 mL) was added isopropylmagnesium chloride 2.0M in THF (79.0 ml, 158 mmol) at 0ºC.The mixture was stirred at 0ºC for 30 min then cooled to -78 ºC and diethyl pyridine-2,4-dicarboxylate (32.0 g, 143 mmol) was added. The mixture was slowly warmed to room temperature overnight. The reaction was cooled to -78

°C,quenched by dropwise addition of NH_4Cl solution(sat'd., aq,) and warmed to room temperature. Most solvent was removed *in vacuo*. The mixture was diluted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Silica gel chromatography eluted with a gradient of 10% EtOAc in hexanes to straight EtOAc afforded ethyl 2-(1-methyl-1H-pyrazole-4-carbonyl)isonicotinate (7.8 g, 21%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (d, 1H), 8.62 (s, 1H), 8.57 (s, 1H), 8.33 (s, 1H), 8.00 (m, 1H), 4.41 (q, 2H), 3.95 (s, 3H), 1.39 (t, 3H). HMBC and nOe experiments confirmed that the product obtained is ethyl 2-(1-methyl-1H-pyrazole-4-carbonyl)isonicotinate and is not ethyl 4-(1-methyl-1H-pyrazole-4-carbonyl)picolinate.



ethyl 2-(1-methyl-1*H*-pyrazole-4-carbonyl)isonicotinate

isolated



ethyl 4-(1-methyl-1*H*-pyrazole-4-carbonyl)picolinate

not isolated

Step C

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine mono tosylate salt (1.02 g, 1.53 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with p-toluenesulfonic acid (1 eq)] in DMSO (1.5 mL) was added ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate (0.56 g, 2.15 mmol, 1.4 eq.) followed by NaOAc (126 mg, 1.53 mmol) followed by tetraethoxysilane (0.344 mL, 0.32 g, 1.52 mmol). The mixture was heated in an oil bath (90 °C) for two days. The mixture was cooled to room temperature and quenched by ice. The mixture was then basified by NaOH (1.0 N, aq. 50 mL). The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a residue, which was purified by silica gel chromatography eluted with a gradient of 10-100% acetone in CH_2Cl_2 to give a mixture of isomers (408 mg). The mixture was separated by chiral SFC (Chiral IA column, 40% MeOH/CO2) to give the two individual isomers.

The slow eluting peak was **7A** (167 mg, 19%). LCMS *m/z* = 562 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 8.81 (d, 1H), 8.02 (s, 1H), 7.81 (d, 1H), 7.70 (m, 2H), 7.50 (d, 1H), 7.39 (d, 1H), 7.37 (s, 1H), 7.33 (m, 1H), 7.29 (s, 1H), 7.13 (t, 1H), 7.08 (t, 2H), 7.03 (t, 1H), 4.32 (m, 2H), 4.09 (m, 1H), 3.80 (s, 3H), 3.19 (m, 1H), 3.15 (m, 1H), 1.29 (t, 3H).

The fast eluting peak was 7**B** (192 mg, 22%). LCMS *m/z* = 562 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD)

δ 8.75 (d, 1H), 8.12 (s, 1H), 7.80 (d, 1H), 7.71 (m, 2H), 7.49 (d, 1H), 7.43 (s, 1H), 7.38 (s, 1H), 7.33 (m, 1H), 7.29 (d, 1H), 7.08 (m, 3H), 7.01 (t, 1H), 4.40 (m, 3H), 3.85 (s, 3H), 3.22 (m, 2H), 1.36 (t, 3H).

The configurations of **7A** and **7B** at the C1 position of β-carboline were determined by nOe experiments.

Step D

Ethyl ester **7A** (36 mg, 0.07 mmol) was treated with LiOH monohydrate (3.1 mg, 0.13 mmol) in MeOH (1 mL) and water (0.2 mL). The mixture was stirred for 2 h at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **3A** (30 mg, 53%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. Accurate Mass $C_{30}H_{24}FN_7O_2$ [M+H] measured 534.2046, calculated 534.2048. ^{1}H NMR (500 MHz, CD_3OD) δ 8.92 (d, 1H), 8.09 (s, 1H), 7.96 (d, 1H), 7.80 (m, 2H), 7.77 (s, 1H), 7.57 (d, 1H), 7.45 (d, 1H), 7.42 (s, 1H), 7.31 (s, 1H), 7.23 (m, 3H), 7.11 (t, 1H), 4.71 (m, 1H), 3.83 (s, 3H), 3.47 (m, 2H).

Step D'

Ethyl ester **7B** (38 mg, 0.07 mmol) was treated with LiOH monohydrate (3.3 mg, 0.14 mmol) in MeOH (1 mL) and water (0.2 mL). The mixture was stirred for 2h at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **3B** (28 mg, 47%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 8.77 (d, 1H), 8.13 (s, 1H), 7.86 (d, 1H), 7.77 (m, 2H), 7.74 (s, 1H), 7.53 (d, 1H), 7.48 (s, 1H), 7.43 (s, 1H), 7.33 (d, 1H), 7.26 (t, 2H), 7.13 (t, 1H), 7.05 (t, 1H), 4.71 (m, 1H), 3.86 (s, 3H), 3.47 (m, 1H), 3.32 (m, 1H).

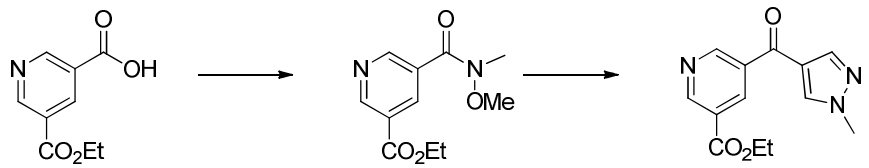
Ethyl 5-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinate (**8A**)

Ethyl 5-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinate (**8B**)

5-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinic acid (**4A**)

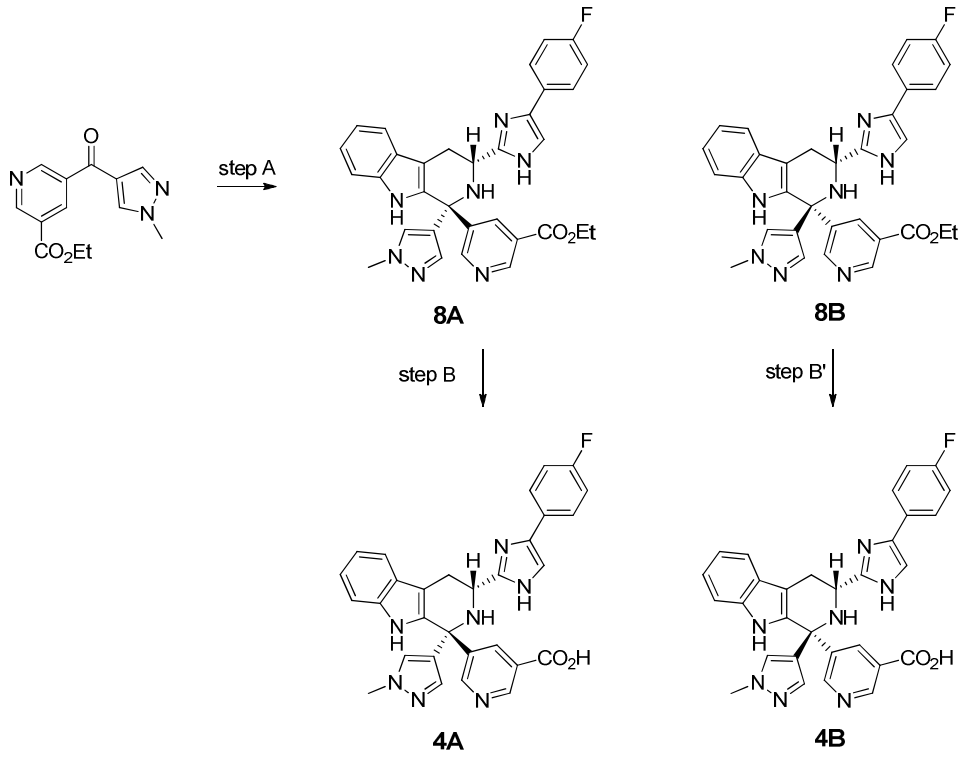
5-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinic acid (**4B**)

Preparation of ethyl 5-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate



5-(ethoxycarbonyl)nicotinic acid (4.0 g, 20.5 mmol) was suspended in CH_2Cl_2 (100 mL). O,N-dimethylhydroxylamine hydrochloride (2.4 g, 24.6 mmol) was added followed by N,N-diisopropylethylamine (4.29 mL, 24.5 mmol), DMAP (0.25 g, 2.01 mml) and EDC (5.9 g, 30.8 mml). The mixture was stirred at room temperature overnight. The reaction mixture was washed with sodium bicarbonate, water, 0.5 N HCl, followed by brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum to provide crude product, which was purified by silica gel chromatography eluted with a gradient of 10-100% (ethyl acetate in hexanes) to give 5-ethyl 5-(methoxy(methyl)carbamoyl)nicotinate (3.8 g, 78%). LC-MS: *m/z* = 239 (M+1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.28 (s, 1H), 9.08 (s, 1H), 8.61 (s, 1H), 4.43 (q, 2H), 3.55(s, 3H), 3.40 (s, 3H), 1.41 (t, 3 H).

Isopropylmagnesium bromide (2M in THF, 7.4 mL, 14.8 mmol) was added to a solution of 4-iodo-1-methyl-pyrazole (2.8 g, 13.5 mmol) in anhydrous THF (50 mL) at 0^{o}C under nitrogen. The reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was cooled to -70°C, and the followed by addition of 5-ethyl 5-(methoxy(methyl)carbamoyl)nicotinate (3.2 g, 13.5 mmol) in anhydrous THF (10 mL) then allowed to warm to room temperature slowly in 4 h. The reaction were quenched by the dropwise addition of saturated aqueous NH_4Cl.and then was diluted with cold 0.5 N HCl and extracted with EtOAc. The organic layer were washed with brine, dried over Na_2SO_4 and concentrated to give the crude product which was then purified by flash column chromatography (10% to 100% ethyl acetate in hex) to give ethyl 5-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate (1.0 g, 29%). LC-MS: *m/z* = 260 (M+1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.39 (d, 1H), 9.19 (d, 1H), 8.70 (t, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 4.46 (q, 2H),), 4.01 (s, 3H),), 1.43 (t, 3H).



Step A

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine mono tosylate salt (1.0 g, 1.50 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with p-toluenesulfonic acid (1 eq)] in DMSO (2 mL) was added ethyl 5-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate (0.51 g, 1.96 mmol, 1.3 eq.) followed by NaOAc (123 mg, 1.50 mmol) followed by tetraethoxysilane (0.337 mL, 0.31 g, 1.50 mmol). The mixture was heated in an oil bath (85 °C) for two days. The mixture was cooled to room temperature and quenched by ice. The mixture was then basified with NaOH solution (1.0 N, aq. 50 mL). The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give a residue, which was purified by silica gel chromatography eluted with a gradient of 10-100% acetone in CH_2Cl_2 to give a mixture of isomers 220 mg. The mixture was separated by chiral SFC (Chiral IA column, 40% MeOH/CO_2) to give two individual isomers.

The slow eluting peak was **8A** (103 mg, 12%). LCMS *m/z* = 562 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.04(d, 1H), 8.80 (d, 1H), 8.47 (m, 1H), 7.69 (m, 2H), 7.52 (d, 1H), 7.46 (s, 1H), 7.38 (s, 1H), 7.36 (d, 1H), 7.31 (m, 1H), 7.14 (t, 1H), 7.07 (m, 3H), 4.35 (m, 2H), 4.04 (m, 1H), 3.84 (s, 3H), 3.18 (m, 2H), 1.31 (t, 3H).

The fast eluting peak was **8B** (86 mg, 10%). LCMS *m/z* = 562 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 8.97 (s, 1H), 8.83 (m, 1H), 8.43 (s, 1H), 7.65 (m, 2H), 7.48 (m, 3H), 7.27 (m, 2H), 7.04 (m, 4H), 4.42 (m, 1H), 4.31 (m, 2H), 3.82 (s, 3H), 3.19 (m, 2H), 1.30 (t, 3H).

The configuration of the C1 position of β-carboline was determined at the stage of **4A** and **4B**.

Step B

Ethyl ester **8A**(70 mg, 0.13 mmol) was treated with LiOH monohydrate (6.0 mg, 0.25 mmol) in MeOH (1 mL) and water (0.3 mL). The mixture was stirred 3 h at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **4A** (55 mg, 50%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.09 (d, 1H), 8.87 (d, 1H), 8.51 (m, 1H), 7.79 (s, 1H), 7.76 (m, 2H), 7.56 (d, 1H), 7.42 (d, 2H), 7.38 (d, 1H), 7.27 (t, 2H), 7.18 (t, 1H), 7.09 (t, 1H), 4.33 (m, 1H), 3.85 (s, 3H), 3.46 (m, 1H), 3.23 (m, 1H). The configuration at the C1 position of β-carboline was determined by nOe experiment.

Step B'

Ethyl ester **8B** (45 mg, 0.08 mmol) was treated with LiOH monohydrate (3.8 mg, 0.16 mmol) in MeOH (1 mL) and water (0.3 mL). The mixture was stirred overnight at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **4A** (26 mg, 37%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.04 (m, 1H), 8.82 (m, 1H), 8.47 (m, 1H), 7.79 (m, 1H), 7.75 (m, 2H), 7.54 (m, 3H), 7.29 (m, 3H), 7.14 (m, 1H), 7.07 (m, 1H), 4.66 (m, 1H), 3.89 (s, 3H), 3.38 (m, 1H), 3.32 (m, 1H). The configuration at the C1 position of β-carboline was determined by nOe experiment.

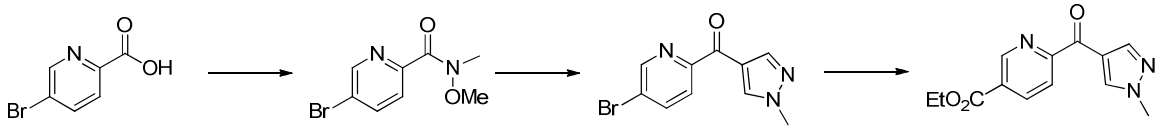
ethyl 6-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinate (**9A**)

ethyl 6-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinate (**9B**)

6-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinic acid (**5A**)

6-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)nicotinic acid (**5B**)

Preparation of ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate

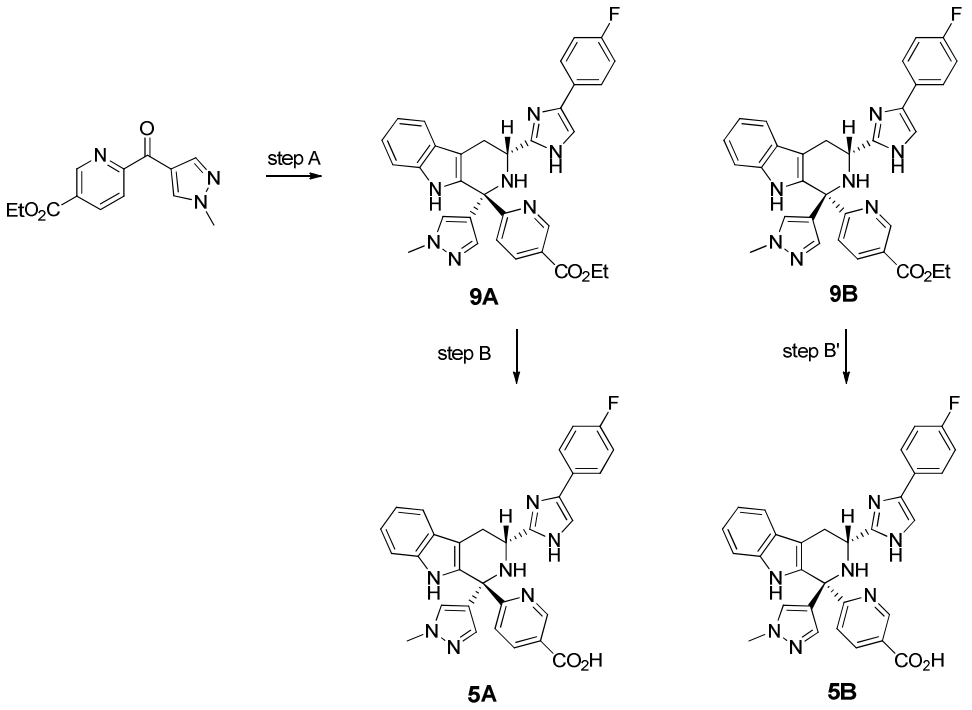


A solution of 5-bromopyridine-2-carboxylic acid (100 g, 0.5 mol), O,N-dimethylhydroxylamine hydrochloride (58 g, 0.6 mol), EDCI (190 g, 1 mol), HOBT (7 g, 0.05 mol), and triethylamine (40 mL, 0.3 mol) in 1.4 L CH_2Cl_2 were stirred at room temperature. The reaction mixture was washed with sodium bicarbonate, water, 0.5 N HCl, followed by brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum to provide crude 5-bromo-N-methyoxy-N-methylpyridine-2-carboxamide (70 g).

Isopropylmagnesium bromide (98 mL, 98 mmol) was added to a solution of 4-iodo-1-methyl-pyrazole (20 g, 98 mmol) in anhydrous THF (200 mL) at 0^{o}C under nitrogen. The reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was cooled to -70°C, and the followed by addition crude 5-bromo-N-methyoxy-N-methylpyridine-2-carboxamide above (20 g, 80 mmol) in anhydrous THF (50 mL) slowly, then allowed to warm to room temperature overnight. The reaction was quenched by the dropwise addition of saturated aqueous NH_4Cl.and then was diluted with cold 0.5 N HCl and extracted with EtOAc. The organic layer were washed with brine, dried over Na_2SO_4 and concentrated to give the crude product which was then purified by flash column chromatography (1:5, ethyl acetate: petroleum ether) to give (5-bromopyridin-2-yl)(1-methyl-1H-pyrazol-4-yl)methanone (17 g). ^{1}H NMR DMSO δ: 3.96 (s, 3 H), 7.97-7.99 (d, 1 H), 8.24(s, 1 H), 8.28-8.30(dd, 1 H), 8.75 (s, H), 8.87-8.88(d, 1 H); LC-MS: *m/z* = 266 (M+1).

To a solution of (5-bromopyridine-2-yl)(1-methyl-pyrazol-4-yl)methanone (1 g, 3.76 mmol) in EtOH 30 mL was added triethylamine (33 mL, 22.6 mmol) and $Pd(PPh_3)_4$ (0.1 g). The reaction mixture was placed under 3 MPa of carbon monoxide, heated to 100^{o}C, and stirred overnight. The reaction mixture was cooled to ambient temperature and filtered and concentrated *in vacuo* to yield a red oil which was then purified by flash column chromatography (1:1, ethyl acetate: petroleum ether) to give ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate. (0.6 g, 62%). ^{1}H NMR DMSO δ: 1.35-1.36(t, 3H), 3.94 (s,

3H), 4.37-4.42 (q, 2H), 8.13-8.15 (d, 1H), 8.24(s, 1H), 8.48-8.50(dd, 1H), 8.79(s, 1 H), 9.23(s, 1H); LC-MS: *m/z* = 260 (M+1).



Step A

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine mono tosylate salt (1.0 g, 1.50 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with p-toluenesulfonic acid (1 eq)] in DMSO (2 mL) was added ethyl 6-(1-methyl-1H-pyrazole-4-carbonyl)nicotinate (0.47 g, 1.81 mmol, 1.2 eq.) followed by NaOAc (123 mg, 1.50 mmol) followed by tetraethoxysilane (0.337 mL, 0.31 g, 1.50 mmol). The mixture was heated in oil bath (90 °C) for two days. The mixture was cooled to room temperature and quenched by ice. The mixture was then basified by NaOH (1.0 N, aq. 50 mL). The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a residue which was purified by silica gel chromatography eluted with a gradient of 10-100% acetone in CH_2Cl_2 to give a mixture of isomers 614 mg. The mixture was separated by chiral SFC (Chiral IA column, 40% MeOH/CO_2) to give the two individual isomers.

The fast eluting peak was **9B** (**330** mg, 39%). LCMS *m/z* = 562 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.03(s, 1H), 8.24 (d, 1H), 7.69 (d, 1H), 7.62 (m, 2H), 7.39 (d, 1H), 7.33 (d, 2H), 7.25 (m, 2H), 7.01 (m, 3H), 6.93 (m, 1H), 4.36 (m, 1H), 4.28 (q, 2H), 3.72 (s, 3H), 3.26 (s, 1H), 3.13 (m, 1H), 1.29 (t, 3H).

The slow eluting peak was **9A** (260 mg, 31%). LCMS *m/z* = 562 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.20(s, 1H), 8.31 (m, 1H), 7.69 (m, 2H), 7.63 (d, 1H), 7.49 (d, 1H), 7.38 (m, 2H), 7.34 (s, 1H), 7.30 (s, 1H), 7.12 (m, 1H), 7.08 (m, 2H), 7.03 (m, 1H), 4.38 (q, 2H), 4.12 (m, 1H), 3.80 (s, 3H), 3.17 (m, 2H), 1.37 (t, 3H).

The configuration of the C1 position of β-carboline was determined at the stage of **5A** and **5B**.

Step B

Ethyl ester **9A**(84 mg, 0.15 mmol) was treated with LiOH monohydrate (9.4 mg, 0.22 mmol) in MeOH (1 mL) and water (0.2 mL). The mixture was stirred 3 h at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **5A** (83 mg, 63%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.34 (m, 1H), 8.42 (m, 1H), 7.85 (s, 1H), 7.82 (m, 2H), 7.70 (d, 1H), 7.56 (d, 1H), 7.47 (s, 1H), 7.45 (d, 1H), 7.32 (s, 1H), 7.21 (m, 3H), 7.10 (m, 1H), 4.95 (m, 1H), 3.83 (s, 3H), 3.61 (m, 1H), 3.49 (m, 1H). The configuration at the C1 position of β-carboline was determined by nOe experiment.

Step B'

Ethyl ester **9B** (85 mg, 0.15 mmol) was treated with LiOH monohydrate (9.5 mg, 0.23 mmol) in MeOH (1 mL) and water (0.2 mL). The mixture was stirred overnight at room temperature. The mixture was concentrated, dissolved in DMSO (1 mL), and purified by RP-HPLC (C18 column) eluted with a gradient of 10-100% acetonitrile in water (with 0.1% TFA) to afford **5B** (85 mg, 64%) as a TFA salt. LCMS *m/z* = 534 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 9.17 (m, 1H), 8.42 (m, 1H), 7.77 (m, 4H), 7.53 (d, 1H), 7.50 (s, 1H), 7.40 (s, 1H), 7.34 (d, 1H), 7.24 (m, 2H), 7.13 (m, 1H), 7.05 (m, 1H), 4.77 (m, 1H), 3.84 (s, 3H), 3.48 (m, 1H), 3.34 (m, 1H). The configuration at the C1 position of β-carboline was determined by nOe experiment.

sstr in vitro assays

Cell line Generation:

Human and mouse sstr3 stable cell-lines were generated in regular CHO or the Flp-In CHO cells (Invitrogen, Cat# R758-07), and were used as single cell clone or stable pools for each species in cAMP assays. The stable CHO cell lines for human sstr1, sstr2, sstr3, sstr4 and sstr5 were made by transfecting the CHO cells with the pSC015 vectors carrying corresponding full-length cDNA followed by antibiotic selection. For generation of Flp-In stable lines, the expression plasmids were transfected using Lipofectamine 2000 (Invitrogen Life Technologies) following manufacturer's instructions followed by antibiotic selection.

sstr Binding Assay Method:

Chinese Hamster Ovary (CHO) cells stably-expressing recombinant receptors were grown in growth media containing alpha-MEM (Gibco#12571 or Hyclone SH30265.02), 10 mM HEPES (Gibco#15630-080), 10% fetal bovine serum (Hyclone) and 1% penicillin-streptomycin (Gibco#15140) with G418 (500 µg/mL, Gibco#10131) for human sstr3, 5 or hygromycin (500 µg/mL, Invitrogen#10687-010) for mouse sstr3. Cells were seeded and grown to near-confluence in 10-layer cell factories for three days. The medium was removed and the monolayers were washed twice with 400 mL PBS, then the cells were detached in 600 mL Enzyme-free dissociation buffer (Specialty Media). The cell suspension was centrifuged at 1000 x g for 10 min and the cell pellet was washed with 40 mL/factory of assay buffer (50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 1 mM EGTA, 10 µg/mL leupeptin, 200 µg/mL bacitracin, 0.5 µg/mL aprotinin, 10 µg/mL pepstatin) then homogenized in 40 mL assay buffer/factory with 8-10 strokes using glass-teflon Glas-Col grinder at setting 40. After gently rocking for 15 min at room temperature, they were re-homogenized as per previous step then centrifuged at 1000 x g for 10 min at 4°C. The resulting supernatant was collected and centrifuged in a Sorvall SS-34 rotor for 20 min at 39,000 x g at 4°C. The final pellet was resuspended in 10 mL assay buffer/factory with five passages sequentially through a syringe with 18-25-gauge needles. Protein content was determined using the Bradford Protein Assay (BioRad) with BSA as a standard. Aliquots were stored at -80 °C.

For competition binding studies, cell membranes expressing human sstr1-4 or mouse sstr3 receptors were incubated for 60-90 min at room temperature in 250 µL assay buffer with 5 µL of serially-diluted compounds in DMSO and 40 µL [^{125}I]SS-14 (100 pM final or 50 pM for sstr2 only; PerkinElmer, NEX446050). Human sstr5 binding was set up as above except incubating with [^{125}I]SS-28 (100 pM final; PerkinElmer, NEX447050MC) for 90-120 min Non-specific binding was determined in wells with the addition of 2 µM unlabeled SS-14 or SS-28. The assay was terminated by vacuum filtration over GF/C filters presoaked in 0.2% PEI and washing with ice cold wash buffer containing 50 mM Tris, pH 7.8. After drying, 50 µL Microscint20 was added prior to sealing the plates for counting the radioactivity using a TopCount (Packard). Data were analyzed using Assay Data Analyzer software (Merck and Co.)

sstr cAMP Functional Assay Method:

The cAMP agonist potency determinations were made using a modification of the PerkinElmer Lance 384 kit (Cat#AD0264). Briefly, each cell line stably-expressing sstr2-5 was grown in media as described in the binding assay Methods section. On the day of assay, cells were detached from flasks with Cellstriper (Cellgro, Cat#25-056-CI) and were resuspended in stimulation assay buffer [HBSS with calcium and magnesium (Invitrogen, Cat#14025-076), 0.05% DTPA-purified BSA (7.5%, PerkinElmer #CR84-100), 0.5 µM IBMX (Sigma-Aldrich# I7018), 5 mM HEPES (Invitrogen #15630080)] with Alexa-labeled antibodies (1/100) as per kit instructions. Cells were seeded into OptiPlate-384 plates at 5000/well in 6 µL. Compounds or native peptides (ss-14 or ss-28), serially diluted across ten half-log doses, were added to the cells in 6 µL along with 6µL forskolin ± peptide as follows: for sstr2 and sstr4 human antagonist assay 5µM forskolin and 2nM ss-14 peptide, for sstr5 human antagonist assay 5µM forskolin and 5nM ss-28, for sstr3 human antagonist assay 3.5µM forskolin and 0.3nM ss-14, for sstr3 mouse antagonist assay 3.5µM forskolin and 0.4nM ss-14. For the agonist assay using human sstr2, 4, and 5, 6µl of challenge buffer with 5µM forskolin were added to the 12 µl of cells and compound or peptide, while human sstr3 was run with 6 µl challenge buffer with 3.5µM forskolin. Plates were incubated for 45 minutes at 37°C and 5% CO_2 followed by addition of 12 µL detection mix for 1 h incubation at room temperature Time-resolved fluorescence was measured using Envision plate reader (PerkinElmer Life and Analytical Sciences, Inc. Waltham, MA) at 665 nm and 615 nm. Resulting cAMP levels were calculated by reading the relative fluorescence units off a cAMP standard curve then agonist

EC_{50}s were subsequently determined from 4-parameter fits by plotting those cAMP levels versus agonist concentration using Assay Data Analyzer software (Merck and Co.).

Protocol for mouse oGTT

C57BL/6 mice (Taconic), 12 weeks old were fasted 6 h. Blood glucose was measured at -60 min with Glucometer prior dosing with compound or vehicle. Mice were oral challenge with 5 g/kg dextrose at time 0, Tail blood glucose was measured at time 0 and 20, 40, 60, and 90 min post dextrose. Mice challenge with water as a background. Calculation: % inhibition is determined as difference from vehicle group AUC with water group.