The Discovery of MK-4256, a Potent SSTR3 Antagonist as a Potential Treatment of Type-2 Diabetes

Supporting Information

Shuwen He*[,†], Zhixiong Ye,[†] Quang Truong,[†] Shrenik Shah,[†] Wu Du,[†] Liangqin Guo,[†] Peter H. Dobbelaar,[†] Zhong Lai,[†] Jian Liu,[†] Tianying Jian,[†] Hongbo Qi,[†] Raman K. Bakshi,[†] Qingmei Hong,[†] James Dellureficio,[†] Alexander Pasternak,[†] Zhe Feng,[†] Reynalda deJesus,[†] Lihu Yang,[†] Mikhail Reibarkh,[†] Scott A. Bradley,[†] Mark A. Holmes,[†] Richard G. Ball,[†] Rebecca T. Ruck,[‡] Mark A. Huffman,[‡] Frederick Wong,[‡] Koppara Samuel,[#] Vijay B. Reddy,[#] Stan Mitelman,[#] Sharon X. Tong,[#] Gary G. Chicchi,[§] Kwei-Lan Tsao,[§] Dorina Trusca,[§] Margaret Wu,[§] Qing Shao,[§] Maria E. Trujillo,[§] George J. Eiermann,[§] Cai Li,[§] Bei Zhang,[§] Andrew D. Howard,[§] Yun-Ping Zhou,[§] Ravi P. Nargund,[†] William K. Hagmann[†]

Departments of [†]Medicinal Chemistry, [‡]Process Research, [#]Drug Metabolism and Pharmacokinetics, and [§]Diabetes Research, Merck Research Laboratories, 126 East Lincoln Avenue, Rahway, NJ 07065, United States

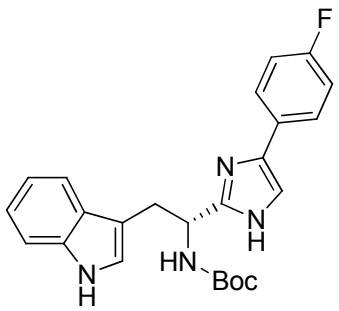
shuwen_he@merck.com

Contents of Supporting Information

General

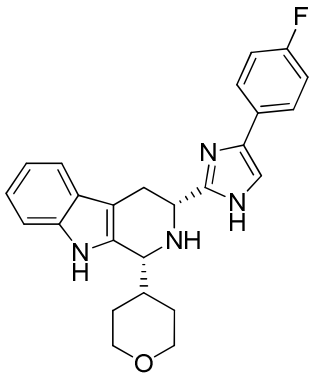
Normal phase column chromatography was carried out in the indicated solvent system (in the percentage of volume) using pre-packed silica gel cartridges for use on the Isco CombiFlash® or Biotage SP1®. Analytical thin layer chromatography (TLC) visualization was performed using 254 nm wavelength ultraviolet 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.

(R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate



A solution of Boc-D-tryptophan (25 g, 82 mmol) and Cs_2CO_3 (13.54 g, 41.6 mmol) in EtOH (180 mL) was stirred for 30 min at RT and evaporated under the reduced pressure to afforad a solid. To this solid in DMF (300 mL) was added 2-bromo-1-(4-fluorophenyl)ethanone (17.83 g, 82 mmol). The mixture was stirred at RT overnight. The reaction mixture was poured into ice-water and extracted with EtOAc(3x). The combined organic layers were washed with water (2x), brine, dried (Na_2SO_4) and concentrated to afford a soapy oil, which was mixed with ammonium acetate (127 g, 1643 mmol) and p-xylene (1L). The mixture was heated under reflux for 3h while attached to a Dean-Stark trap. The reaction was cooled and diluted with EtOAc/water. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated to give a solid, which was triturated to afford (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate as a pale yellow solid (25.7 g, 74%). LCMS *m/z*=421.2 $(M+H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.84 (s, 1H), 10.74 (s, 1H), 7.77 (m, 2H), 7.54 (d, 1H), 7.45 (s, 1H), 7.29 (d, 1H), 7.14 (t, 2H), 7.10 (d, 1H), 7.03 (t, 1H), 6.99 (s, 1H), 6.95 (t, 1H), 4.87 (m, 1H), 3.34 (m, 1H), 3.13 (m, 1H), 1.32 (s, 9H).

(3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(tetrahydro-2H-pyran-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole(2)

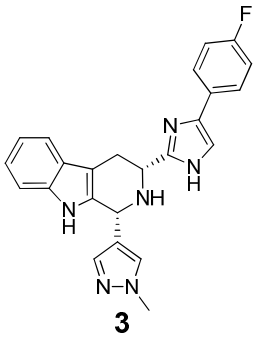


2

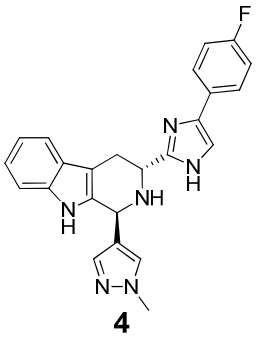
To a stirred solution of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate (100 mg, 0.24 mmol) in DCM (2 mL) was added TFA (2 mL) at RT. The mixture was stirred at RT for 30 min, and then the volatile was removed

in vacuo. To the resulting residue was added DCM (2 mL), followed by addition of tetrahydro-2H-pyran-4-carbaldehyde (54.3 mg, 0.48 mmol) at RT. The mixture was stirred at RT overnight. The reaction was concentated to give a residue which was purified by prep TLC eluted with 90% DCM: 9% MeOH: 1% NH_4OH to afford (3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1-(tetrahydro-2H-pyran-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**2**) as a white solid (65 mg, 65%). LCMS *m/z*=417.2 $(M+H)^+$. ^1H NMR (CD_3OD, 500MHz) δ 7.72 (m, 2H), 7.42 (d, 1H), 7.36 (s, 1H), 7.31 (d, 1H), 7.1 (m, 3H), 6.97 (m, 1H), 4.32 (s, 1H), 4.23 (dd, 1H), 4.04 (d, 1H), 3.94 (d, 1H), 3.52 (t, 1H), 3.43 (t, 1H), 3.10 (d, 1H), 2.94 (t, 1H), 2.34 (m, 1H), 1.88 (m, 1H), 1.74 (m, 1H), 1.66 (d, 1H), 1.20 (d, 1H).

(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]indole (3) and (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]indole (4)



3

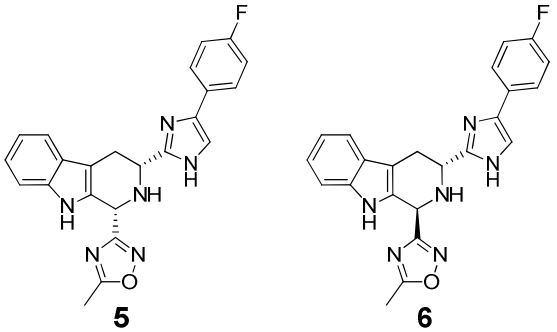


4

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (200 mg, 0.56 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] in MeOH (5 mL) was added 1-methyl-1H-pyrazole-4-carboxaldehyde (74 mg, 0.67 mmol) followed by a few drops of TFA. The mixture was stirred at RT overnight and then neutralized with 7 N ammonia in methanol (3 mL). The solvent was then removed under reduced pressure. The residue was purified by prep TLC eluated with 10% MeOH/DCM+1% NH_4OH to afford individual diastereomers. The structures were

determined by comparing NOE of C1 and C3 protons. Less polar isomer (69 mg, 30%) was (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]indole (**3**). LCMS *m/z*=413.1 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 7.70 (m, 2H), 7.58 (s, 1H), 7.52 (s, 1H), 7.45 (d, 1H), 7.33 (s, 1H), 7.27 (d, 1H), 7.07 (m, 3H), 7.00 (m, 1H), 5.38 (s, 1H), 4.40 (dd, 1H), 3.85 (s, 3H), 3.17 (m, 2H). More polar isomer (40 mg, 17%) was (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]indole (**4**). LCMS *m/z*=413.1 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 7.68 (m, 2H), 7.48 (d, 1H), 7.41 (s, 1H), 7.39 (s, 1H), 7.29 (s, 1H), 7.29 (d, 1H), 7.07 (m, 3H), 7.01 (m, 1H), 5.36 (s, 1H), 4.38 (dd, 1H), 3.80 (s, 3H), 3.18 (m, 2H).

3-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2,3,4,9-tetrahydro-1H-pyrido [3,4-b]indol-1-yl)-5-methyl-1,2,4-oxadiazole (5) and 3-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)-5-methyl-1,2,4-oxadiazole (6)

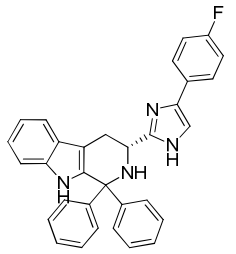


5 **6**

To a solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (200 mg, 0.56 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] in MeOH (5 mL) was added 5-methyl-1,2,4-oxadiazole-3-carboxaldehyde (75 mg, 0.67 mmol) followed by a few drops of TFA. The mixture was stirred at RT overnight and then neutralized with 7 N ammonia in methanol (3 mL). The solvent was then removed under reduced pressure. The residue was purified by prep TLC eluted with 10% MeOH/DCM+1% NH_4OH to afford individual diastereomers. The structures were

determined by comparing NOE of C1 and C3 hydrogens. Less polar isomer (71 mg, 31%) was 3-((1S,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2,3,4,9-tetrahydro-1H-pyrido [3,4-b]indol-1-yl)-5-methyl-1,2,4-oxadiazole (**5**). LCMS *m/z*=415.1 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 7.72 (m, 2H), 7.48 (d, 1H), 7.36 (s, 1H), 7.31 (d, 1H), 7.10 (m, 3H), 7.01 (t, 1H), 5.69 (s, 1H), 4.48 (dd, 1H), 3.23 (ddd, 1H), 3.13 (m, 1H), 2.61 (s, 3H). More polar isomer (116 mg, 50%) was 3-((1R,3R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)-5-methyl-1,2,4-oxadiazole (**6**). LCMS *m/z*=415.1 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 7.72 (m, 2H), 7.50 (d, 1H), 7.33 (s, 1H), 7.31 (d, 1H), 7.10 (m, 3H), 7.01 (t, 1H), 5.53 (s, 1H), 4.68 (dd, 1H), 3.25 (dd, 1H), 3.11 (ddd, 1H), 2.58 (s, 3H).

(R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1,1-diphenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (7)

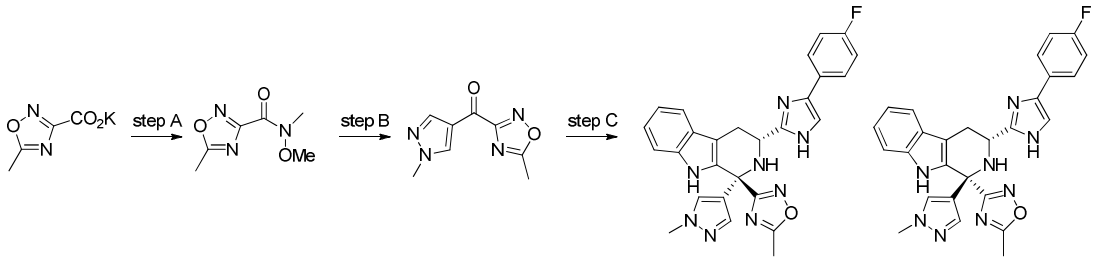


7

(R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (50 mg, 0.14 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] was dissolved in minimum amount of pyridine (several drops), then benzophenone (38.3 mg, 0.210 mmol)was added. The mixture was heated at oil bath overnight at 100ºC. To this mixture was added tetraisopropoxytitanium (80 mg, 0.280 mmol). The reaction was heated at 70ºC overnight followed by 150ºC overnight. The reaction was cooled to RT, diluted with water, and extracted with EtOAc. The combined organic was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel MPLC eluted with EtOAc-hexanes (10% to 100%) to afford (R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1,1-diphenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**7**) as a solid (13 mg, 19%).

LCMS *m/z*=485.1 $(M+H)^+$. ^1H NMR (500 MHz, CD_3OD, TFA salt): δ 7.74 (m, 3H), 7.52 (d, 1H), 7.46 (d, 2H), 7.40-7.22 (m, 11H), 7.11 (t, 1H), 7.04 (t, 1H), 4.51 (dd, 1H), 3.38 (dd, 1H), 3.27 (m, 1H).

3-((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)-5-methyl-1,2,4-oxadiazole (8, MK-4256) and 3-((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)-5-methyl-1,2,4-oxadiazole (9)



8 (MK-4256) **9**

Step A

To a supension of potassium 5-methyl-1,2,4-oxadiazole-3-carboxylate (5 g, 30.1 mmol) in DCM (75 ml) containing a drop of DMF was added oxalyl chloride (4.3 mL, 6.24 g, 49.1 mmol) over a few minutes at 0ºC. The reaction was stirred at RT for 30 min. The cloudy mixture was concentrated. The residue was diluted with DCM (ca. 50 mL) and reconcentrated to give the crude acid chloride, which was diluted with DCM (25 mL) and added over a few minutes to a vigorously stirred mixture of N,O-dimethylhydroxylamine hydrochloride (4.6 g, 47.2 mmol), K_2CO_3 (19 g, 137 mmol), water (50 mL) and DCM (50 mL) at 0ºC. The stirring was continued at 0ºC for 30 min. The organic layer was separated and dried with Na_2SO_4 and concentrated to give a colorless liquid, which was purified by silica gel MPLC eluted with straight ether to afford N-methoxy-N,5-dimethyl-1,2,4-oxadiazole-3-carboxamide (4.5 g, 87%). LCMS *m/z*=172.0 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): 3.78 (s, 3H), 3.37 (s, 3H), 2.65 (s, 3H).

Step B

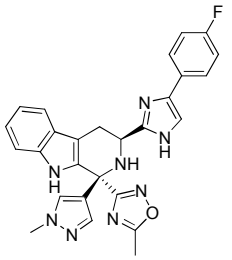
To a solution of 1-methyl-4-iodo-1H-pyrazole (3 g, 14.42 mmol) in THF (40 mL) was added isopropylmagnesium chloride 2.0M in THF (8.00 ml, 16.01 mmol) at 0ºC.The mixture was stirred at 0ºC for 1h then cooled to -78ºC and N-methoxy-N,5-dimethyl-1,2,4-oxadiazole-3-carboxamide (3.21 g, 18.75 mmol) was added. The mixture was slowly warmed to RT in 1.5h. The reaction was cooled to -78ºC and quenched by dropwise addition of NH_4Cl(sat,. aq) and warmed to RT, and stored in a refrigerator overnight. The reaction was diluted cold 1N aqueous HCl, extracted with EtOAc 4 times. The combined organic layers were washed with brine and dried (Na_2SO_4). Silica gel chromatography eluted with a gradient of 10% EtOAc in hexanes to straight EtOAc afforded (5-methyl-1,2,4-oxadiazol-3-yl)(1-methyl-1H-pyrazol-4-yl)methanone (0.5 g, 18%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41(s, 1H), 8.29 (s, 1H), 3.99(s, 3H), 2.71 (s, 3H).

Step C

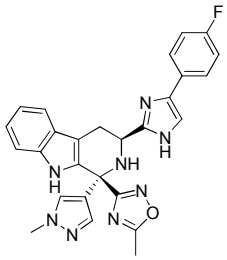
A mixture of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (370 mg, 1.04 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] in pyridine (4 mL) was treated with (5-methyl-1,2,4-oxadiazol-3-yl)(1-methyl-1H-pyrazol-4-yl)methanone (219 mg, 1.141 mmol). The reaction mixture was heated under N_2 (oil bath 70ºC) for 2 days followed by being heated (oil bath 85ºC) for another 3 days. The reaction mixture was concentrated and azeotroped with toluene. The residue was purified with preparative TLC eluted with 10% MeOH in DCM to give a mixture of isomers, which were separated by Chiral HPLC (Chiral OD column eluted with 20% IPA in heptane) to afford individual diastereomers. With analytical Chiral OD column eluted with 20% IPA in heptane, the retention times for the two isomers were 18.13 min and 24.62 min, respectively. The structures were determined by comparing NOE of C3 proton and the aromatic protons on the N-methylpyrazole ring. The fast eluting diastereomer (52 mg, 10%) was 3-((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)-5-methyl-1,2,4-oxadiazole (**8, MK-4256**).$[\alpha]_D$ = +24.2, c=10 mg/mL in MeOH. LC-MS: m/z 495.3 $(M + H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 7.74 (m, 2H), 7.65 (s, 1H), 7.52 (m, 2H), 7.37 (m, 2H), 7.13

(m, 3H), 7.04 (t, 1H), 4.47 (dd, 1H), 3.87 (s, 3H), 3.24 (dd, 1H), 3.16 (dd, 1H), 2.63 (s, 3H). ^{13}C NMR (150.8 MHz, CD_3OD) δ 178.0, 173.0, 162.0, 150.2, 139.7, 138.1, 137.1, 132.4, 130.6, 126.5, 126.4, 124.4, 122.0, 119.0, 118.2, 115.2, 112.4, 111.3, 109.1, 55.5, 50.2, 37.8, 27.9, 11.1. (Note: two carbons have coinciding chemical shift of 130.6 ppm). Accurate Mass $C_{27}H_{23}FN_8O$ [M+H] measured 495.2068, calculated 495.2052. The slow eluting diastereomer (40 mg, 8%) was 3-((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)-5-methyl-1,2,4-oxadiazole (**9**). LC-MS: m/z 495.3 $(M + H)^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 7.73 (m, 2H), 7.54 (d, 1H), 7.48 (s, 1H), 7.43 (s, 1H), 7.40 (d, 1H), 7.36 (brs, 1H), 7.13 (m, 3H), 7.06 (t, 1H), 4.40 (dd, 1H), 3.84 (s, 3H), 3.26 (dd, 1H), 3.16 (dd, 1H), 2.63 (s, 3H).

3-((1S,3S)-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)-5-methyl-1,2,4-oxadiazole (10) and 3-((1R,3S)-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)-5-methyl-1,2,4-oxadiazole (11)



10

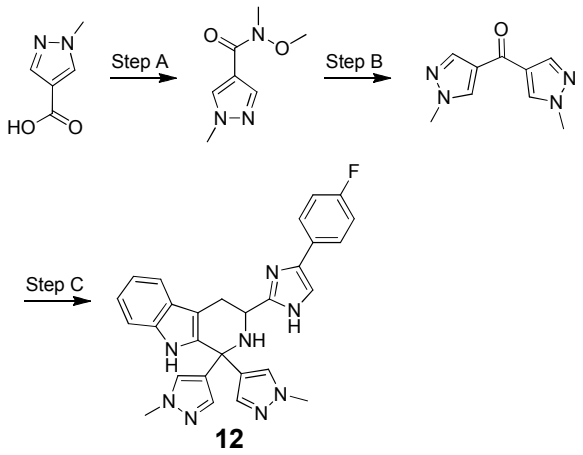


11

Prepared according to the procedures described for **8(MK-4256)** and **9**, starting from Boc-L-tryptophan instead of Boc-D-tryptophan. Compounds **8** and **10** are a pair of enantiomers, while compounds **9** and **11** are a pair of enantimers. 3-((1S,3S)-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)-5-methyl-1,2,4-oxadiazole (**10**). LCMS *m/z*=495.2 $(M+H)^+$. ^{1}H NMR of **10** is identical to **8 (MK-4256)**. 3-((1R,3S)-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)-5-methyl-1,2,4-oxadiazole (**11**). LCMS *m/z*=495.2 $(M+H)^+$. ^{1}H NMR of **11** is identical to **9**.

(R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1,1-bis(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (12)



12

Step A

A solution of 1-methyl-1H-pyrazole-4-carboxylic acid (10 g, 79 mmol) in CH_2Cl_2 (150 ml) and DMF (0.307 ml, 3.96 mmol) was cooled to 0ºC. Oxalyl chloride(8.33 ml, 95 mmol) was added dropwise over 10 min. The reaction mixture was warmed to RT and stirred for 1h. This reaction solution was added into a cool solution N,O-dimethylhydroxylamine hydrochloride (12.38 g, 127 mmol) and K_2CO_3 (49.3 g, 357 mmol) in 40mL water. The mixture was stirred at RT overnight. The organic layer was separated, washed with brine, dried (Na_2SO_4), filtered, and concentrated to give the crude product. MPLC on silica gel eluted with 5% to 100% acetone in hexane afforded N-methoxy-N,1-dimethyl-1H-pyrazole-4-carboxamide (11.6 g, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.91 (s, 1H), 3.92 (s, 3H), 3.71 (s, 3H), 3.31 (s, 3H).

Step B

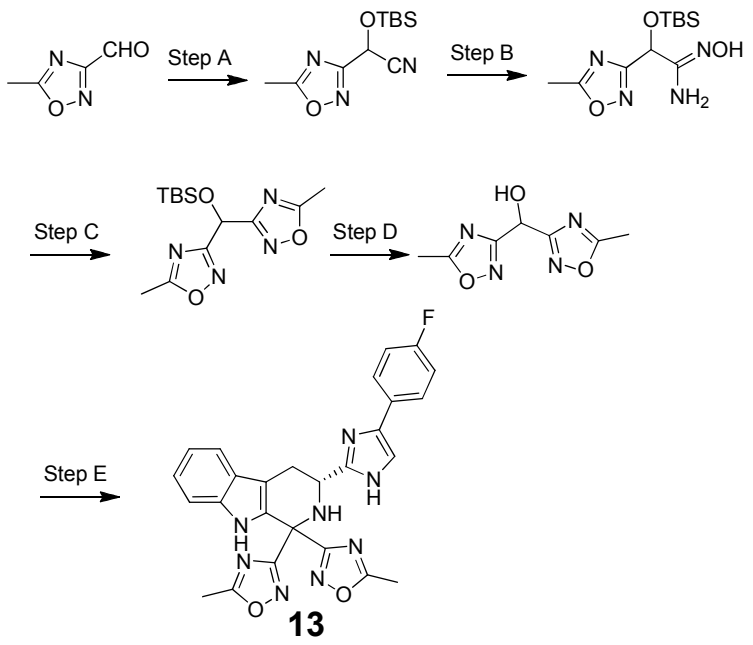
To a solution of 1-methyl-4-iodo-1*H*-pyrazole (4 g, 19.23 mmol) in THF (50 mL) was added isopropylmagnesium chloride (2.0M in THF, 10.58 ml, 21.15 mmol) at 0ºC. The

mixture was stirred at 0ºC for 1h then cooled to -78ºC and N-methoxy-N,1-dimethyl-1H-pyrazole-4-carboxamide (2.147 g, 12.69 mmol) was added. The mixture was slowly warmed to RT in 2 h. MPLC on silica gel eluted with 10% to 100% acetone in hexane to afford bis(1-methyl-1H-pyrazol-4-yl)methanone (561 mg, 15%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 2H), 7.86 (s, 2H), 3.84 (s, 6H).

Step C

A solution of (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (200 mg, 0.56 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] and tetraethoxysilane (250 µl, 1.121 mmol) in pyridine (2 mL) was treated by bis(1-methyl-1H-pyrazol-4-yl)methanone (117 mg, 0.617 mmol). The mixture was heated under N_2 (oil bath temperature 100ºC) for 22 h. Additional tetraethoxysilane (500 µl, 2.242 mmol) was added. The reaction was heated overnight (oil bath temperature 120ºC). The reaction was cooled to RT, treated with EtOAc and $NaHCO_3$ (sat. aq.). The organic layer was separated, washed with brine, and dried (Na_2SO_4). MPLC on silica gel eluted with 10% to 100% acetone in hexane afforded the crude product. The crude product was purified by prep TLC eluted with 6% MeOH in dichloromethane to afford (R)-3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-1,1-bis(1-methyl-1H-pyrazol-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**12**) (35 mg, 13%). LCMS *m/z*=493.3 $(M+H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 7.67 (t, 2H), 7.39-7.49 (m, 5H), 7.29-7.33 (m, 2H), 7.04-7.11 (m, 3H), 7.00 (t, 1H), 4.22 (dd, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.11-3.16(m, 2H).

(R)-3,3'-(3-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1,1-diyl)bis(5-methyl-1,2,4-oxadiazole) (13)



Step A

To a solution of 5-methyl-1,2,4-oxadiazole-3-carbaldehyde (6 g, 53.5 mmol) and tert-butyldimethylsilyl cyanide (9.08 g, 64.2 mmol) in THF (60 mL) was added 2,5,8,9-tetraaza-1 phosphabicyclo[3.3.3] undecane 2,8,9-tris(1-methylethyl) (1.744 mL, 5.35 mmol) at 0ºC. The reaction was stirred at 0ºC for 2h. The reaction was diluted with EtOAc then treated ice and 1N HCl (aq). The organic was separated, washed $NaHCO_3$ (sat. aq), brine, dried (Na_2SO_4), and concentrated. The residue was purified by MPLC on silica gel column eluted with a gradient of 5% to 50% EtOAc in hexanes to afford 2-((tert-butyldimethylsilyl)oxy)-2-(5-methyl-1,2,4-oxadiazol-3-yl)acetonitrile as an oil (8.4g, 62%). LCMS *m/z*=254.2 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 5.65 (s, 1H), 2.62 (s, 3H), 0.90 (s, 9H), 0.22 (s, 3H), 0.17 (s, 3H).

Step B

A solution of 2-((tert-butyldimethylsilyl)oxy)-2-(5-methyl-1,2,4-oxadiazol-3-yl) acetonitrile (4 g, 15.79 mmol) and hydroxylamine (2.90 ml, 47.4 mmol) in EtOH (50 ml) was heated at 50ºC for 7h. The reaction was cooled to RT, concentrated in vacuo to remove volatiles. The residue was diluted with water and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to

give crude 2-((tert-butyldimethylsilyl)oxy)-N'-hydroxy-2-(5-methyl-1,2,4-oxadiazol-3-yl)acetimidamide, which was used in the next step without purification. LCMS *m/z*=287.3 $(M+H)^+$.

Step C

To a solution of 2-((tert-butyldimethylsilyl)oxy)-N'-hydroxy-2-(5-methyl-1,2,4-oxadiazol-3-yl)acetimidamide (2.11 g, 7.37 mmol) in toluene (10 ml) was added acetic anhydride (0.834 ml, 8.84 mmol) at RT. After stirring at RT for 1.5h, the reaction was heated at 110ºC (bath temperature) for 45min., and then at 140ºC (bath temperature) at reflux for 3h. The mixture was cooled to RT and treated with sodium bicarbonate (1.485 g, 17.68 mmol) followed by some silica gel. The mixture was concentrated in vacuo and the residue was loaded on a silica gel column, which was eluted with a gradient of 5% EtOAc to 50% EtOAc in hexanes to 3,3'-(((tert-butyldimethylsilyl)oxy)methylene)bis(5-methyl-1,2,4-oxadiazole) (970 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.10 (s, 1H), 2.59 (s, 6H), 0.90 (s, 9H), 0.14 (s, 6H).

Step D

To a solution of 3,3'-(((tert-butyldimethylsilyl)oxy)methylene)bis(5-methyl-1,2,4-oxadiazole) (893 mg, 2.88 mmol) in THF (20 mL) was added TBAF (1. M in THF, 4.32 mL, 4.32 mmol) at 0ºC. The mixture was stirred at 0ºC for 1h. The reaction was quenched by NH_4Cl (sat. aq.) was concentrated in vacuo to remove THF, diluted with water (10mL), and extracted with EtOAc (6x20 mL). The combined EtOAc extractions were dried (Na_2SO4), filtered, and concentrated to give a residue. MPLC on silica gel column eluted with a gradient of 5% acetone to 100% acetone in hexanes afford bis(5-methyl-1,2,4-oxadiazol-3-yl)methanol (348 mg, 62%). ^{1}H NMR (500 MHz $CDCl_3$) δ 6.00 (s, 1H), 2.47 (s, 6H).

Step E

To a solution of bis(5-methyl-1,2,4-oxadiazol-3-yl)methanol (327 mg, 1.667 mmol) in DCM (18 mL) was added manganese dioxide (2898 mg, 33.3 mmol). The mixture was stirred at RT for 30min. TLC (1:1 Hex:acetone) indicated that starting material alcohol

was consumed. The reaction was diluted with MeOH (18 mL) and filtered. The filtrate was concentrated and the residue was treated with (R)-1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)-ethanamine HCl salt (595 mg, 1.67 mmol) [prepared by treatment of (R)-tert-butyl (1-(4-(4-fluorophenyl)-1H-imidazol-2-yl)-2-(1H-indol-3-yl)ethyl) carbamate with HCl (4 M in dioxane)] followed by pyridine (5 mL). The mixture was stirred at RT for 4 days. The reaction was concentrated and then azeotroped with toluene one time. The residue was purifed by preparative TLC eluted with 7.5% MeOH in dichloromethane to afford the product (69 mg, 8.3%). LCMS *m/z*=497.3 $(M+H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (dd, 2H), 7.47 (d, 1H), 7.37 (d, 1H), 7.26 (s, 1H), 7.10 (t, 1H), 7.03 (t, 2H), 6.99 (t, 1H), 4.53 (dd, 1H), 3.23 (dd, 1H), 3.13 (dd, 1H), 2.47 (s, 3H), 2.45 (s, 3H).

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.).

Protocols for ipGTT and oGTT

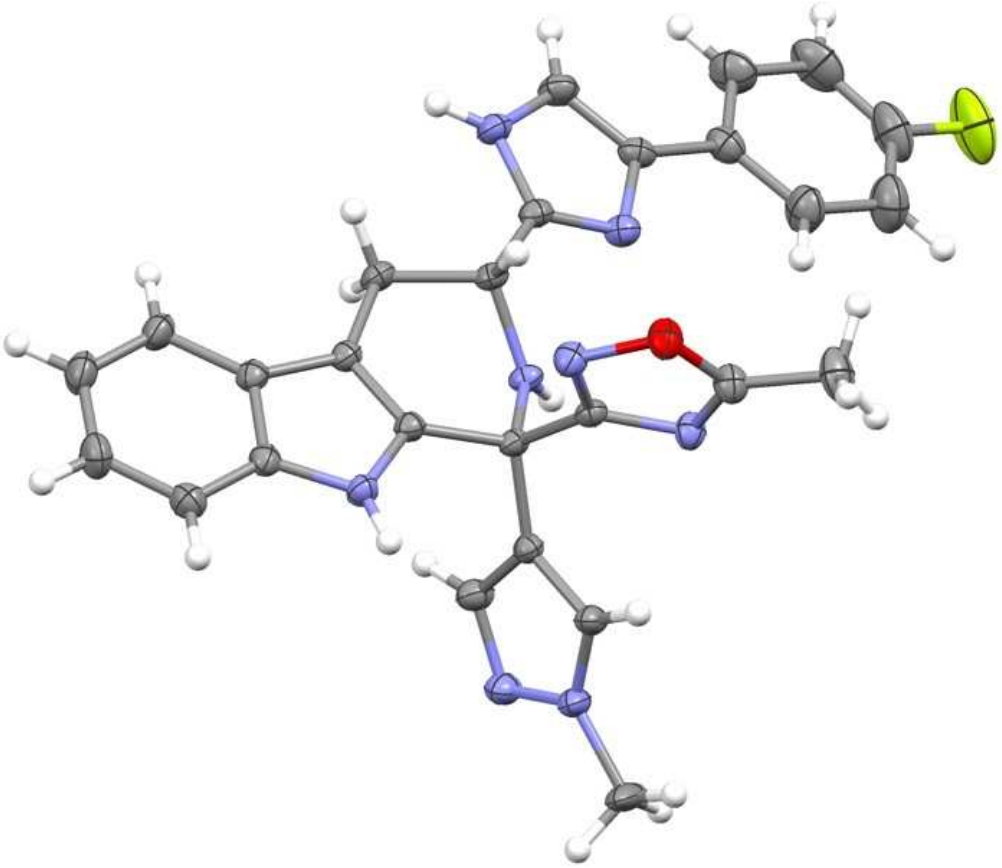
Mouse oGTT procedure

C57BL/6 mice (Taconic), 12 weeks old were fasted 6 hrs. Blood glucose was measured at -60 min with Glucometer prior oral 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.

Mouse ipGTT

Procedure was same as oGTT except that mice received ip injection with 2 g/kg dextrose.

X-ray crystallography data for **8** (**MK-4256**)



Compound $C_{27}H_{23}F\,N_8O$, C_7H_8, (a toluene solvate), *Mr* = 586.662, monoclinic, *P*21, *a* = 12.6771(11), *b* = 7.9412(7), *c* = 15.1961(13) Å, *β*=94.555(2)°, *V* = 1525.0(2) Å3, *Z* = 2, *Dx* = 1.278 gcm-3, monochromatized radiation λ(Mo) = 0.71073 Å, μ = 0.09 mm-1, *F*(000) = 616, *T* = 100 K. Data were collected on a Bruker CCD diffractometer to a θ limit of 28.32° which yielded 21050 reflections. There are 7549 unique reflections with 6906 observed at the 2σ level. The structure was solved by direct methods (SHELXS-97, Sheldrick, G.M. *Acta Crystallogr.*, 2008, A64, 112-122) and refined using full-matrix least-squares on *F2* (SHELXL-97, Sheldrick, G.M. *Acta Crystallogr.*, 2008, A64, 112-122). The compound crystallized as a toluene solvate in which the toluene is disordered over two positions in an approximately equal ratio. The final model was refined using 433 parameters and all 7549 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are: *R* = 0.040 (based on 6906 reflections with $I > 2\sigma(I)$), *wR* = 0.103, *S* = 1.03 with (Δ/σ)max = 0.01. The maximum

peak height in a final difference Fourier map is 0.431 eÅ-3 and this peak is without chemical significance. CCDC nnnnnn 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.