Supporting Information

Discovery of indazoles as potent, orally-active dual neurokinin 1 receptor antagonists and serotonin transporter inhibitors for the treatment of depression

Andrew P. Degnan, George O. Tora, Hong Huang, David A. Conlon, Carl D. Davis, Umesh M. Hanumegowda, Xiaoping Hou, Yi Hsiao, Joanna Hu, Rudolph Krause, Yu-Wen Li, Amy E. Newton, Rick L. Pieschl, Joseph Raybon, Thorsten Rosner, Jung-Hui Sun, Matthew T. Taber, Sarah J. Taylor, Michael K. Wong, Huiping Zhang, Nicholas J. Lodge, Joanne J. Bronson, John E. Macor, and Kevin W. Gillman

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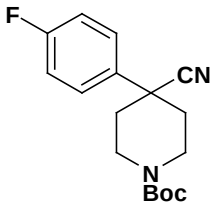
Chemistry Experimental

General: All commercially available reagents and solvents were used without further purification unless otherwise stated. All reactions were carried out under an inert atmosphere of dry nitrogen in oven or flame-dried glassware unless otherwise stated. Flash column chromatography was performed using 40-60 μm Silica Gel 60 (EMD Chemicals, Inc.) as the stationary phase. ^{1}H-NMR spectra were recorded on a Bruker DRX-500 instrument operationg at 500 MHz with tetramethylsilane or residual protiated solvent used as a reference. ^{13}C-NMR were recorded on a Bruker DRX-500 instrument operating at 125 MHz with residual ^{12}C solvent used as a reference. Low resolution mass spectra were recorded using a Waters Micromass ZQ with electrospray ionization. High resolution mass spectra were recorded using a Waters Micromass LCT time of flight mass spectrometer with electrospray ionization. Full experimental procedures for compounds **1**-**3** can be found in WO2007121389. Full experimental procedures for compounds **4**-**8** as well as alternate procedures for the preparation of **9** can be found in WO2009009411. All final compounds (**1**-**9**) were assayed for purity by long gradient HPLC using UV detection at 220 nm and 254 nm. All were determined to have purity in excess of 95%.

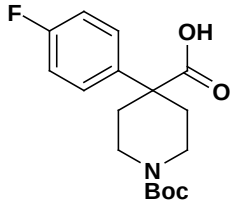
tert-Butyl 4-cyano-4-(4-fluorophenyl)piperidine-1-carboxylate



2-(4-Fluorophenyl)acetonitrile (1.35 g, 10.0 mmol) and *tert*-butyl bis(2-chloroethyl)carbamate (2.42 g, 10.0 mmol) were combined in dimethylformamide (30 mL) and cooled to 0˚C. The reaction was treated with sodium hydride (760 mg, 30.0 mmol) in several portions. The ice bath was removed and the reaction heated at 60˚C for 24 h. After cooling to room temperature, the mixture was poured into ice water and

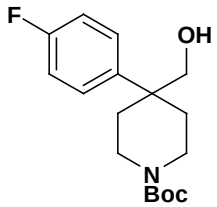
extracted with ethyl acetate (2X). The organic layers were pooled together, washed with brine (2X), dried over sodium sulfate, and concentrated. Column chromatography on silica gel (15% ethyl acetate/hexanes) gave 1.65 g (54%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.39-7.44 (m, 2H), 7.04-7.10 (m, 2H), 4.23-4.27 (m, 2H), 3.12-3.21 (m, 2H), 2.03-2.07 (m, 2H), 1.82-1.92 (m, 2H), 1.45 (s, 9H). Mass spec.: 327.11 $(MNa)^+$.

1-*(tert*-Butoxycarbonyl)-4-(4-fluorophenyl)piperidine-4-carboxylic acid



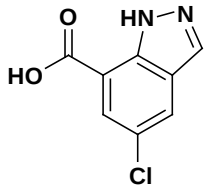
tert-Butyl 4-cyano-4-(4-fluorophenyl)piperidine-1-carboxylate (0.35 g, 1.15 mmol) was dissolved in ethanol (3.0 mL) and sodium hydroxide (50% in water, 3.0 mL). The reaction mixture was then heated at reflux for 6 h. After cooling to room temperature, the mixture was concentrated *in vacuo* to remove most of the ethanol. The residue was poured into water/ethyl acetate. The product was extracted with water (2X) and the organics discarded. The aqueous layers were pooled together and acidified to pH 2.0 with 1 N hydrochloric acid. The resulting precipitate was filtered and dried *in vacuo* for several hours to afford 0.24 g (64%) as a white powder. ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.46-7.48 (m, 2H), 7.07-7.11 (m, 2H), 3.94-3.98 (m, 2H), 3.10 (m, 2H), 2.50-2.53 (m, 2H), 1.78-1.83 (m, 2H), 1.48 (s, 9H). Mass spec.: 346.20 $(MNa)^+$.

tert-Butyl 4-(4-fluorophenyl)-4-(hydroxymethyl)piperidine-1-carboxylate (**12**)



1-(*tert*-Butoxycarbonyl)-4-(4-fluorophenyl)piperidine-4-carboxylic acid (9.5 g, 29.3 mmol) was suspended in tetrahydrofuran (60 mL) and cooled to 0˚C. To this solution was added borane tetrahydrofuran complex (1 M in tetrahydrofuran, 59 mL, 59 mmol) cautiously over 15 min. The reaction mixture was allowed to warm to room temperature overnight and then heated at reflux for 24 h. The mixture was cooled to 0˚C, treated with excess methanol, diluted with ethyl acetate, washed with 1 N sodium hydroxide (2X), then brine (2X), dried over sodium sulfate, and concentrated. Column chromatography on silica gel (40% ethyl acetate/hexanes) gave 6.6 g (72%) as a white powder. ^{1}H-NMR ($CDCl_3$, 500 MHz) 7.24-7.29 (m, 2H), 7.00-7.05 (m, 2H), 3.66-3.71 (m, 2H), 3.49 (s, 2H), 2.96-3.05 (m, 2H), 2.06-2.10 (m, 2H), 1.69-1.77 (m, 2H), 1.40 (s, 9H). Mass spec.: 310.21 $(MH)^+$.

5-Chloro-1H-indazole-7-carboxylic acid (**11**)

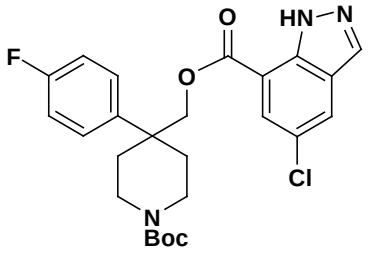


Step 1. To 2-amino-5-chloro-3-methylbenzoic acid (20 g, 108 mmol) in a 2-L 3-neck round-bottomed flask was added 8 M HCl (135 mL, 1078 mmol) (conc. HCl 96 mL and water 32 mL) and stirred to become a fine slurry. The reaction mixture was cooled to -11 °C under acetone-ice water bath (internal temperature = -4 °C), followed by slow addition of a solution of sodium nitrite (8.18 g, 119 mmol) in water (50 mL). The reaction mixture was stirred at -4 °C for 30 minutes to become a homogeneous light brown solution. The reaction was monitored by LC until starting material was consumed. Sodium acetate trihydrate (205 g, 1509 mmol) was added slowly to adjust to pH 6, and the internal temperature was kept below 0 °C. At 0 °C, 2-methyl-2-propanethiol (12.27 mL, 109 mmol) was added through an addition funnel slowly. The mixture was gradually warmed to room temperature and stirred for 2 h until disappearance of the diazonium ion by LC. The resulting precipitates were collected, then thoroughly washed with water (4 x 150 mL), and dried *in vacuo* overnight to afford 2-(*tert*-

butylthiodiazenyl)-5-chloro-3-methylbenzoic acid (30.6 g, 107 mmol, 99 % yield) as a light yellow solid. It was used directly in the next step.

Step 2. To a stirred solution of potassium *tert*-butoxide (47.9 g, 427 mmol) in DMSO (200 mL) at -5 °C was added a solution of 2-(*tert*-butylthiodiazenyl)-5-chloro-3-methylbenzoic acid (30.6 g, 107 mmol) in DMSO (80 mL) slowly through an addition funnel. Internal temperature was maintained below 15 °C. After addition, the reaction mixture was gradually warmed to room temperature and stirred until the reaction was complete (~ 2 h). The reaction mixture was cooled to 0 °C, slowly diluted with ice water, neutralized with 4 N HCl to pH 1~2. Precipitates were collected, thoroughly washed with water (4 x 150 mL) and dried *in vacuo* for 3 days to afford 5-chloro-1H-indazole-7-carboxylic acid (20.6 g, 98% yield) as an off white solid. ^{1}H-NMR (d_6-DMSO, 500 MHz) δ 13.29 (bs, 1H), 8.18 (s, 1H), 8.14 (d, *J* = 1.8 Hz, 1H), 7.86 (d, *J* = 2.1 Hz, 1H); ^{13}C-NMR (d_6-DMSO, 126 MHz) δ ppm 165.6, 136.5, 133.6, 128.0, 125.5, 124.8, 123.9, 115.5. Mass spec.: 196.97 $(MH)^+$.

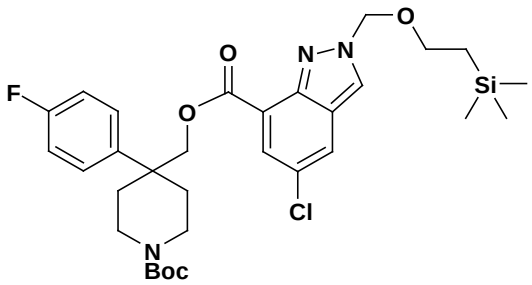
(1-(*tert*-Butoxycarbonyl)-4-(4-fluorophenyl)piperidin-4-yl)methyl 5-chloro-1H-indazole-7-carboxylate (**13**)



To a suspension of 5-chloro-1H-indazole-7-carboxylic acid (4.32 g, 22.0 mmol), *tert*-butyl 4-(4-fluorophenyl)-4-(hydroxymethyl)piperidine-1-carboxylate (6.19 g, 20.0 mmol), and dimethylaminopyridine (0.73 g, 6.0 mmol) in dichloromethane (100 mL) at 0 ^{o}C was added 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (5.75 g, 30.0 mmol) in one portion. The mixture was stirred briefly in an ice bath, and then allowed to slowly warm to room temperature. After 28 h, the reaction mixture was poured into water (200 mL) and diluted with diethyl ether (500 mL). The ethereal layer

was washed with saturated sodium bicarbonate (2 x 300 mL), water (2 x 300 mL), brine (1 x 300 mL), then dried over sodium sulfate, and concentrated to give 10.02 g of a white foam. This was triturated with diethyl ether (100 mL) and stirred at room temperature for 30 min. The white solid was filtered to give 7.86 g (79% yield) of **13**. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 11.32 (bs, 1H), 8.03 (s, 1H), 7.89 (d, *J* = 1.5 Hz, 1H), 7.80 (d, *J* = 1.5 Hz, 1H), 7.41 (dd, *J* = *J* = 8.9, 5.2 Hz, 2H), 7.09 (dd, *J* = 8.6, 8.5 Hz, 2H), 4.42 (s, 2H), 3.79 (bs, 2H), 3.11 (m, 2H), 2.26 (m, 2H), 1.92 (m, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ ppm 164.9, 161.8 (d, *J* = 247 Hz), 155.0, 137.3, 134.4, 129.1, 128.8 (d, *J* = 7.7 Hz), 126.04, 125.97, 125.5, 115.9 (d, *J* = 21 Hz), 113.3, 79.8, 73.0, 39.8, 32.3, 28.5. Mass spec.: 488.21 $(MH)^+$.

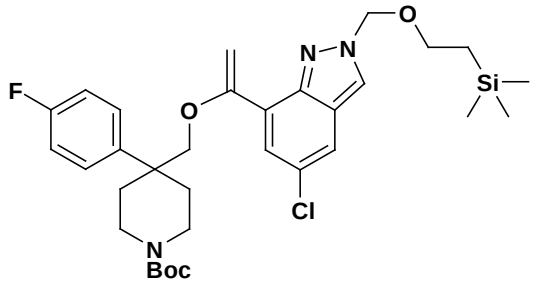
(1-(*tert*-Butoxycarbonyl)-4-(4-fluorophenyl)piperidin-4-yl)methyl 5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazole-7-carboxylate (**14**)



To a solution of (1-(*tert*-butoxycarbonyl)-4-(4-fluorophenyl)piperidin-4-yl)methyl 5-chloro-1H-indazole-7-carboxylate (85 g, 174 mmol) and N-cyclohexyl-N-methylcyclohexanamine (54.4 g, 279 mmol) in tetrahydrofuran (500 mL) at room temperature was added (2-(chloromethoxy)ethyl)trimethylsilane (47.9 g, 287 mmol). The reaction was slightly exothermic up to 28 °C after 30 min of stirring. Water bath was used to cool back to room temperature and stirring continued for 16 h. There was still 2% of unreacted starting material observed by HPLC. An additional SEMCl (900 mg) and N-cyclohexyl-N-methylcyclohexanamine (1.1 g) was added. After another 16 h of stirring at room temperature, no more starting material was observed. The reaction mixture was treated with 143 mL of 2 M ammonia in methanol and stirred at room temperature for 30 min. Then, it was diluted with diethyl ether (800 mL), washed with 1

M potassium bisulfate (500 mL), water (500 mL), and brine (500 mL), dried over sodium sulfate, and concentrated to an oil. This oil was dissolved in diethyl ether (100 mL). Hexanes (1400 mL) was then added slowly through an addition funnel to give a white suspension. This suspension was stirred at room temperature for 16 h. The product **14** was obtained as a white solid (93 g, 86% yield) after filtration and drying in vacuum at room temperature for 16 h. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.18 (s, 1H), 7.86 (d, *J* = 2.1 Hz, 1H), 7.82 (d, *J* = 1.8 Hz, 1H), 7.44 (dd, *J* = 8.6, 5.6 Hz, 2H), 7.03 (dd, *J* = 8.9, 8.6 Hz, 2H), 5.77 (s, 2H), 4.33 (s, 2H), 3.80 (br, 2H), 3.63 (t, *J* = 8.2 Hz, 2H), 3.07 (m, 2H), 2.28 (m, 2H), 2.05 (m, 2H), 1.42 (s, 9H), 0.93 (t, *J* = 8.2 Hz, 2H), -0.05 (s, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ ppm 164.5, 161.7 (d, *J* = 247 Hz), 155.0, 144.4, 137.5, 131.8, 129.1 (d, *J* = 7.7 Hz), 126.8, 125.0, 124.4, 122.7, 121.1, 115.5 (d, *J* = 21 Hz), 82.6, 79.6, 72.7, 68.0, 40.8, 40.1 (br), 32.1, 28.5, 18.1, -1.3. Mass spec.: 618.28 $(MH)^+$.

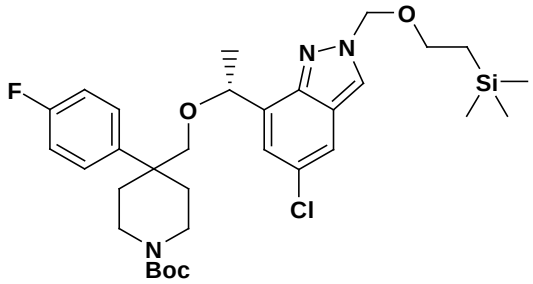
tert-Butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)vinyloxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**15**)



To a chilled suspension (0-5 °C) of titanocenedichloride (73 g, 286 mmol) in toluene (700 mL) was added methylmagnesium chloride in THF (3 M, 215 mL, 645 mmol) dropwise through an addition funnel over 1 h under nitrogen. The reaction mixture was stirred vigorously below 5 °C for 1 h. The reaction mixture was quenched by addition of 255 mL of a 6% ammonium chloride solution (reverse addition) at 0-5 °C. The resulting heterogeneous mixture was vigorously stirred for 15 min prior to separation of the layers. The bottom heterogeneous aqueous layer was filtered through a celite pad and added to a separatory funnel containing toluene and water. The organic layer was separated, washed with cold water and brine, dried over sodium sulfate, filtered, and concentrated to a

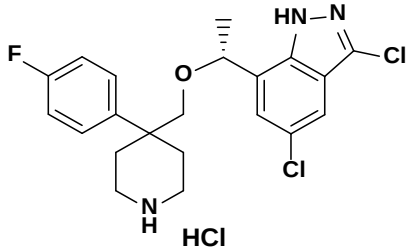
certain weight (208 g). This material was assayed by ^{1}H NMR to contain 21.7 wt% in 73% yield. A total of 105 g of this solution was treated with (1-(*tert*-butoxycarbonyl)-4-(4-fluorophenyl)piperidin-4-yl)methyl 5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazole-7-carboxylate (27.4 g, 44.3 mmol), α,α-dimethylphenethyl acetate (6.39 g, 33.2 mmol), then dichloro titanocene (0.65 g, 2.55 mmol). The reaction mixture was heated to 80 °C and stirred for 6 h. The reaction was monitored by HPLC until ca. 1-2% of unreacted starting material remained. The reaction mixture was cooled to room temperature and treatehed with sodium bicarbonate (10.8 g), methanol (173 mL), and water (6.4 mL). The mixture was heated to 40 °C and stirred for 14 h. A dark green suspension was observed. The resulting suspension was filtered, and the filtrate was concentrated to a yellow solution (27 g in 52 g of toluene solution, 99% yield). This solution was used as is in the subsequent asymmetric hydrogenation. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.05 (s, 1H), 7.58 (d, *J* = 1.8 Hz, 1H), 7.44 (dd, *J* = 8.9, 5.5 Hz, 2H), 7.33 (d, *J* = 1.8 Hz, 1H), 7.10 (dd, *J* = 8.9, 8.5 Hz, 2H), 5.95 (d, *J* = 2.1 Hz, 1H), 5.69 (s, 2H), 4.59 (d, *J* = 2.1 Hz, 1H), 3.83 (s, 2H), 3.80 (bs, 2H), 3.64 (t, *J* = 8.6 Hz, 2H), 3.10 (m, 2H), 2.31 (m, 2H), 1.99 (m, 2H), 1.45 (s, 9H), 0.93 (t, *J* = 8.2 Hz, 2H), -0.04 (s, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ ppm 161.6 (d, *J* = 246 Hz), 155.0, 144.3, 138.21, 138.19, 128.9 (d, *J* = 8.6 Hz), 127.8, 126.9, 124.7, 123.6, 122.4, 119.2, 115.5 (d, *J* = 20 Hz), 90.0, 82.0, 79.6, 75.8, 67.8, 41.1, 40.2 (br), 32.5, 28.6, 17.9, -1.4. Mass spec.: 616.36 $(MH)^{+}$.

(R)-*tert*-Butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)ethoxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**16**)



In a glove box, a glass round bottom flask was charged with diacetato[(S)-(+)-2,2'-bis(di-p-tolylphosphino)-1,1'-binaphthyl]ruthenium(II) (2.6 g, 2.9 mmol), *tert*-butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)vinyloxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**15**) (36.5g as 58.4 g of 65 wt% in toluene, 59.3 umol), and 1,2-dichloroethane (196 mL). The vessel was sealed under nitrogen, removed from the glove box, and connected to a gas handling manifold. Under rigorous stirring, the flask was purged 3 times with 30 psig hydrogen before finally pressurizing the flask to 100 psig with hydrogen. The reaction mixture was stirred at 22 ^{o}C under hydrogen pressure for 16 h. The flask was depressurized, purged 3 times with 15 psig nitrogen, concentrated, and purified by ISCO (0-20% EtOAc/hexanes) to give 33.0 g (85% yield) of **16** as a viscous oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.00 (s, 1H), 7.48 (d, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 8.5, 5.2 Hz, 2H), 7.03 (dd, *J* = 8.9, 8.6 Hz, 2H), 6.77 (s, 1H), 5.67 (d, *J* = 10.7 Hz, 1H), 5.64 (d, *J* = 10.7 Hz, 1H), 4.93 (q, *J* = 6.4 Hz, 1H), 3.72 (m, 2H), 3.59 (t, *J* = 8.4 Hz, 2H), 3.37 (d, *J* = 9.2 Hz, 1H), 3.34 (d, *J* = 9.2 Hz, 1H), 3.05 (m, 2H), 2.19 (m, 1H), 2.08 (m, 1H), 1.94 (m, 1H), 1.87 (m, 1H), 1.44 (s, 9H), 1.43 (d, *J* = 6.4 Hz, 3H), 0.91 (dd, *J* = 9.2, 7.3 Hz, 2H), -0.06 (s, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ ppm 161.5 (d, *J* = 245 Hz), 155.1, 145.6, 138.9, 135.8, 128.9 (d, *J* = 7.7 Hz), 128.3, 122.82, 122.76, 122.2, 117.6, 115.2 (d, *J* = 21 Hz), 81.9, 79.4, 77.9, 73.9, 67.6, 41.3, 40.3, 32.3, 32.0, 28.6, 22.8, 17.9, -1.4. Mass spec.: 618.39 $(MH)^{+}$.

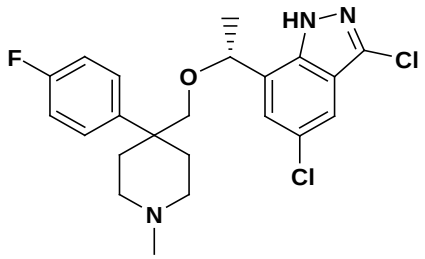
(R)-3,5-Dichloro-7-(1-((4-(4-fluorophenyl)piperidin-4-yl)methoxy)ethyl)-1H-indazole hydrochloride (**17**)



To a solution of (R)-*tert*-butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)ethoxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (7.10 g, 11.48 mmol) in 2-propanol (42 mL) was added *N*-chlorosuccinimide (2.30 g, 17.23 mmol) at

room temperature. The mixture was warmed to 50 °C in an oil bath and stirred for 30 min. The reaction mixture was cooled in an ice bath. To this was added 6 N HCl in 2-propanol (9.57 mL, 57.4 mmol). The reaction mixture was warmed to 70 °C and held there for 2 h. After cooling to room temperature, the resulting precipitate was collected in a büchner funnel, washed with 2-propanol, dried under vacuum to give 4.69 g (88% yield) of **17** as a white solid. ^{1}H-NMR (DMSO-d_6, 400MHz) δ 13.55 (s, 1H), 8.90 (br s, 2H), 7.58 (d, *J* = 2.0 Hz, 1H), 7.43 (br dd, *J* = 8.7, 5.4 Hz, 2H), 7.17 (br t, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 1.8 Hz, 1H), 4.77 (br d, *J* = 6.3 Hz, 1H), 3.39 (br d, *J* = 9.3 Hz, 1H), 3.23 (br d, *J* = 9.1 Hz, 1H), 3.21 - 2.97 (m, 2H), 2.82 - 2.55 (m, 2H), 2.40 - 2.17 (m, 2H), 2.17 - 1.92 (m, 2H), 1.34 (d, *J* = 6.3 Hz, 3H). Mass spec.: 422.1 $(MH)^+$.

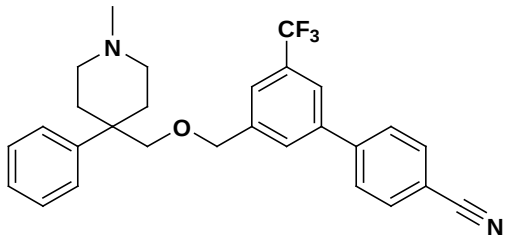
(*R*)-3,5-Dichloro-7-(1-((4-(4-fluorophenyl)-1-methylpiperidin-4-yl)methoxy)ethyl)-1H-indazole (**9**)



To a mixture of (R)-4-((1-(3,5-dichloro-1H-indazol-7-yl)ethoxy)methyl)-4-(4-fluorophenyl)piperidinium chloride (19.03 g, 41.5 mmol) in 1,2-dichloroethane (300 mL) was added *N*-ethyl-*N*-isopropylpropan-2-amine (5.90 g, 45.6 mmol). The reaction mixture was mechanically stirred at room temperature for 15 min and then cooled to 0-2 °C in an ice bath. To this was added formaldehyde, 37 wt% in water (4.04 g, 49.8 mmol). The reaction mixture was stirred for 15 min to give a clear yellow solution. To this was added ground STAB-H (14.07 g, 66.4 mmol) in portions over 5 min. After 7.5 h, 1.1% of starting material remained. The reaction mixture was cooled to 0-2 °C. Additional formaldehyde, 37 wt% in water (0.34 g, 4.15 mmol) and STAB-H (0.88 g, 4.15 mmol) were added. After 22 h, the reaction mixture was cooled to 0 °C in an ice bath, then 1 N NaOH (300 mL) was added. The layers were separated. The aqueous layer was extracted with DCM (100 mL). The combined organic layers were washed with water

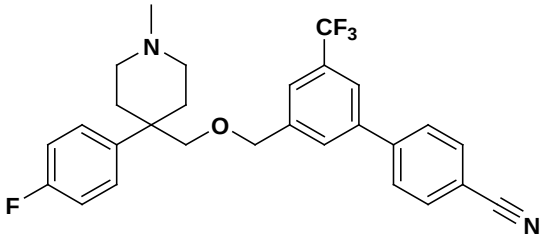
(400 mL), then was treated with MeOH (40 mL), washed with brine (400 mL), dried (Na_2SO_4), and concentrated to give 17.85 g (97% yield) of **9** as a yellow foam. This was further purified by recrystallization from IPA/H_2O to give **9** as an off-white solid (80% recovery yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.50 (bs, 1H), 7.51 (d, *J* = 1.8 Hz, 1H), 7.26 (dd, *J* = 8.8, 5.2 Hz, 2H), 7.07 (dd, *J* = 8.8, 8.6 Hz, 2H), 7.02 (d, *J* = 1.8 Hz, 1H), 4.50 (q, *J* = 6.6 Hz, 1H), 3.43 (d, *J* = 8.7 Hz, 1H), 3.29 (d, *J* = 8.7 Hz, 1H), 2.59 (m, 1H), 2.53 (m, 1H), 2.29 (m, 1H), 2.21 (s, 3H), 2.20 (m, 1H), 2.12 (m, 1H), 2.09 (m, 1H), 1.99 (m, 1H), 1.90 (m, 1H), 1.41 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 161.5 (d, *J* = 247 Hz), 139.1, 137.0, 134.3, 128.6 (d, *J* = 7.8 Hz), 128.3, 126.9, 125.2, 122.1, 117.7, 115.6 (d, *J* = 21 Hz), 78.9, 78.1, 51.8, 51.7, 46.2, 40.3, 32.9, 32.6, 22.1. ^{19}F-NMR ($CDCl_3$, 471 MHz) δ -116.4 (s). Mass spec.: 436.1 $(MH)^+$. $[\alpha]_D$ = +20.3 (c=0.40, $CHCl_3$).

3'-(((1-Methyl-4-phenylpiperidin-4-yl)methoxy)methyl)-5'-(trifluoromethyl)biphenyl-4-carbonitrile (**1**)



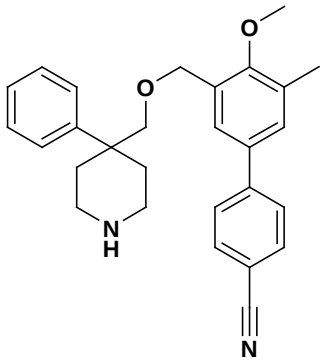
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.74-7.76 (m, 2H), 7.66 (s, 1H), 7.58-7.60 (m, 2H), 7.44 (s, 1H), 7.41 (s, 1H), 7.36-7.38 (m, 2H), 7.30-7.33 (m, 2H), 7.18-7.21 (m, 1H), 4.45 (s, 2H), 3.48 (s, 2H), 2.58-2.60 (m, 2H), 2.17-2.28 (m, 4H), 2.21 (s, 3H), 1.98-2.08 (m, 2H). Mass spec.: 465.11 $(MH)^+$.

3'-(((4-(4-Fluorophenyl)-1-methylpiperidin-4-yl)methoxy)methyl)-5'-(trifluoromethyl)biphenyl-4-carbonitrile (**2**)



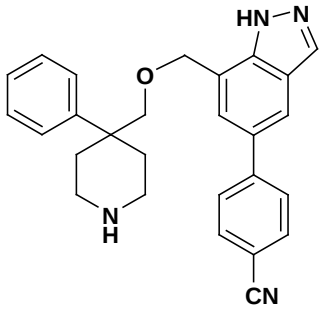
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.76-7.74 (m, 2H), 7.67 (s, 1H), 7.59-7.57 (m, 2H), 7.39-7.41 (m, 2H), 7.30-7.33 (m, 2H), 6.97-7.00 (m, 2H), 4.46 (s, 2H), 3.44 (s, 2H), 2.54-2.56 (m, 2H), 2.16-2.25 (m, 4H), 2.20 (s, 3H), 1.97-1.99 (m, 2H). Mass spec.: 483.33 $(MH)^+$.

4'-Methoxy-3'-methyl-5'-(((4-phenylpiperidin-4-yl)methoxy)methyl)biphenyl-4-carbonitrile (**3**)



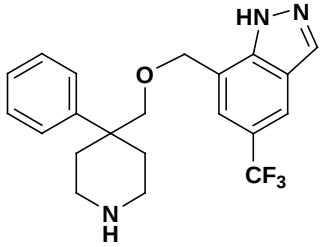
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.69 (s, 1H), 7.68 (s, 1H), 7.55 (s, 1H), 7.53 (s, 1H), 7.37 (s, 1H), 7.36 (s, 1H), 7.27-7.30 (m, 3H), 7.17-7.20 (m, 2H), 4.46 (s, 2H), 3.61 (s, 3H), 3.50 (s, 2H), 2.89-2.92 (m, 2H), 2.72-2.76 (m, 2H), 2.31 (s, 3H), 2.16-2.19 (m, 2H), 1.88-1.93 (m, 2H). ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 157.1, 145.4, 144.4, 134.7, 132.5, 132.4, 131.7, 129.2, 128.3, 127.6, 127.4, 126.1, 125.8, 119.1, 110.6, 80.2, 68.2, 60.9, 42.7, 41.9, 33.6, 16.2. Mass spec.: 427.42 $(MH)^+$. Accurate mass spec.: m/z 427.2378 $[MH]^+$, Δ = 1.8 ppm.

4-(7-(((4-Phenylpiperidin-4-yl)methoxy)methyl)-1H-indazol-5-yl)benzonitrile (**4**)



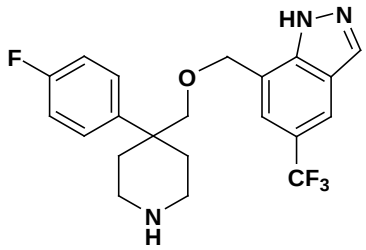
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.02 (s, 1H), 7.79 (s, 1H), 7.64 (m, 4H), 7.22-7.50 (m, 7H), 4.73 (s, 2H), 3.57 (s, 2H), 3.41 (s, 1H), 2.90 (m, 2H), 2.75 (m, 2H), 2.19 (m, 2H), 1.88 (m, 2H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 145.9, 144.1, 138.6, 134.9, 132.7, 132.0, 129.0, 127.9, 127.1, 126.8, 124.4, 123.7, 121.8, 119.1, 119.0, 110.4, 80.6, 72.0, 42.6, 41.7, 33.6. Mass spec.: 423.18 $(MH)^+$. Accurate mass spec.: m/z 423.2206 $[MH]^+$, Δ = 5.0 ppm.

7-(((4-Phenylpiperidin-4-yl)methoxy)methyl)-5-(trifluoromethyl)-1H-indazole (**5**)



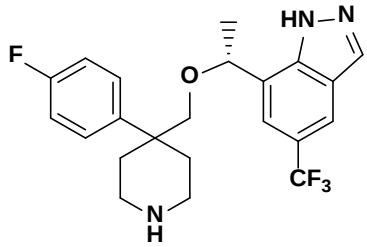
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.04 (s, 1H), 7.92 (s, 1H), 7.45 (m, 2H), 7.32-7.42 (m, 3H), 7.26 (s, 1H), 4.70 (s, 2H), 3.57 (s, 2H), 2.90 (m, 2H), 2.76 (m, 2H), 2.20 (m, 2H), 1.86 (m, 2H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 144.1, 139.6, 135.2, 129.0, 127.0, 126.9, 124.7 (q, J = 272 Hz), 123.1 (q, J = 33 Hz), 122.9, 121.9, 120.3, 118.4, 80.7, 71.8, 50.4, 42.6, 41.7, 33.8. Mass spec.: 390.08 $(MH)^+$. Accurate mass spec.: m/z 390.1801 $[MH]^+$, Δ = 2.0 ppm.

7-(((4-(4-Fluorophenyl)piperidin-4-yl)methoxy)methyl)-5-(trifluoromethyl)-1H-indazole (**6**)



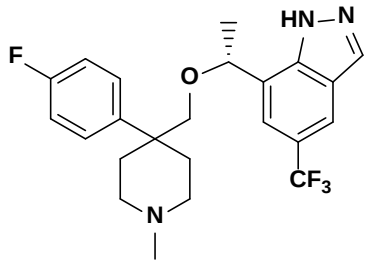
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.06 (s, 1H), 7.94 (s, 1H), 7.20-7.40 (m, 3H), 7.07 (m, 2H), 4.71 (s, 2H), 3.50 (s, 2H), 3.43 (s, 2H), 2.88 (m, 2H), 2.72 (m, 2H), 2.12 (m, 2H), 1.85 (m, 2H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 162.5, 160.5, 139.6, 139.5, 135.3, 128.64, 128.58, 124.7 (q, J = 272 Hz), 123.2 (q, J = 32 Hz), 122.9, 121.8, 120.6, 118.5 (q, J = 3.8 Hz), 115.8, 115.6, 80.3, 71.6, 50.5, 42.4, 41.4, 33.7. Mass spec.: 408.09 $(MH)^+$. Accurate mass spec.: m/z 408.1680 $[MH]^+$, Δ = 4.7 ppm.

7-(1-((4-(4-Fluorophenyl)piperidin-4-yl)methoxy)ethyl)-5-(trifluoromethyl)-1H-indazole (**7**)



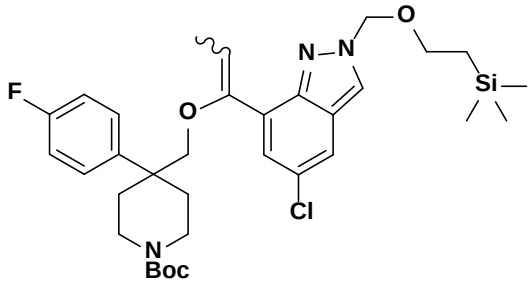
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 10.21 (bs, 1H), 8.06 (s, 1H), 7.92 (s, 1H), 7.28 (m, 2H), 7.22 (s, 1H), 7.06 (m, 2H), 4.59 (q, J = 6.4 Hz, 1H), 3.36 (q_{AB}, J_{AB}=8.9 Hz, 2H), 2.92 (m, 1H), 2.85 (m, 1H), 2.76 (m, 1H), 2.69 (m, 1H), 2.22 (m, 1H), 2.05 (m, 1H), 1.91 (m, 1H), 1.81 (m, 1H), 1.44 (d, J = 6.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 162.5, 160.6, 139.5, 138.2, 135.3, 128.70, 128.65, 127.3, 124.7 (q, J = 272 Hz), 123.5, 123.3, 119.8 (q, J = 2.9 Hz), 118.2 (q, J = 3.8 Hz), 115.6, 115.4, 79.0, 78.4, 42.6, 42.5, 41.4, 33.9, 33.7, 22.2. Mass spec.: 422.17 $(MH)^+$.

7-(1-((4-(4-Fluorophenyl)-1-methylpiperidin-4-yl)methoxy)ethyl)-5-(trifluoromethyl)-1H-indazole (**8**)



^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.95 (bs, 1H), 8.05 (s, 1H), 7.92 (s, 1H), 7.27 (m, 2H), 7.21 (s, 1H), 7.06 (m, 2H), 4.60 (q, *J* = 6.4 Hz, 1H), 3.47 (s, 1H), 3.43 (d, *J* = 8.9 Hz, 1H), 3.26 (d, *J* = 8.9 Hz, 1H), 2.59 (m, 1H), 2.53 (m, 1H), 2.30 (m, 1H), 2.20 (s, 3H), 1.85-2.25 (m, 5H), 1.44 (d, *J* = 6.7 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 162.6, 160.6, 139.2, 138.1, 135.3, 128.8, 128.7, 127.3, 124.7 (q, *J* = 272 Hz), 123.5, 123.3, 119.8 (q, *J* = 2.9 Hz), 118.1 (q, *J* = 4.8 Hz), 115.6, 115.5, 78.8, 78.5, 51.9, 51.7, 46.2, 40.3, 32.8, 32.5, 22.2. Mass spec.: 436.07 $(MH)^+$.

tert-Butyl 4-(((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)prop-1-en-1-yl)oxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (ethylidene byproduct from Petasis methylenation)



Step 1: To a suspension of titanocenedichloride (13.6 g, 53.4 mmol) in toluene (443 mL) at 0 °C was added methyllithium (1.6M in ether, 83 mL, 133 mmol) dropwise. The reaction was stirred at 0 °C for 1 h. The reaction was quenched by addition of 190 mL of a 6%

ammonium chloride solution. The layers were separated and the organic layer dried over magnesium sulfate. The solution was filtered and concentrated to ca. 1/4 its original volume. To this was added (1-(*tert*-butoxycarbonyl)-4-(4-fluorophenyl)piperidin-4-yl)methyl 5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazole-7-carboxylate (**14**) (11 g, 17.8 mmol) as a solid in one portion. The resulting solution was heated at 80 °C for 3 h under a reflux condenser. The reaction was concentrated to remove most of the toluene. The reaction was diluted with several volumes of hexanes to precipitate some of the titanocene byproducts with stirring. To decompose the remaining titanocene, silica gel was added as a solid with vigorous stirring to the suspension at 0 °C. The resulting suspension was filtered and the resulting pad washed with 25% EtOAc/Hex. The mother liquor was concentrated not quite to dryness and loaded onto a silica gel column (10% EtOAc/Hex). After several volumes, the gradient was ramped to 15% EtOAc/Hex and then to 20% EtOAc/Hex to give *tert*-butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)vinyloxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**15**) (4.8 g, 7.8 mmol, 44 % yield) as a faint yellow amorphous foam solid. ^{1}H-NMR indicated that it contained ca. 15% of the ethylidene byproduct (*tert*-butyl 4-(((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)prop-1-en-1-yl)oxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate). The two products were inseparable by column chromatography and the mixture was used without additional purification in Step 2.

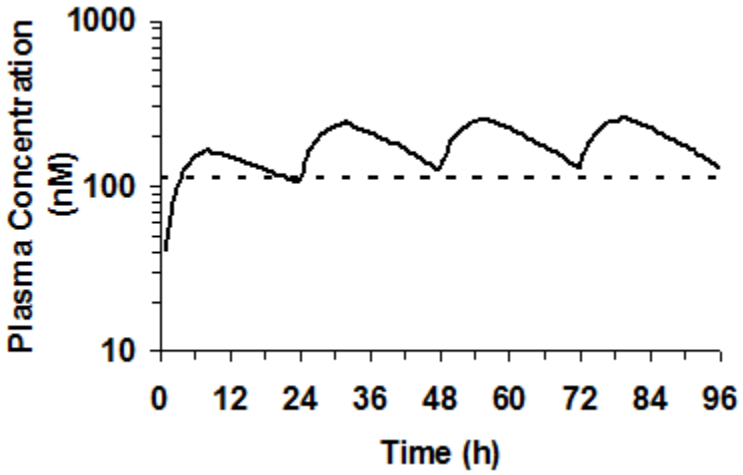
Step 2: A solution of *tert*-butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)vinyloxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**15**) (4.8 g, 7.8 mmol) tainted with ca. 15% *tert*-butyl 4-(((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)prop-1-en-1-yl)oxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate in 1,2-dichloroethane (60 mL) was transferred to a parr bottle and degassed by bubbling nitrogen through the solution for 2 h. The solution was treated with diacetato[(S)-(+)-2,2'-bis(di-p-tolylphosphino)-1,1'-binaphthyl]ruthenium(II) (0.5 g, 0.56 mmol) quickly under a stream of nitrogen. The resulting solution was bubbled with nitrogen for an additional 5 min and placed on a parr shaker. The reaction was vacuum/pressurized 5 times with hydrogen and then the pressure was brought to 60 psig and shaken for 72 h. The crude reaction mixture was filtered through a plug of silica gel which was washed with several volumes of 25% EtOAc/Hex

and concentrated. The resulting residue was purified by column chromatography (12% EtOAc/Hex → 18% EtOAc/Hex → 25% EtOAc/Hex) until all of the product was off the column. The ethylidene byproduct eluted first, but there were many mixed fractions. A pure fraction of (R)-*tert*-Butyl 4-((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)ethoxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate (**16**) (2.95 g) and mixed fractions totaling 1.4 g. ^{1}H-NMR, ^{13}C-NMR, and LC/MS of the ethylidene byproduct were consistent with the structure shown above as a single geometric isomer around the olefin. A series of 1D NOE's were performed, but results were inconclusive as to the geometry at the olefin. *tert*-Butyl 4-(((1-(5-chloro-2-((2-(trimethylsilyl)ethoxy)methyl)-2H-indazol-7-yl)prop-1-en-1-yl)oxy)methyl)-4-(4-fluorophenyl)piperidine-1-carboxylate: ^{1}H-NMR ($CDCl_3$, 500MHz) δ 8.01 (s, 1H), 7.46 (d, *J* = 1.5 Hz, 1H), 7.40 (dd, *J* = 8.5, 5.2 Hz, 2H), 7.10 (t, *J* = 8.5 Hz, 2H), 6.67 (q, *J* = 7.0 Hz, 1H), 6.61 (d, *J* = 1.8 Hz, 1H), 5.67 (s, 2H), 3.78 (br. s., 2H), 3.67 - 3.57 (m, 4H), 3.12 (t, *J* = 10.8 Hz, 2H), 2.30 (d, *J* = 12.2 Hz, 2H), 2.03 - 1.92 (m, 2H), 1.73 (d, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 0.97 - 0.88 (m, 2H), -0.04 (s, 9H). ^{13}C-NMR ($CDCl_3$, 126MHz) δ 161.6 (d, *J* = 246 Hz), 155.0, 149.7, 144.6, 138.2 (d, *J* = 2.9 Hz), 129.0 (d, *J* = 7.7 Hz), 127.9, 126.7, 123.6 (d, *J* = 8.6 Hz), 122.6, 118.2, 117.4, 115.6, 115.4, 81.8, 79.5, 79.3, 67.7 (s, 2C), 65.9, 41.4, 40.6 (br), 39.7 (br), 32.2 (br.), 28.6, 17.8, 15.4, 11.4, -1.3. Mass spec.: 630.38 $(MH)^+$.

Table S-1. Summary of solution pharmacokinetic parameters of compound 9

	mouse	rat	dog	cyno
IV dose (mg/kg)	1 mg/kg	1 mg/kg	0.25 mg/kg	0.5 mg/kg
Cl (ml/min/kg)	23	52.1	5.6	13.8
Vss (L/kg)	12.3	13.8	5.7	3.6
$T_{½}$ (h)	5.4	3.3	18.6	8.5
AUClast (nM·h)	1577	647	1315	1347
Oral dose (mg/kg)	10 mg/kg	3 mg/kg	1 mg/kg	10 mg/kg
Cmax (nM)	2174	211	213	548
Tmax (h)	8	7.3	12	8
AUClast (nM·h)	36700	2892	3975	16302
Fpo (%)	232%	149%	76%	62%

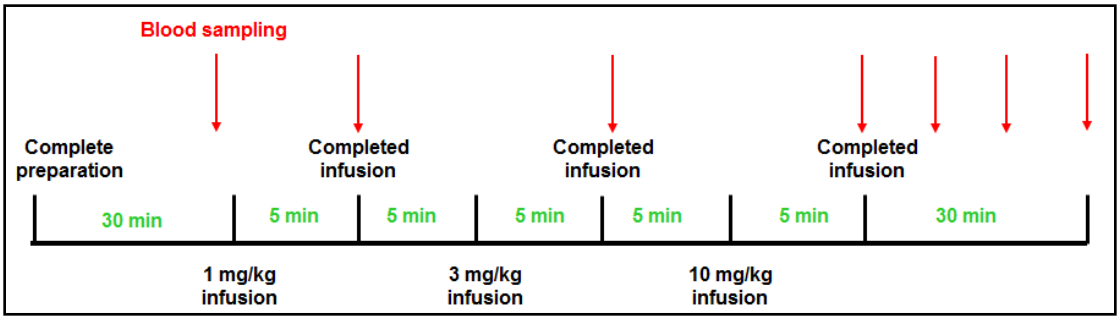
Figure S-1. Human pharmacokinetic projection of compound 9



Dose (mg/kg)	Cmax,ss (µM)	$AUC_{0-\tau}$ (µM·h)	$C_{min,ss}$ (µM)
2	0.26	4.8	0.13

Figure S-2. Study design and exposure data for rabbit cardiovascular (CV) safety study with compound 9.

Protocol: Incrementing IV (5 min infusion) doses of 1, 3, and 10 mg/kg of compound **9** in each rabbit as a solution in PEG 400:EtOH:water (1:1:1), 10 mg/mL. Blood sampling: baseline, immediately after each infusion, and at 10, 20, and 30 min after the last infusion.



Exposure of compound 9 in rabbit CV study (µM)

Animal No.	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg	10 min	20 min	30 min
rabbit1	0	0.58	1.61	3.66	1.25	1.17	0.98
rabbit2	0	0.59	1.15	3.55	1.06	0.93	0.91
rabbit3	0.01	0.52	6.28	3.23	0.98	0.87	0.99
mean	**0**	**0.57**	**3.02**	**3.48**	**1.09**	**0.99**	**0.96**
SD	0	0.04	2.84	0.22	0.14	0.16	0.04
free drug*	**0**	**0.01**	**0.06**	**0.07**	**0.02**	**0.02**	**0.02**

*Based on 98% protein binding in rat

Effects of compound 9 on QT intervals

	Compound 9			Vehicle		
Dose (mg/kg)	**1**	**3**	**10**	**1**	**3**	**10**
QTcf, ms	-3±1	-5±2	-8±3	1±0	2±0	1±0
QTcv, ms	2±1	-4±1	-9±2	1±0	1±1	1±1

*Data are Mean±SEM expressed as delta changes from pre-dose baseline averaged from n=3 animals for each group. QTcf and QTcv: QT interval corrected for changes in heart rate using Fridericia and Van De Water formulae, respectively.

Table S-2. Exposure data from 2-week rat toxicology study with compound 9

	Day	M 10 mg/kg	F 10 mg/kg	M 30 mg/kg	F 30 mg/kg	M 100 mg/kg	F 100 mg/kg	M 300 mg/kg	F 300 mg/kg
Tmax (h)	**1**	4	4	4	4	8	8	24	24
	14	8	8	4	4	8	8	NA	NA
Cmax (uM)	**1**	0.56	0.95	2.30	2.44	5.32	4.86	7.76	11.1
	14	1.39	1.89	5.14	7.84	17.0	18.5	NA	NA
AUC (uM*h)	**1**	9.02	12.6	35.3	46.9	99.7	99.7	143	164
	14	23.8	37.1	90.6	137	351	356	NA	NA
Multiples Over Human Cmax	**1**	2	4	9	9	20	19	30	43
	14	5	7	20	30	65	71	NC	NC
Multiples Over Human AUC	**1**	2	3	7	10	21	21	30	34
	14	5	8	19	28	73	74	NC	NC

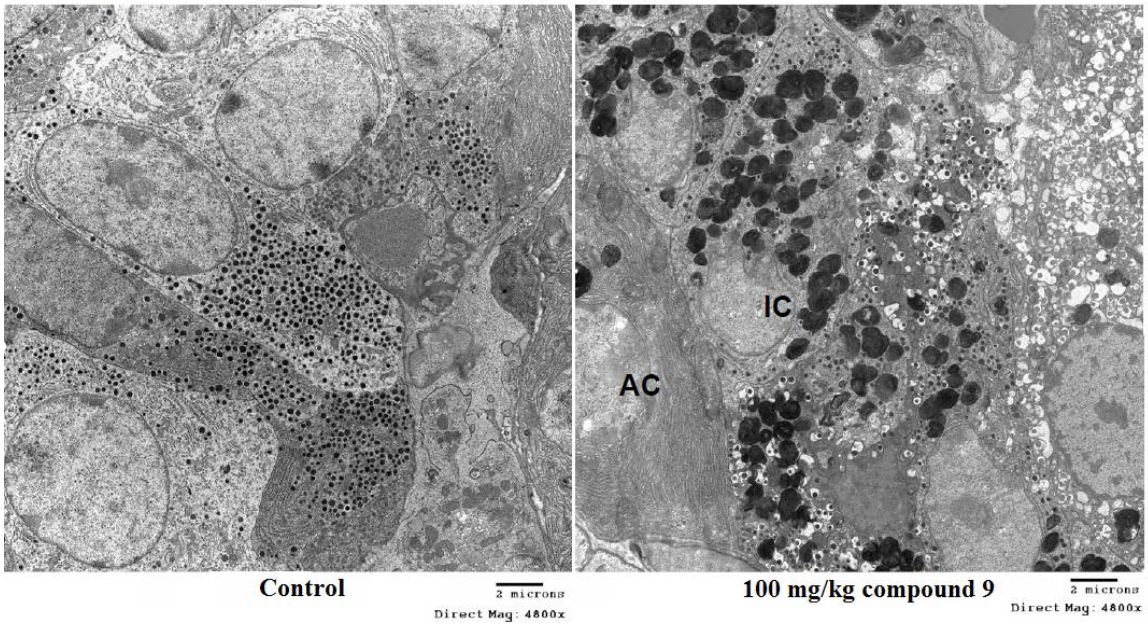
Projected human efficacious Cmax and AUC are 0.26 uM and 4.8 uM·h, respectively. 6 male/female rats/group.

Table S-3. Summary of microscopic findings from 2-week rat toxicology study with compound 9

Dose	Findings
≥ 10 mg/kg	– Apoptosis/vacuolar degeneration (vagina/uterus) (1 animal each at 10 mpk)
≥ 30 mg/kg	– Epithelial vacuolation and histiocytosis (multiple tissues) – Degeneration/necrosis (skeletal muscle; females only at 30 mpk) – Lymphoid depletion (spleen, lymph nodes) – Vacuolation (adrenal gland; females only at 30 mpk) – Vacuolation/apoptosis to degeneration (ovary)
≥ 100 mg/kg	– Vacuolar degeneration (liver, kidneys, male mammary gland) – Cell loss (chief, parietal)/regenerative hyperplasia (stomach) – Vacuolation (pancreas, prostate, seminal vesicle, female mammary gland) – Apoptosis (liver, prostate) – Lymphoid depletion (thymus) – Secretory depletion (epididymides, prostate gland, seminal vesicle) – Increased mitotic figures (liver, kidney) – Adipocyte atrophy (female mammary gland)
≥ 300 mg/kg	– Not tolerated. All rats euthanized between day 6-8 for humane reasons

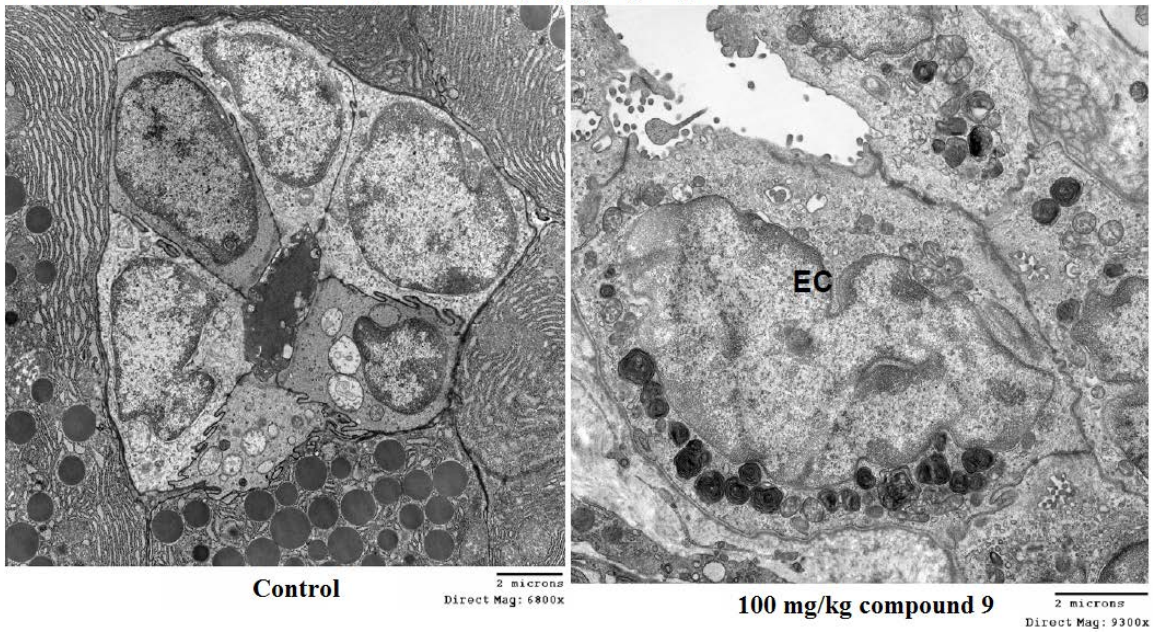
Figure S-3. Representative transmission electron microscopy slides from control and drug-treated rats in 2-week toxicology study

a. Pancreas: Islet cell (IC) and acinar cell (AC) phospholipidosis



Control 100 mg/kg compound 9

b. Pancreas: Intercalated duct epithelial cell (EC) phospholipidosis



Control 100 mg/kg compound 9

c. Pancreas: Acinar cell (AC), smooth muscle cell (SMC), and islet cell (IC) phospholipidosis

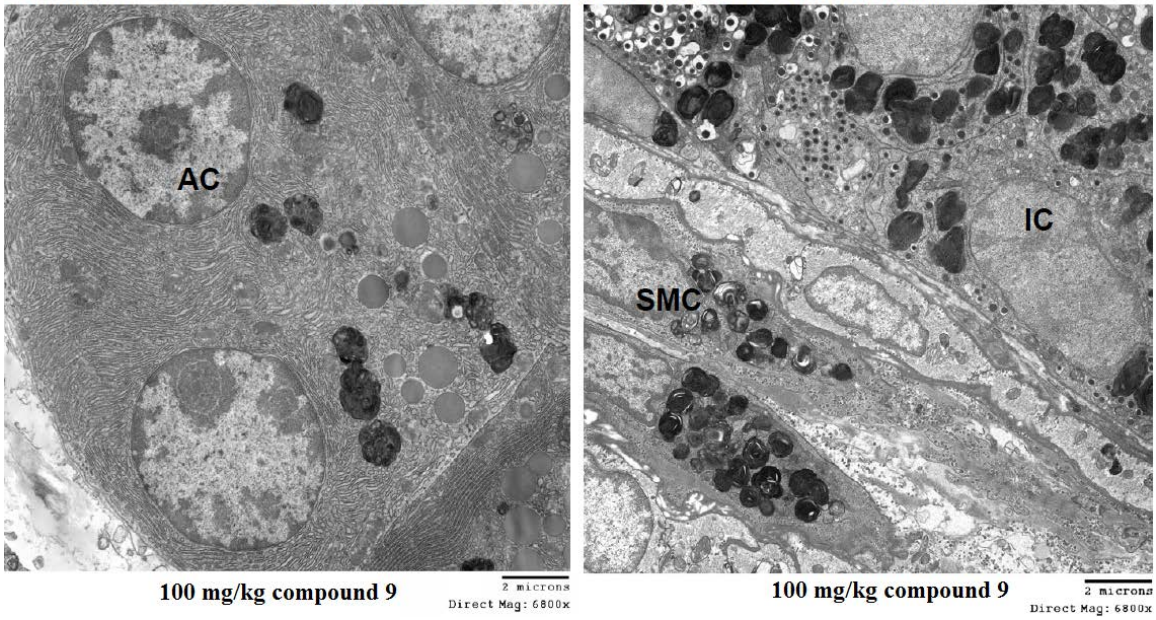


Table S-4. Exposure data from single dose toxicokinetics study in male rats

Dose (mg/kg)	**30**	**100***	**300**
Cmax (µM)	2.9	7.3	9.1
Tmax (h)	17	17	24
AUC_{0-24} (µM·h)	44	89	158
Liver conc 24h (µM)	92	185	NA

*telemeterized rats used in 100 mg/kg cohort.

Table S-5. Exposure data from single dose toxicokinetics study in mice

	Female				**Male**		
Dose (mg/kg)	**30**	**100**	**300**		**30**	**100**	**300**
Cmax	5.2	15	22		6.4	15	29
Tmax	4.0	24	48		24	24	72
AUC_{0-72} (µM·h)	222	791	1412		294	805	1477
AUC_{0-24} (µM·h)	107	245	353		114	246	362
Plasma conc 24h (µM)	4.6	15	22		6.4	15	18
Liver conc 24h (µM)	194	212	1943		80.5	100	142
Heart conc 24h (µM)	24	80.4	149		26.0	118	133
Brain conc 24h (µM)	15	49	69		9.9	38	50

Biology Experimental

NK-1, SERT Binding assays: Setup. Crude membrane suspensions were prepared for the NK1 and SERT radioligand binding assays from U373 cells or recombinant HEK-293 cells expressing hSERT, respectively. Cells were harvested from T-175 flasks as follows. The medium is removed from the flasks and the cells rinsed with HBSS without Ca and without Mg. The cells are then incubated for 5-10 minutes in 10 mM Tris-Cl, pH 7.5, 5 mM EDTA before the cells are lifted with a combination of pipetting and scraping, as needed. To prepare membranes, the cell suspension is collected into centrifuge bottles and homogenized for 30 seconds with a Polytron homogenizer. The suspension is centrifuged for 30 min @ 32,000 x g, 4^{O}C, then the supernatant is decanted and the pellet resuspended and homogenized in 50 mM Tris-Cl, pH 7.5, 1 mM EDTA for 10 seconds. The suspension is then centrifuged again for 30 min @ 32,000 x g, 4^{O}C. The supernatant is decanted and the pellet resuspended in 50 mM Tris-Cl, pH 7.5, 1 mM EDTA and briefly homogenized. A Bradford assay (Bio-rad) is performed and the membrane preparation diluted to 2 mg/ml with 50 mM Tris-Cl, pH 7.5, 1 mM EDTA. Aliquots are prepared, and then frozen and stored at -80^{O}C.

NK1 radioligand binding assay. Compounds are dissolved in 100% DMSO at a concentration 100x the desired highest assay concentration, serially diluted 1:3 in 100% DMSO, and 0.6 ul/well of each solution is dispensed to a Nunc polypropylene, round bottom, 384 well plate. 100% inhibition is defined with 0.6 ul/well of 1 mM L-733,060 (Sigma L-137) dissolved in DMSO. 30 ul/well of a 2x U373 membrane preparation (267 ug/ml in 100 mM Tris-Cl, pH 7.5, 6 mM $MgCl_2$, 0.2% (v/v) Sigma mammalian protease inhibitor cocktail (Sigma P-8340), and 4 ug/ml chymostatin, Sigma C-7268) and 30 ul/well of a 2x radioligand solution (400 pM [^{125}I]Substance P (Perkin Elmer NEX-190) in 1% (w/v) BSA (Sigma A-2153), 0.1 mg/ml bacitracin, Sigma B-0125) are added to the well and the reaction incubated for 1 hour at room temperature. The contents of the assay plate are then transferred to a Millipore Multiscreen$_{HTS}$ GF/B filter plate which has been pretreated with 0.5% PEI for at least one hour. The plate is vacuum filtered and washed

with 7 washes of 100 ul/well of 20 mM Tris-Cl, pH 7.5, 0.5% (w/v) BSA chilled to 4^{O}C. The filtration and washing is completed in less than 90 s. The plates are air-dried overnight, 12 ul/well of MicroScint scintillation fluid added, and the plates counted in a Trilux.

SERT radioligand binding assay. Compounds are dissolved in 100% DMSO at a concentration 100x the desired highest assay concentration, serially diluted 1:3 in 100% DMSO, and 0.4 ul/well of each solution is dispensed to a Nunc polypropylene, round bottom, 384 well plate. 100% inhibition is defined with 0.4 ul/well of 1 mM fluoxetine (Sigma F-132) dissolved in DMSO. 20 ul/well of a 2x HEK-hSERT membrane preparation (15 ug/ml in 50 mM Tris-Cl, pH 7.5, 120 mM NaCl, 5mM KCl) and 20 ul/well of a 2x radioligand solution (520 pM [^{125}I]RTI-55 (Perkin-Elmer NEX-272) in 50 mM Tris-Cl, pH 7.5, 120 mM NaCl, 5mM KCl) are added to each well and the reaction incubated for 1 hour at room temperature. The contents of the assay plate are then transferred to a Millipore Multiscreen$_{HTS}$ GF/B filter plate which has been pretreated with 0.5% PEI for at least one hour. The plate is vacuum filtered and washed with 7 washes of 100 ul/well of 50 mM Tris-Cl, pH 7.5, 120 mM NaCl, 5mM KCl chilled to 4^{O}C. The filtration and washing is completed in less than 90 s. The plates are air-dried overnight, 12 ul/well of MicroScint scintillation fluid added, and the plates counted in a Trilux.

Data analysis. The raw data are normalized to percent inhibition using control wells defining 0% (DMSO only) and 100% (selective inhibitor) inhibition which are run on each plate. Each plate is run in triplicate, and the concentration response curve thus generated is fit using the four-parameter dose response equation, Y=Bottom + (Top-Bottom)/(1+10^((LogIC$_{50}$-X)*HillSlope)) in order to determine the IC$_{50}$ value for each compound. The radioligand concentration chosen for each assay corresponds to the K_d concentration determined through saturation binding analysis for each assay.