Discovery of N-(indazol-3-yl)-piperidine-4-carboxylic acids as RORγt Allosteric Inhibitors for Autoimmune Diseases

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Abbreviations

DCM: dichloromethane

DMF: dimethylformamide

KOH: potassium hydroxide

EtOAc: ethyl acetate

DMAP: 4-dimethylaminopyridine

TBDPS-Cl: *tert*-butyldimethylphenylsilyl chloride

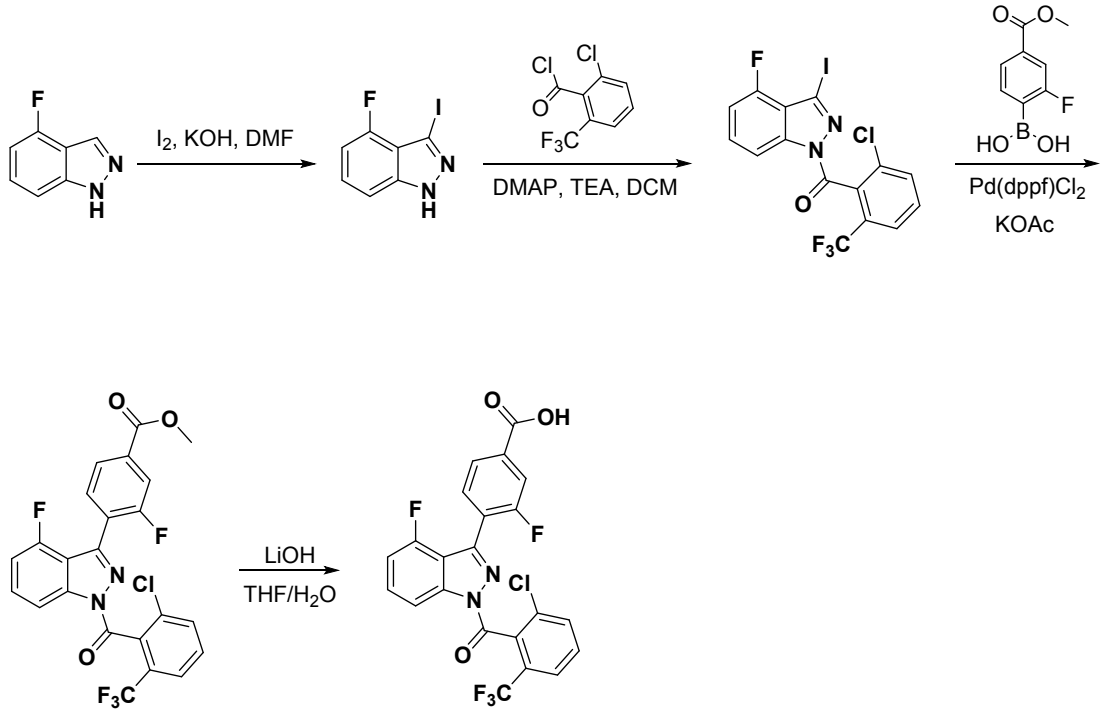
MTBE: methyl *tert*-butyl ether

TR-FRET: time-resolved fluorescence energy transfer

PBMC: peripheral blood mononuclear cell

PCR: polymerase chain reaction

Preparation of 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1*H*-indazol-3-yl)-3-fluorobenzoate (12)



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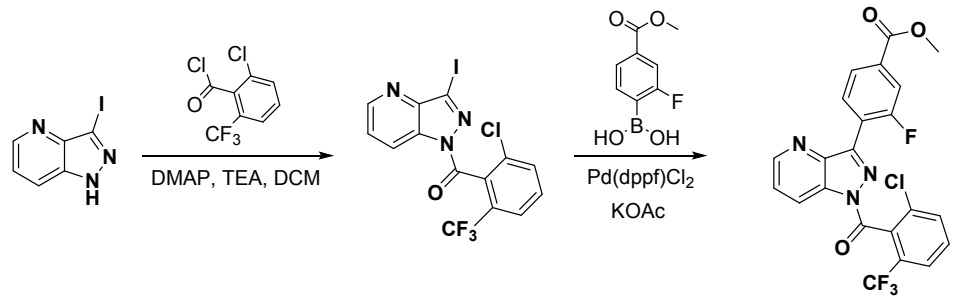
Step1: To a solution of 4-fluoroindazole (5.0 g, 37 mmol) in DMF (80 mL) at rt was added I_2 (18.6 g, 74 mmol) and KOH (7.73 g, 138 mmol) respectively. The reaction mixture was stirred at rt for 2 h, and TLC showed complete conversion. The reaction mixture was poured into aq. $NaHSO_3$ (10%, 200 mL) and extracted with EtOAc (200 mLx3). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude solid was washed with hexanes to give the desired product as a yellow solid (8.33 g, 86%). LCMS calc'd for $[(C_7H_4FIN_2)+H]^+$: 263, found: 263.

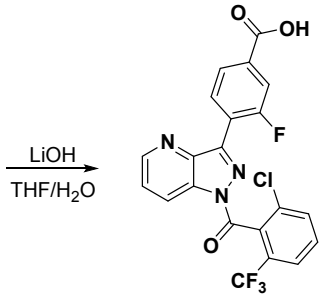
Step2: To a mixture of 4-fluoro-3-iodo-1*H*-indazole (5.24 g, 20 mmol), 2-chloro-6-(trifluoromethyl)benzoyl chloride (4.86 g, 20 mmol), DMAP (2.44 g, 20 mmol) and DCM (30 mL) was added triethylamine (5.8 mL, 40 mmol) drop wise. After the addition, the reaction mixture was kept stirring at rt for 14 h. The mixture was diluted with H_2O and extracted with DCM. The combined organics were washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel flash chromatography (2-10% EtOAc/hexanes) to give the desired (7.8 g, 83%). LCMS calc'd for $[(C_{15}H_6ClF_4IN_2O)+H]^+$: 469, found: 469.

Step3: A mixture of (2-chloro-6-(trifluoromethyl)phenyl)(4-fluoro-3-iodo-1*H*- indazol-1-yl)methanone (300 mg, 0.64 mmol), 2-fluoro-4-(methoxycarbonyl)phenylboronic acid (190 mg, 0.96 mmol), Pd(dppf)Cl_2 (52 mg, 0.064 mmol) and KOAc (190 mg, 1.92mmol) in dioxane (10 mL) and H_2O (2 mL) was heated at 90 ^oC for 2 h under microwave. The reaction mixture was cooled down, diluted with H_2O and extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4, concentrated. The crude residue was purified by silica gel flash chromatography (0-5% EtOAc/hexanes) to give the desired product as a yellow solid (180 mg). LCMS calc'd for $[(C_{23}H_{12}ClF_5N_2O_3)+H]^+$: 495, found: 495.

Step 4: To a solution of methyl 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-fluorobenzoate (180 mg, 0.36 mmol) in THF (5 ml) and H_2O (5 ml) was added LiOH (350 mg, 1.44 mmol). The reaction mixture was stirred at rt for 14 h, acidified with 2N HCl to pH = 3~4. The mixture was concentrated to give a white solid, which was rinsed with H_2O to furnish the desired product (160 mg, purity >95%). LCMS calc'd for $[(C_{22}H_{10}ClF_5N_2O_3)+H]^+$: 481, found: 481. ^1H NMR (DMSO-d6, 600MHz) δ 13.6 (br s) 8.41 (1H, d, J = 8.5 Hz); 8.04 (1H, d, J = 8.2 Hz); 7.99 (1H, d, J = 8.2); 7.91 (2H, m); 7.86 (1H, t, J = 8.2 Hz); 7.85 (1H, dd, J = 10, 1.4 Hz); 7.70 (1H, t, J = 7.5 Hz); 7.47 (1H, dd, J = 10.2, 8.1 Hz); ^{13}C NMR (DMSO-d6): 166.2 (d, J =2.5 Hz); 164.8; 160.1 (d, J = 250 Hz); 155.3 (d, J = 255 Hz); 144.4 (d, J = 3.1); 141.5 (d, J = 6.3 Hz); 135.4 (d, J = 6.9); 134.3; 133.9 (d, J = 8.1); 133.2; 132.3;; 132.1 (q, J = 2.1 Hz) ; 131.9; 128.1 (q, J = 32.1 Hz); 126.0 (q, J = 3.9 Hz); 126.0 (d, J = 3.9 Hz); 123.3 (q, J = 274 Hz); 122.8 (d, J = 14.8 Hz); 117.0 (d, J = 21.5 Hz); 115.0 (d, J = 19.6 Hz); 112.6 (d, J = 18.6 Hz); 111.8 (d, J = 3.9 Hz);

Preparation of 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-1H-pyrazolo[4,3-b]pyridin-3-yl)-3-fluorobenzoic acid (14).





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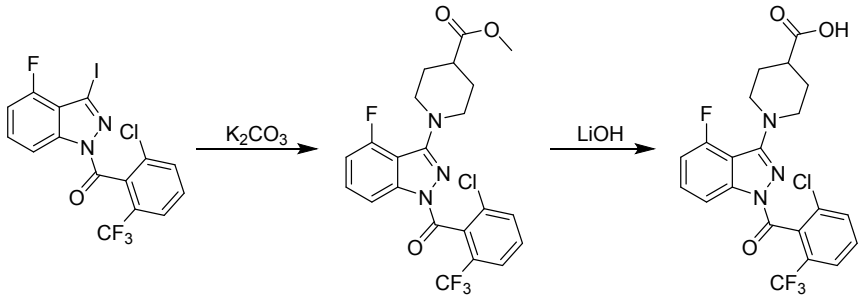
Compound **14** was synthesized from commercially available 3-iodo-1H-pyrazolo[4,3-b]pyridine by following a similar route as described for **12**. LCMS calc'd for $[(C_{21}H_{20}ClF_4N_3O_3)+H]^+$: 464, found: 464. HRMS calc'd for $[(C_{21}H_{20}ClF_4N_3O_3)+H]^+$: 464.0420, found: 464.0431. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (dd, J = 4.5, 1.4 Hz, 1H), 8.91 (dd, J = 8.5, 1.4 Hz, 1H), 8.39 (t, J = 7.5 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.96 (dd, J = 8.0, 1.6 Hz, 1H), 7.90 – 7.86 (m, 2H), 7.81 (dd, J = 10.7, 1.5 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 166.29, 165.04, 159.88 ($^1J_{CF}$=256.1 Hz), 150.60, 146.28, 142.74, 135.19, 134.27, 133.24, 133.02, 132.43, 132.05, 131.73, 128.32, 126.02, 125.85, 125.55, 123.50, 122.43 ($^1J_{CF}$=272.9 Hz), 121.37, 117.50.

Preparation of 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)piperidine-4-carboxylic acid (20)



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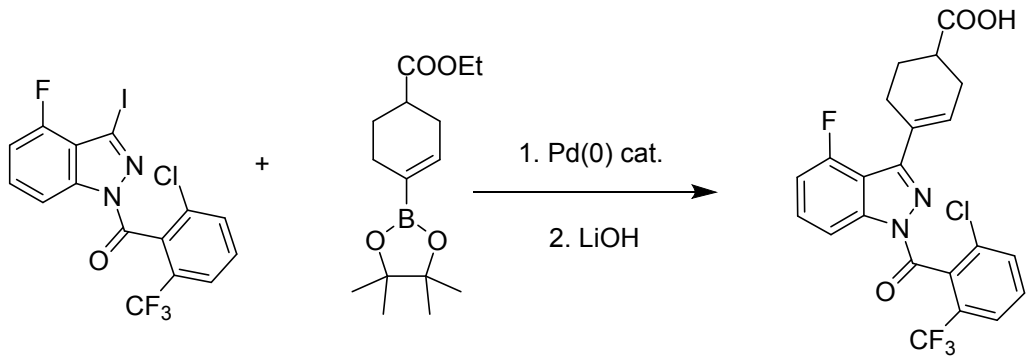
Step 1. To a mixture of (2-chloro-6-(trifluoromethyl)phenyl)(4-fluoro-3-iodo-1H-indazol-1-yl) methanone (2.0 g, 4.2 mmol) and methyl piperidine-4-carboxylate (3.06 g, 21.4 mmol) in DMF (20 mL) was added K_2CO_3 (1.77 g, 12.8 mmol). The mixture was stirred at 130 ^oC under N_2 for 10 hrs. The mixture was diluted with water (500 mL) and extracted with EtOAc (100 mL*3). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified with column (petane : EtOAc = 8 : 1 to 5 : 1) to give the title compound as white solid (1.1 g, yield: 42%). LCMS calc'd for $[(C_{22}H_{18}ClF_4N_3O_3)+H]^+$: 484, found: 484.

Step 2. To a solution of methyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)piperidine-4-carboxylate (5.2 g, 10.8 mmol) in THF / H_2O (60 mL / 10 mL) was added $LiOH{\cdot}H_2O$ (901 mg, 21.5 mmol) and under N_2 at 6 ^oC. The mixture was stirred at ambient temperature for 10 hrs. After TLC showed the reaction was completed, the mixture was diluted (300 mL) and acidified with aqueous HCl. The solution was extracted with EtOAc (50 mL * 3). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified with prep-HPLC (acetonitrile + 0.75‰ trifluoroacetic acid in water) to give the title compound (4.1 g, yield: 93%, purity >95%). LCMS calc'd for $[(C_{21}H_{16}ClF_4N_3O_3)+H]^+$: 470, found: 470.

Preparation of racemic ethyl 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1*H*-indazol-3-yl)cyclohex-3-enecarboxylate (21)



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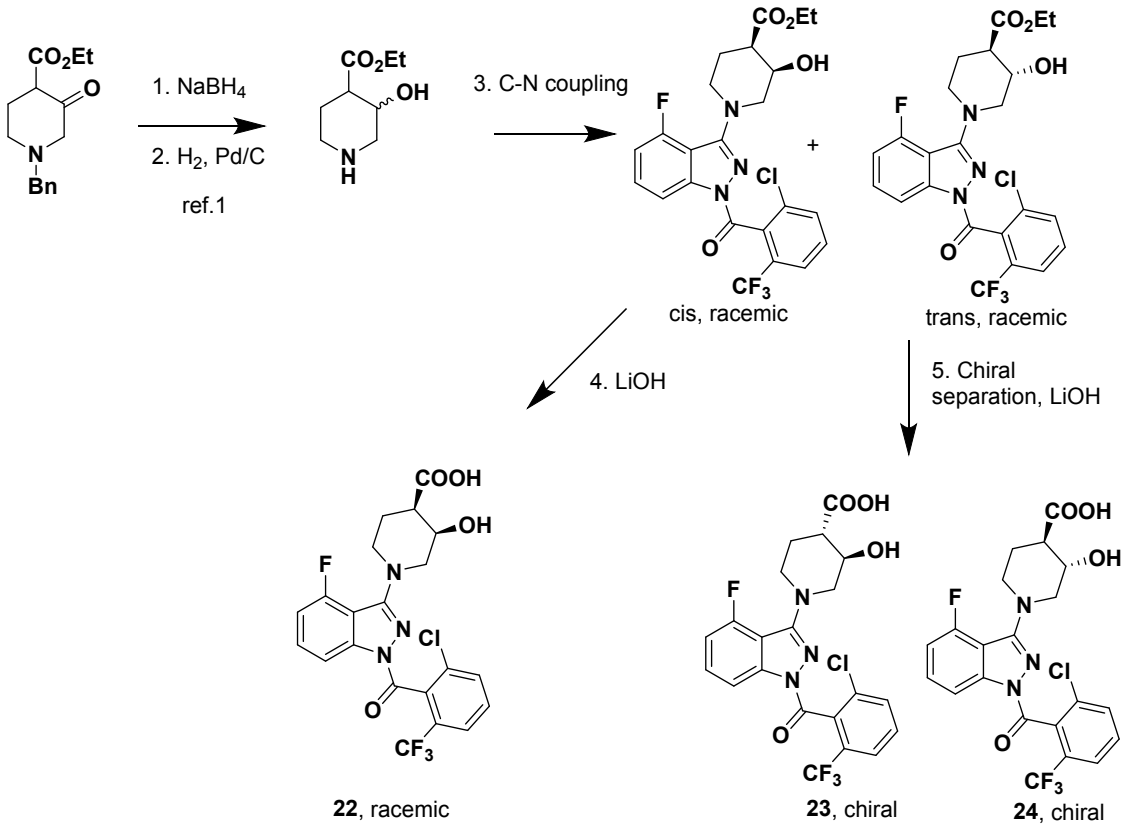
Step 1. To a mixture of (2-chloro-6-(trifluoromethyl)phenyl)(4-fluoro-3-iodo-1*H*-indazol-1-yl)methanone (1 g, 2.1 mmol) in THF (40 mL) and H_2O (10mL) was added ethyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cyclohex-3-enecarboxylate (897 mg, 3.2 mmol) and Na_2CO_3 (667 mg, 6.3 mmol). The mixture was purged with nitrogen followed by the addition of $Pd(dppf)Cl_2$ (726 mg,0.63 mmol). The reaction mixture was heated at 80 ^oC for 10 h. The resulting mixture was cooled down, filtered through Celite. The filtrate was diluted with H_2O, and extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was purified by silica gel flash chromatography (0-10% EtOAc/hexanes) to give the desired product (300 mg, 29%) as a brown oil. LCMS calc'd for $[(C_{24}H_{19}ClF_4N_2O_3)+H]^+$: 495, found: 495.

Step2. To a mixture of ethyl 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro -1*H*-indazol-3-yl)cyclohex-3-enecarboxylate from previous step (100 mg, 0.36 mmol) in in 5 ml THF/5 ml H_2O was added LiOH (350 mg, 1.44 mmol). The mixture was stirred at rt for 14h, and acidified with 2N HCl. The mixture was concentrated under reduced pressure to afford the desired product. LCMS calc'd for $[(C_{22}H_{15}ClF_4N_2O_3)+H]^+$: 467, found: 467

Preparation of (3R,4R and 3S, 4S)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid (22), (3R,4S)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid (23) and (3S,4R)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid (24)



Step1: To a solution of 1-benzyl-3-oxo-piperidine-4-carboxylic acid ethyl ester (1.4 g, 5.4 mmol) in ethanol (13.4 ml) at 0 °C was added $NaBH_4$ (0.203 g, 5.36 mmol) portionwise.[1] After addition, the reaction mixture was kept stirring for additional 30 mins. Then the reaction mixture was concentrated under reduced pressure, diluted with EtOAc and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purification by flash chromatography (10-60% EtOAc/hexanes) to give 0.52g (37%) of ethyl 1-benzyl-3-hydroxypiperidine-4-carboxylate as a mixture of cis and trans isomer (ratio ~2.3:1), LCMS calc'd for $[(C_{15}H_{21}NO_3)+H]^+$: 264, found: 264.

Step2: To a flask containing a solution of ethyl 1-benzyl-3-hydroxypiperidine-4-carboxylate (0.52g, 1.98 mmol) in ethanol (9.9 ml) was added palladium hydroxide on carbon (0.069 g, 0.099 mmol). The mixture was stirred at rt for 14h with a hydrogen balloon. Then the mixture was filtered through celite and rinsed with EtOAc. The filtrate was concentrated and used directly for next step.

Step3: To a flask was added (2-chloro-6-(trifluoromethyl)phenyl)(4-fluoro-3-iodo-1H-indazol-1-yl)methanone (500mg, 1.067 mmol), ethyl 3-hydroxypiperidine-4-carboxylate (crude from step 2,

314 mg, 1.81 mmol), DMF (5.4 ml), copper(i) iodide (31 mg, 0.16 mmol), cesium carbonate (869 mg, 2.67 mmol) and 2-isobutyrylcyclohexanone (53.9 mg, 0.320 mmol). The mixture was degassed for 5min, then sealed and heated at 90 °C for 12h. The mixture was cooled down, diluted with EtOAc and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, concentrated. The residue was purified by flash chromatography (10-70% EtOAc/hexances) to give ~100 mg of product, which was contaminated by some minor impurity. The material was re-purified by prep-TLC (5% EtOAc/DCM, develop twice) to give two racemic isomers. Cis-isomer: 56 mg (yield 10%), less polar. Trans-isomer, 32 mg (yield 5.8%), more polar. LCMS calc'd for $[(C_{23}H_{20}ClF_4N_3O_4)+H]^+$: 514, found: 514.

Step 4: To a solution of racemic cis-isomer (3R,4R and 3S,4S)-ethyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate (18mg, 0.035 mmol, cis-isomer from step 3) in THF (1 ml) /MeOH (0.5 ml)was added lithium hydroxide (0.175 ml, 0.175 mmol). The mixture was stirred at rt for 2h. The mixture was acidified with 2N HCl to pH=3~4, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by singleton (reverse HPLC, 0-100% CH3CN/H2O with 0.1% TFA) to give racemic (3R,4R and 3S, 4S)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid **22** (14 mg, yield 82%). LCMS calc'd for $[(C_{21}H_{16}ClF_4N_3O_4)+H]^+$: 486, found: 486. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.25 (d, J = 8.3 Hz, 1H), 7.91 (dt, J = 33.0, 6.8 Hz, 2H), 7.82 – 7.64 (m, 2H), 7.29 (dd, J = 11.0, 8.2 Hz, 1H), 4.06 (s, 1H), 3.78 – 3.05 (m, 4H), 2.94 (d, J = 12.6 Hz, 1H), 2.70 (q, J = 11.0 Hz, 1H), 2.05 – 1.76 (m, 1H), 1.58 – 1.41 (m, 1H).

Step5: 32mg of racemic trans-isomer from step 3 was subjected to chiral separation [Chiral cel OJ-H, 21 x 250 (mm), 10% MeOH as modifier in CO_2] to afford two enantiomers. Enantiomer 1: 13 mg, retention time 3.31min and 3.60 min (two rotamers), (3R,4S)-ethyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate.

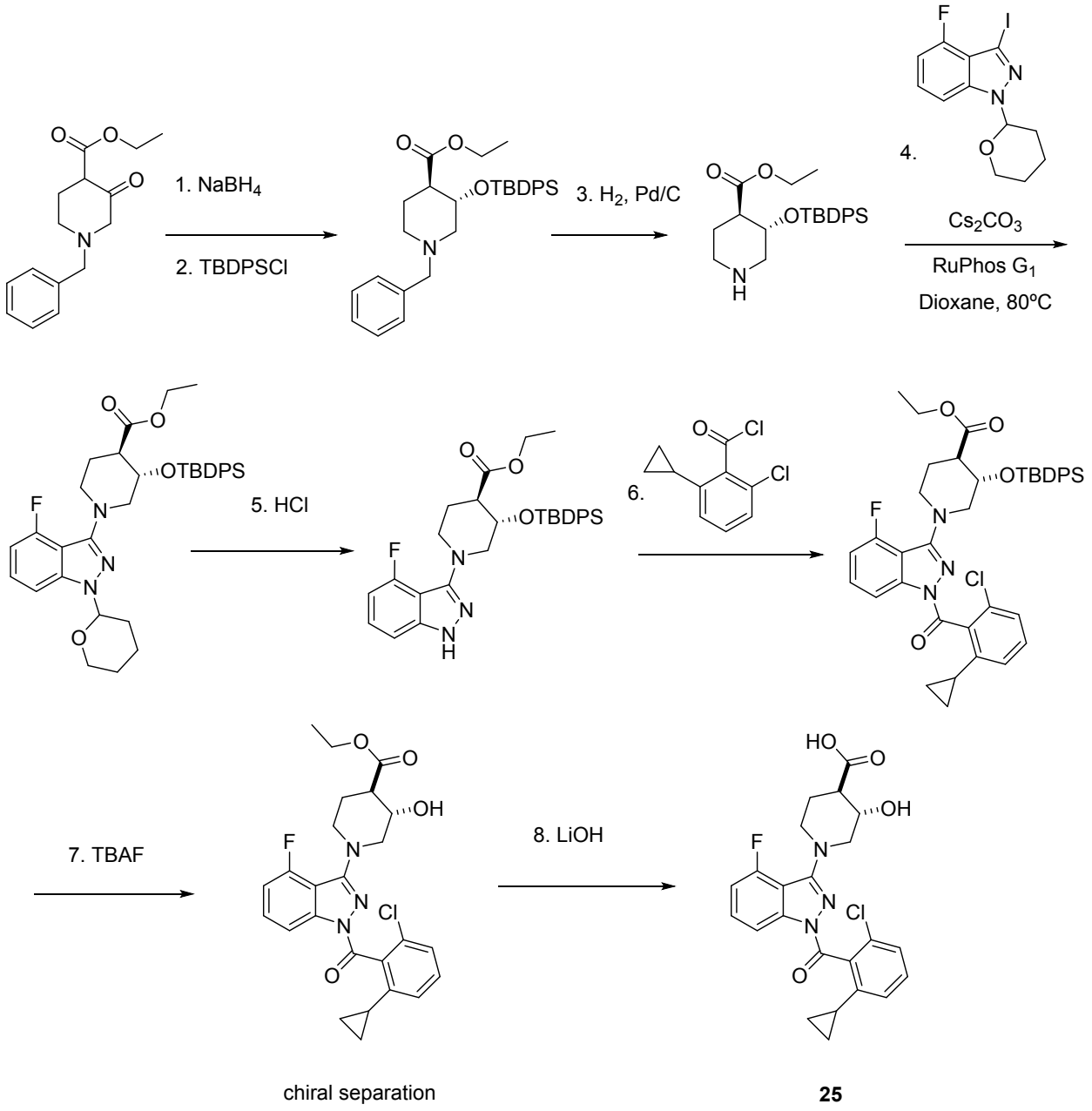
Enantiomer 2: 10mg, retention time 5.17 min and 6.31 min (two rotamers), (3S,4R)-ethyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate.

To a solution of (3R,4S)-ethyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate (13 mg, 0.025 mmol, enantiomer 1 from chiral separation)

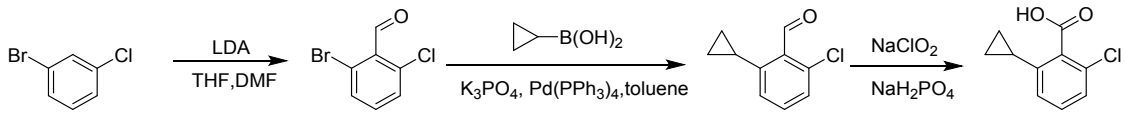
in THF (1 ml) /MeOH (0.5 ml)was added lithium hydroxide (0.253 ml, 0.253 mmol) . The mixture was stirred at rt for 2h. The mixture was acidified with 2N HCl to pH=3~4, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated. The residue was purified by reverse HPLC (50-85% CH_3CN/H_2O+0.1% TFA) . The desired fraction was collected, diluted with EtOAc and H_2O. The organic layer was washed with brine, dried over $MgSO_4$, concentrated to give (3R,4S)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid **23** (10 mg, 83%). LCMS calc'd for $[(C_{21}H_{16}ClF_4N_3O_4)+H]^+$: 486, found: 486. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.30 (d, J = 8.3 Hz, 1H), 7.77 (dd, J = 11.5, 8.1 Hz, 2H), 7.66 (td, J = 8.1, 3.7 Hz, 2H), 7.17 (dd, J = 10.9, 8.2 Hz, 1H), 3.86 (ddq, J = 28.5, 11.7, 5.3 Hz, 2H), 3.74 – 3.65 (m, 1H), 2.82 – 2.63 (m, 1H), 2.63 – 2.52 (m, 1H), 2.38 – 2.24 (m, 1H), 1.94 – 1.83 (m, 1H), 1.78 (ddt, J = 21.0, 13.7, 6.3 Hz, 1H).

To a solution of (3S,4R)-ethyl 1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate, (10 mg, 0.019 mmol, enantiomer 2 from chiral separation) in THF (1 ml) was added lithium hydroxide (0.195 ml, 0.195 mmol) . The mixture was stirred at rt for 2h. The mixture was acidified with 2N HCl to pH=3~4, extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated. The residue was purified by reverse HPLC (50-85% CH_3CN/H_2O+0.1% TFA) . The desired fraction was collected, diluted with EtOAc and H_2O. The organic layer was washed with brine, dried over $MgSO_4$, concentrated to give (3S,4R)-1-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid **24** (8 mg, yield 84%) . LCMS calc'd for $[(C_{21}H_{16}ClF_4N_3O_4)+H]^+$: 486, found: 486. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.30 (d, J = 8.3 Hz, 1H), 7.77 (dd, J = 11.5, 8.1 Hz, 2H), 7.66 (td, J = 8.1, 3.7 Hz, 2H), 7.17 (dd, J = 10.9, 8.2 Hz, 1H), 3.86 (ddq, J = 28.5, 11.7, 5.3 Hz, 2H), 3.74 – 3.65 (m, 1H), 2.82 – 2.63 (m, 1H), 2.63 – 2.52 (m, 1H), 2.38 – 2.24 (m, 1H), 1.94 – 1.83 (m, 1H), 1.78 (ddt, J = 21.0, 13.7, 6.3 Hz, 1H).

Preparation of (3S,4R) -1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid (25)



1) Preparation of 2-chloro-6-cyclopropylbenzoic acid



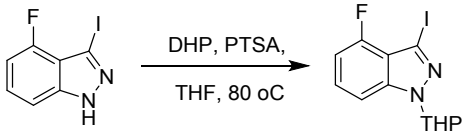
Step 1. To a solution of 1-bromo-3-chlorobenzene (81.5 g, 0.43 mol) in 500 mL of THF was added LDA (255 mL, 0.51 mol) solution dropwise at -78 ºC for 1h, the reaction mixture was stirred at the same temperature for 1h. After the addition of N,N-dimethylformamide (50 mL, 0.64 mmol)

at -78 °C dropwise, the reaction mixture was stirred at -78 °C for additional 2 hrs. TLC showed the reaction was completed (Pentane:EtOAc=10:1), then the mixture was quenched by sat.NH_4Cl solution (100 mL) and extracted with EtOAc (300 mL*3). The combined organic layers were washed with brine (100 mL*2), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Pentane:EtOAc = 100:1) to give 50 g (55%) of the title compound as a yellow solid. LCMS calc'd for $[(C_7H_4BrClO)+H]^+$: 219, found: 219.

Step 2. To a solution of 2-bromo-6-chlorobenzaldehyde (27g, 123 mmol) in 540 mL of toluene/ H_2O (8:1) were added K_3PO_4 (98.5 g, 369 mmol), cyclopropylboronic acid (16 g, 185 mmol) and $Pd(PPh_3)_4$ (2.8 g, 246 mmol) under N_2. The mixture was stirred at 100 °C for 12h. After the reaction finished (monitored with TLC, Pentane:EtOAc = 20:1), the mixture was cooled to 25 °C and filtered. The filtration was concentrated in vacuo. The residue was purified by column chromatography on silica gel (Pentane:EtOAc = 100:1) to give 15 g (55 %) of the title compound as a yellow oil. LCMS calc'd for $[(C_{10}H_9ClO)+H]^+$: 181, found: 181.

Step 3. To a solution of 2-chloro-6-cyclopropylbenzaldehyde (14 g, 77.7 mmol), NaH_2PO_4 (65 g, 544 mmol) and NH_2SO_3H (26 g, 271 mmol) in 300 mL of THF/H_2O (2:1) was added $NaClO_2$ (17.5 g, 194 mmol) in 50 mL of water dropwise at 0 °C. The mixture was stirred at the same temperature for 3h. After the reaction finished (monitored with TLC, Pentane:EtOAc = 5:1), the mixture was diluted with 100 mL of water and extracted with EtOAc (300 mL*3). The combined organic layers were washed with water (100 mL*2), brine (100 mL*2), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Pentane:EtOAc = 10:1 to pure DCM) to give 13 g (86.7%) of the title compound as a yellow solid. LCMS calc'd for $[(C_{10}H_9ClO_2)+H]^+$: 197, found: 197.

2) Preparation of 4-fluoro-3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole



To a solution of 4-fluoro-3-iodo-1H-indazole (10 g, 38.1 mmol) in 150 mL of THF was added dihydropyran (DHP) (11.5 g, 122.4 mmol) and PTSA (776 mg, 4 mmol). The reaction mixture heated to reflux for 6h. After the reaction was finished, The reaction mixture was poured into

water. The mixture was extracted with EtOAc (300 mL * 3) and the extracts were washed with brine, dried over Na_2SO_4 and concentrated to afford crude**,** the crude product was purified by silica gel chromatography eluted with Pentane:EtOAc = 50:1 to 5:1 to afford the title compound (7 g, yield: 54%) as a yellow solid. LCMS calc'd for $[(C_{12}H_{12}FIN_2O)+H]^+$: 347, found: 347.

3) Preparation of (3S,4R) -1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylic acid

Step 1. A solution of ethyl 1-benzyl-3-oxopiperidine-4-carboxylate, HCl (20.0 g, 67.2 mmol) in MeOH (200 ml) in a 500 ml 3-neck round bottle flask equipped with thermocouple was cooled to 0ºC and charged with sodium borohydride (7.62 g, 201 mmol) portionwise over a period of 75 min, avoiding excessive gas evolution. Then cooling bath was removed and the reaction mixture was kept stirring at rt for 2.5 hr. The mxiture was cooled to 0ºC again, quenched dropwise with 200 ml H_2O and extracted into EtOAc. The combined organic layers were washed with water, brine, and dried over Na_2SO_4, filtered and concentrated in vacuo to afford crude ethyl 1-benzyl-3-hydroxypiperidine-4-carboxylate. LCMS calc'd for $[(C_{15}H_{21}NO_3)+H]^+$: 264, found: 264.

Step 2. A solution of ethyl 1-benzyl-3-hydroxypiperidine-4-carboxylate (16.95 g, 63.5 mmol) from previous step and imidazole (13.15 g, 193 mmol) in DMF (85 ml) was cooled to 0ºC, followed by the addition of TBDPS-Cl (15 ml, 58.4 mmol). The reaction mixture was stirred at rt for 65 hr, quenched with 100 ml water slowly and extracted with MTBE. The combined organics were washed with H_2O, brine, dried over Na_2SO_4, filtered, concentrated in vacuo onto SiO_2 and purified via flash chromatography (Silicycle 40g, 0-15% EtOAc/Hexanes) to afford ethyl *trans*-1-benzyl-3-((tert-butyldiphenylsilyl)oxy)piperidine-4-carboxylate (10.3 g, 27% over 2 steps). LCMS calc'd for $[(C_{31}H_{39}NO_3Si)+H]^+$: 502, found: 502.

Step 3. A solution of ethyl *trans*-1-benzyl-3-((tert-butyldiphenylsilyl)oxy)piperidine-4-carboxylate (10.3 g, 20.4 mmol) and AcOH (5.85 ml, 102 mmol) in ethanol (50 ml) was evacuated and backfilled nitrogen (3x), charged with Pd-C (2.08 g, 1.955 mmol), evacuated and backfilled with hydrogen (3x). The reaction mixture was stirred at rt for 14 hr under a balloon of hydrogen, filtered through celite, and rinsed with CH_2Cl_2. The filtrate was concentrated filtrate in vacuo, then taken up in 100 ml EtOAc. The mixture was stirred vigorously with 200 ml sat aq $NaHCO_3$. The organic layer was separated and washed with sat aq $NaHCO_3$, water and brine, dried over Na_2SO_4,

filtered and concentrated filtrate in vacuo to provide ethyl *trans*-3-((tert-butyldiphenylsilyl)oxy)piperidine-4-carboxylate (7.96g, 94%). LCMS calc'd for $[(C_{24}H_{33}NO_3Si)+H]^+$: 412, found: 412.

Step 4. A mixture of 4-fluoro-3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (5.00 g, 14.5 mmol), ethyl *trans*-3-((tert-butyldiphenylsilyl)oxy)piperidine-4-carboxylate (7.96 g, 17.60 mmol), Cs_2CO_3 (14.12 g, 43.3 mmol) and RuPhos G1 Precat (953 mg, 1.17 mmol) in dioxane (35 ml) was sparged with N_2, sealed and The mixture was heated to 80ºC for 20 h, filtered through celite, and rinsed with EtOAc. The filtrate was concentrated organics in vacuo onto SiO_2 and purified via flash chromatography (10-40% EtOAc/Hexanes) to provide ethyl *trans*-3-((tert-butyldiphenylsilyl)oxy)-1-(4-fluoro-1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)piperidine-4-carboxylate (8.0 g, 96%). LCMS calc'd for $[(C_{36}H_{44}FN_3O_4Si)+H]^+$: 630, found: 630.

Step 5. A solution of ethyl *trans*-3-((tert-butyldiphenylsilyl)oxy)-1-(4-fluoro-1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)piperidine-4-carboxylate (8.0 g, 12.8 mmol) in DCM (56 ml) and methanol (16 ml) in a 250 ml 3-neck RBF equipped with addition funnel and thermocouple was cooled to ~5ºC internal temperature, followed by the addition of concentrated HCl (10.5 ml, 128 mmol) dropwise. After addition, the reaction mixture was kept stirring at rt for 51 h. The mixture was then diluted with water (temperature rose to ~30ºC), and the organic layer was separated. The aqueous layer was extracted with DCM. The combined organics were washed with sat aq $NaHCO_3$, brine, dried over Na_2SO_4, filtered, concentrated in vacuo and purified via flash chromatography (10-50% EtOAc/Hexanes) to afford ethyl *trans*-3-((tert-butyldiphenylsilyl)oxy)-1-(4-fluoro-1H-indazol-3-yl)piperidine-4-carboxylate (4.9 g, 71%) LCMS calc'd for $[(C_{31}H_{36}FN_3O_3Si)+H]^+$: 546, found: 546. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (4H, dd, J = 14.4, 6.8 Hz), 7.33 (6H, m), 7.07 (1H, d, J = 8.5 Hz), 6.60 (1H, dd, J = 10.8, 7.9 Hz), 4.27 (1H, m), 3.98 (2H, m), 3.75 (1H, dd, J = 12.0, 3.8 Hz), 3.69 (1H, d, J = 12.4 Hz), 2.99 (1H, m), 2.89 (1H, m), 2.63 (1H, dt, J = 11.8, 4.1 Hz), 2.04 (1H, d, J = 10.9 Hz), 1.91 (1H, m), 1.16 (3H, m), 0.97 (9H, s).

Step 6: To a flask was containing (3S,4R and 3R,4S)-ethyl 3-((tert-butyldiphenylsilyl)oxy)-1-(4-fluoro-1H-indazol-3-yl)piperidine-4-carboxylate (200 mg, 0.37 mmol) was added DIPEA (256 µl, 1.47 mmol), DMAP (22 mg, 0.18 mmol), DCM (1.2 ml), and 2-chloro-6-cyclopropylbenzoyl

chloride (158 mg, 0.73 mmol, prepared and used as crude through reacting the corresponding benzoic acid with oxalyl chloride in the presence of catalytic amount of DMF followed by concentration) and the resulting solution was allowed to stir at room temperature overnight. The reaction mixture was then concentrated and the residue was purified by flash chromatography (EtOAc/Hexanes 0-65%) to give desired product as a colorless solid. (167 mg, 62%) LCMS calc'd for $[(C_{41}H_{43}ClFN_3O_4Si)+H]^+$: 724, found: 724.

Step 7: To a vial was added (3S,4R and 3R,4S)-ethyl 3-((tert-butyldiphenylsilyl) oxy)-1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)piperidine-4-carboxyl ate (165 mg, 0.23 mmol), THF (2.3 ml), and TBAF (456 µl, 0.456 mmol) and the solution was heated to 50°C for 2 hours. The reaction mixture was cooled and diluted with saturated ammonium chloride. The mixture was diluted with ethyl acetate, washed with aqueous ammonium chloride and brine. Aqueous layers were back extracted once with ethyl acetate. The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc/Hexane 10-75%) to give desired product, which was separated by chiral separation to give the desired enantiomer (3S,4R)-ethyl 1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate (Peak 1, 19.6 mg, 17%). LCMS calc'd for $[(C_{25}H_{25}ClFN_3O_4)+H]^+$: 486, found: 486.

Step 8: To a flask was added (3S,4R)-ethyl 1-(1-(2-chloro-6-cyclopropylbenzoyl)-4-fluoro-1H-indazol-3-yl)-3-hydroxypiperidine-4-carboxylate (19.6 mg, 0.040 mmol), lithium hydroxide (9.7 mg, 0.40 mmol), THF (538 µl), and H_2O (269 µl) and the solution was allowed to stir at room temperature for 2 h. The reaction mixture was acidified with 2N HCl and then washed with EtOAc. The combined organic layers were dried With Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was purified by Prep-HPLC (Acetonitrile/Water + 0.10% TFA 50-95%) to get the desired product **25** (10.7 mg, 57%, purity >95%) as a colorless solid. LCMS calc'd for $[(C_{23}H_{21}ClFN_3O_4)+H]^+$: 458, found: 458. HRMS calc'd for $[(C_{23}H_{21}ClFN_3O_4)+H]^+$: 458.1277, found: 458.1290. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (d, J = 8.3 Hz, 1H), 7.74 (td, J = 8.2, 4.8 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.37 (dt, J = 8.0, 1.5 Hz, 1H), 7.31 (dd, J = 11.0, 8.1 Hz, 1H), 7.05 (d, J = 7.7 Hz, 1H), 3.75 – 3.65 (m, 2H), 3.61 – 3.55 (m, 1H), 2.71 (tt, J = 12.6, 2.9 Hz, 1H), 2.57 – 2.52 (m, 1H), 2.20 (ddd, J = 12.9, 9.4, 4.1 Hz,

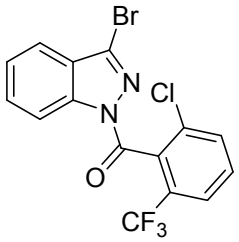
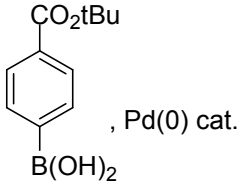
1H), 1.87 (tt, *J* = 10.0, 3.8 Hz, 1H), 1.72 – 1.67 (m, 1H), 1.64 (ddd, *J* = 12.6, 8.4, 3.9 Hz, 1H), 0.88 – 0.79 (m, 1H), 0.72 (tdd, *J* = 7.6, 6.3, 3.4 Hz, 2H), 0.65 – 0.59 (m, 1H). ^{13}C NMR (151 MHz, DMSO) δ 176.08, 166.29, 154.50 ($^{1}J_{CF}$ = 251.5 Hz), 154.33, 142.73, 142.65, 136.12, 132.89, 131.30, 129.77, 126.51, 123.90, 112.26, 111.61, 109.85, 67.27, 55.59, 50.03, 48.84, 27.01, 13.72, 8.69, 8.54.

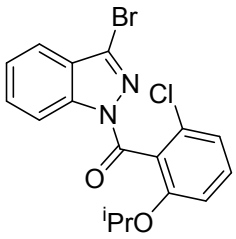
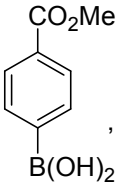
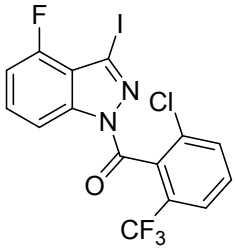
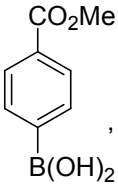
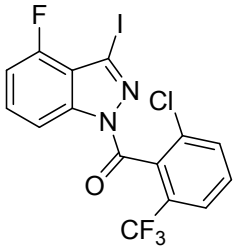
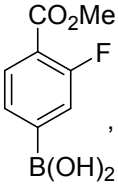
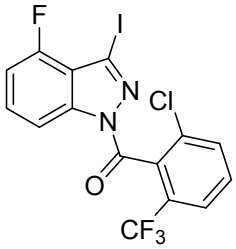
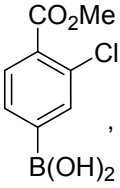
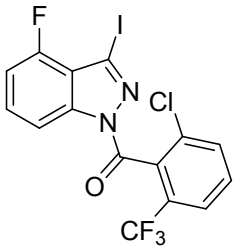
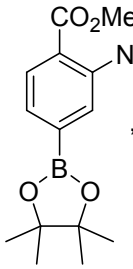
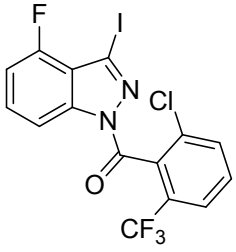
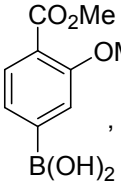
Synthesis of 4-(1-(2,6-dichlorobenzyl)-1H-indazol-3-yl)benzoic acid (**2**).

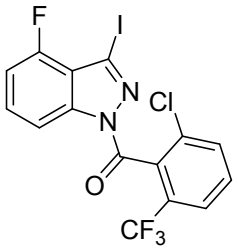
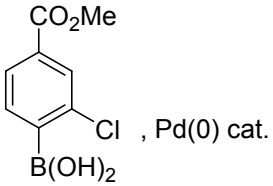
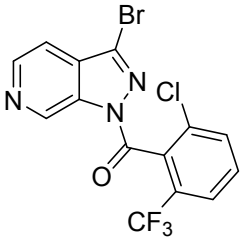
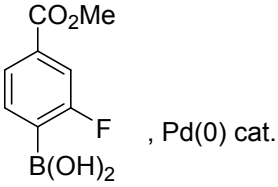
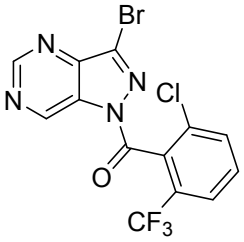
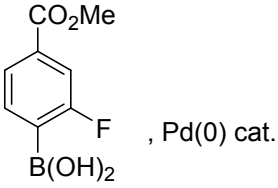
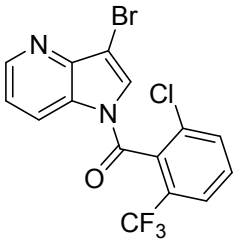
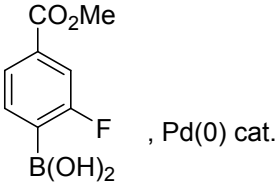
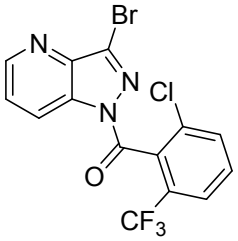
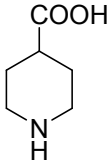
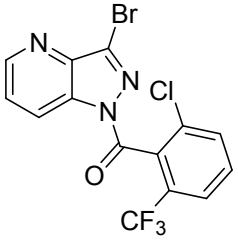
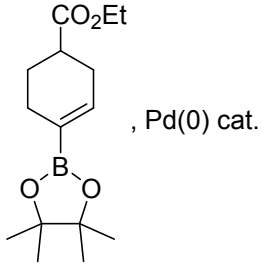
Compound **2** could be synthesized by following a similar procedure as described in WO2014/028591 for example 1B by reacting methyl 4-(1H-indazol-3-yl)benzoate with 2-(bromomethyl)-1,3-dichlorobenzene followed by ester hydrolysis . LCMS calc'd for $[(C_{21}H_{14}Cl_2N_2O_3)+H]^+$: 397, found: 397. Purity >95%.

Synthesis of compounds 3, 4, 6, 7, 8, 9, 11, 13, 15, 16, 17, 18, 19.

Synthesis of compounds **3-4, 6-9, 11, 13, 15-17** was carried out similarly according to the method described for both **12, 14,** and **5**[2] by employing the corresponding commercially available boronic acids or pinacol boronic esters, aryl halides, and bis-o-substituted benzoyl chlorides. Synthesis of compound **18** and **19** was carried out by employing the corresponding aza-indazole iodide according to the method described for **20** and **21** respectively. All the final compounds showed >95% purity as indicated by LCMS.

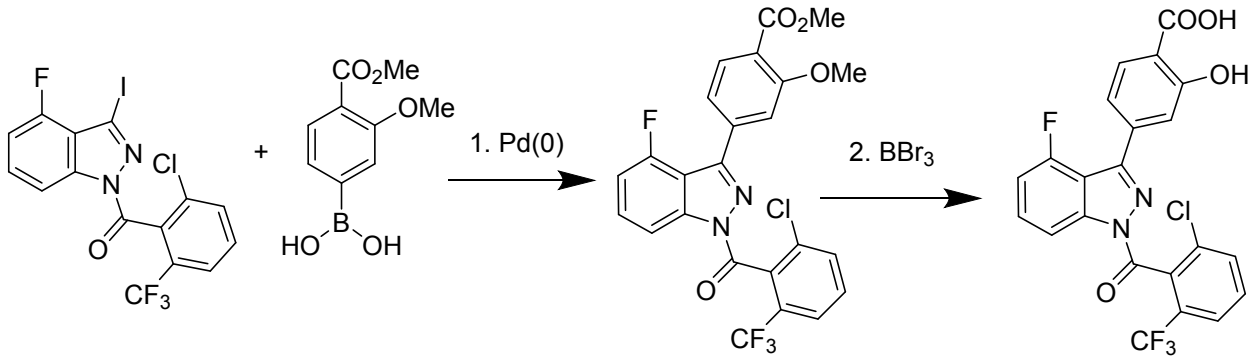
Compound #	Aryl halide	Coupling reagent and conditions	LCMS Calc'd for $[M+H]^+$	LCMS Found for $[M+H]^+$
3*			411	411

4		 , Pd(0) cat.	435	435
6		 , Pd(0) cat.	463	463
7		 , Pd(0) cat.	481	481
8		 , Pd(0) cat.	497	497
9		 , Pd(0) cat.	478	478
11		 , Pd(0) cat.	493	493

13		 , Pd(0) cat.	497	497
15		 , Pd(0) cat.	464	464
16		 , Pd(0) cat.	465	465
17		 , Pd(0) cat.	463	463
18			453	453
19		 , Pd(0) cat.	450	450

*Final ester deprotection was carried out in the presence of TFA instead of base.

Preparation of 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-2-hydroxybenzoic acid (10)



10

To a solution of intermediate methyl 4-(1-(2-chloro-6-(trifluoromethyl)benzoyl)-4-fluoro-1H-indazol-3-yl)-2-methoxybenzoate (prepared according to the procedures described for compound 11) (100 mg, 0.19 mmol) in DCM (10 mL) was added BBr_3 (1M, 0.37 mL, 0.37 mmol) at rt, and the mixture was kept stirring for 48 h. Then MeOH (~10 mL) was added to quench excess BBr_3 and the mixture was concentrated under reduced pressure. The residue was purified by prep HPLC to give the desired product **10** as a yellow solid. LCMS calc'd for $[(C_{22}H_{11}ClF4N_2O_4)+H]^+$: 479, found: 479. H NMR (400 MHz, MeOD) δ 8.46 (1H, d, J=8.4 Hz), 7.95-7.76 (5H, m), 7.35-7.29 (3H, m). Purity >95%.

Biological Assays[2]

RORg-LBD SRC-1 cofactor peptide interaction assay – The potency of small molecule RORg ligands was assessed by monitoring their effect on the association of a LXXLL-motif containing steroid receptor coactivator (SRC1) peptide. Compound (10 mM DMSO stock) was serially diluted in 3-fold steps using an Agilent Bravo liquid handler. Diluted compound or DMSO (25 nL) were transferred into a black Greiner 384 well plate (Cat#781076) using a LabCyte Echo acoustic dispenser. To each well of the plate was added 15 uL of 3.75 nM RORg-LBD in receptor buffer (50 mM Tris-HCl pH 7.0, 50 mM potassium chloride, 1 mM EDTA, 0.1% delipidated bovine serum albumin, 1 mM dithiothreitol, 1.25 nM Anti-His W1024 Europium chelate antibody (Perkin Elmer) and 3% (v/v) of lysate from ~24000 Sf9 cells). Compounds were allowed to incubate with receptor for 15 minutes and then 5 uL of peptide in detection buffer or detection buffer alone were added. Detection buffer (5x) consists of 50 mM Tris-HCL pH 7.0, 50 mM potassium chloride, 1 mM EDTA, 0.1% delipidated bovine serum albumin, 1 mM dithiothreitol and 20 nM streptavidin-APC (Perkin Elmer). When peptide (Biotin-SPSSHSSLTERHKILHRLLQEGSP) was included, its concentration in the 5x stock was 250 nM. The plate was then incubated overnight at 4 degrees. The following morning the plate was warmed to room temperature and read using an Envision plate reader (Perkin Elmer). TR-FRET signal was defined as the ratio of the fluorescence emission at 615 nm to 665 nm following excitation at 337 nm. The percent activity of each dilution was determined as the ratio of background corrected signal to the background corrected signal of wells receiving only DMSO. IC50 values were determined by fitting percent activity data to a four-parameter logistic dose response equation in GraphPad Prism (GraphPad Software).

Chimeric RORg-GAL4 reporter assay- The coding sequence of RORg aa97-518 was cloned in frame with the DNA binding domain of the yeast GAL4 protein within the CMV-promoter-driven pCDNA3.1 vector. This vector, along with the GAL4 UAS-luciferase reporter vector pGL4.31 (Promega), were used to transfect HEK293T cells. Briefly, $1x10^7$ cells in 10 mL of DMEM high glucose media with 10% FBS were transfected with a mixture consisting of 10 ug of each plasmid and 60 uL of TransIT-293 (Mirus Bio) in 1.5 mL of Optimem (Invitrogen). Following transfection, cells were transferred to one T75 flask and incubated overnight at 37 °C and 5% CO_2. Compound dilutions are prepared as above and 50 nL was transferred to a 384-well Greiner white tissue-culture treated plate (catalog #781080) using an Echo acoustic dispenser (LabCyte). Cells were

harvested and resuspended at $0.8x10^6$ cells per mL in DMEM high glucose media with 10% FBS. To each well of the plate was added 25 uL of cell suspension and the cells incubated overnight at 37°C and 5% CO_2. After 20-22 hours, the plates were brought to room temperature and 25 uL of Steady-Glo luciferase reagent (Promega) was added to each well. The luminescent signal was measured on an Envision plate reader. Determination of compound IC50 was performed as described above

PBMC Th17 polarization and IL-17 production assay- Test compounds were prepared as 10 mM stocks in DMSO and serially diluted 1:3 to provide an 8-concentration titration. The compounds (200 nL of each dilution) were acoustically dispensed into 96-well Costar 3912 assay plate. Frozen human PBMCs from a single donor were and diluted to a density of $5x10^5$cells/ml with growth media (RPMI 1640/ 10% FBS/ pen/strep). Stimulatory cytokines were added to final concentrations of 25 ng/mL IL-1B, 10 ng/mL IL-23, 0.5 ng/mL IL-2, 10 ng/mL IL-6 (all cytokines from R&D Systems). Additionally, T-Activator CD3/28 Dynabeads (Invitrogen) were added to a concentration of 100,000 beads per mL. The stimulated cells were immediately dispensed into the assay plate containing serially diluted compound at a volume of 200 uL cells per well. Cell plates were then incubated at at 37°C and 5% CO_2 for 4 days. Culture media (100 uL) was harvested from each well and IL-17 expression was measured by ELISA (R&D Systems) according to the manufacturer's instructions. Cell viability was assessed by addition of 100 uL of CellTiter-Glo (Promega) to each well of the cell assay plate followed by luminescence detection on an Envision plate reader (PerkinElmer).

Bcl-xL PD study protocol[3]

Single cell suspension was prepared from freshly harvested thymus from mice (Male C57Bl/6, 7wks old from Taconic). Cell were checked for viability and counted using Vi-cell XR counter (Beckman Coulter). DNA samples were prepared as per vendor's instructions using Qiagen RNeasy Mini Kit (#74104) to generate RNA and Bio-Rad iScript to synthesize cDNA (#170-8891). Quantitative real-time PCR was performed in a QuantStudio™ 7 Flex Real-Time PCR System, according to manufacturer's instructions (Thermo Fisher Scientific Inc.). Bcl-XL expressions were measured with TaqMan® Gene Expression Assays (Actb Mm00607939 and Bcl2l1 Mm00437783) (Thermo Fisher Scientific Inc.). The gene expression in individual treated animal was compared to the average expression in the tissues from vehicle treated control group, and expressed as percent inhibition relative to control group.

Note: All procedures were approved by the Animal Care and Use Committee of Merck & Co., Inc. (Boston, MA) and carried out at Merck & Co., Inc. All experiments adhered to the ''Guide for the Care and Use of Laboratory Animals''(Guide for the Care and Use of Laboratory Animals. 8th Edition; The National Academies Press: Washington, DC, 2011).

Table 1: Data collection and refinement statistics of 25**

PDB code	Compound 28
Data collection	
Space group	$P6_122$
Cell dimensions *a, b, c* (Å)	108.4, 108.4, 104.3
Resolution (Å)	48.11-2.87 (*3.03-2.87)
R_{merge}	0.065 (0.88)
$I/\sigma I$	33.5 (3.7)
Completeness (%)	100.0 (100.0)
Redundancy	18.8 (19.4)
Refinement	
Resolution (Å)	48.1-2.9
No. reflections	8712
R_{work} / R_{free}	0.20/0.26
No. atoms	
Protein	1987
Ligand	32
Solvent	5
B-factors	
Protein (Å^2)	86.6
Ligands (Å^2)	78.6
Solvent (Å^2)	71.4
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.14

*Values in parentheses are for highest-resolution shell

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Data from Panlabs profiling of 25

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
104010	Cholinesterase, Acetyl, ACES	343529	hum	2	10 µM	6	
116020	Cyclooxygenase COX-1	343546	hum	2	10 µM	0	
118010	Cyclooxygenase COX-2	343547	hum	2	10 µM	9	
140010	Monoamine Oxidase MAO-A	343496	hum	2	10 µM	8	
140120	Monoamine Oxidase MAO-B	343495	hum	2	10 µM	-14	
163200	Peptidase, CASP3 (Caspase 3)	343533	hum	2	10 µM	-2	
163500	Peptidase, CASP8 (Caspase 8)	343534	hum	2	10 µM	0	
163600	Peptidase, CASP9 (Caspase 9)	343535	hum	2	10 µM	0	
112510	Peptidase, CTSG (Cathepsin G)	343531	hum	2	10 µM	15	
152000	Phosphodiesterase PDE3	343532	hum	2	10 µM	-12	
176610	Protein Serine/Threonine Kinase, MAPK14 (p38α)	343539	hum	2	10 µM	-9	
171000	Protein Serine/Threonine Kinase, MAPK3 (ERK1)	343537	hum	2	10 µM	26	
171315	Protein Serine/Threonine Kinase, MARK3	343538	hum	2	10 µM	8	
178010	Protein Serine/Threonine Kinase, PKC, Non-Selective	343540	rat	2	10 µM	10	
170020	Protein Tyrosine Kinase, EGF Receptor	343689	hum	2	10 µM	-10	
174990	Protein Tyrosine Kinase, Insulin Receptor	343688	hum	2	10 µM	8	
195000	Tyrosine Hydroxylase	343541	rat	2	10 µM	12	
200510	Adenosine A_1	343558	hum	2	10 µM	5	
200610	Adenosine A_{2A}	343559	hum	2	10 µM	3	
203100	Adrenergic α_{1A}	343466	rat	2	10 µM	8	
203200	Adrenergic α_{1B}	343467	rat	2	10 µM	13	
203400	Adrenergic α_{1D}	343468	hum	2	10 µM	15	
203630	Adrenergic α_{2A}	343647	hum	2	10 µM	-11	
203710	Adrenergic α_{2B}	343648	hum	2	10 µM	6	
203810	Adrenergic α_{2C}	343649	hum	2	10 µM	-1	
204010	Adrenergic β_1	343478	hum	2	10 µM	1	
204110	Adrenergic β_2	343479	hum	2	10 µM	3	
204200	Adrenergic β_3	343480	hum	2	10 µM	-1	
204460	Adrenomedullin AM_1	343450	hum	2	10 µM	2	
206000	Androgen (Testosterone)	343517	hum	2	10 µM	6	
210030	Angiotensin AT_1	343605	hum	2	10 µM	7	
211000	Atrial Natriuretic Factor (ANF)	343575	gp	2	10 µM	10	
212510	Bradykinin B_1	343613	hum	2	10 µM	5	

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
212620	Bradykinin B_2	343518	hum	2	10 µM	-2	
217030	Cannabinoid CB_1	343563	hum	2	10 µM	7	
217100	Cannabinoid CB_2	343564	hum	2	10 µM	15	
217510	Chemokine CCR1	343615	hum	2	10 µM	-12	
244500	Chemokine CXCR2 (IL-8R$_B$)	343619	hum	2	10 µM	2	
244550	Chemokine CXCR4	343458	hum	2	10 µM	4	
218020	Cholecystokinin CCK_1 (CCK_A)	343522	hum	2	10 µM	8	
218130	Cholecystokinin CCK_2 (CCK_B)	343678	hum	2	10 µM	-7	
219150	Corticotropin Releasing Factor CRF_1	343576	hum	2	10 µM	2	
219500	Dopamine D_1	343561	hum	2	10 µM	-3	
219700	Dopamine D_{2S}	343562	hum	2	10 µM	0	
224010	Endothelin ET_A	343469	hum	2	10 µM	9	
224110	Endothelin ET_B	343470	hum	2	10 µM	-16	
226010	Estrogen ERα	343524	hum	2	10 µM	0	
226810	$GABA_A$, Chloride Channel, TBOB	343471	rat	2	10 µM	8	
226630	$GABA_A$, Ro-15-1788, Hippocampus	343612	rat	2	10 µM	7	
228510	$GABA_B$, Non-Selective	343472	rat	2	10 µM	-2	
232030	Glucocorticoid	343644	hum	2	10 µM	4	
232600	Glutamate, AMPA	343473	rat	2	10 µM	4	
232700	Glutamate, Kainate	343526	rat	2	10 µM	5	
237000	Glutamate, Metabotropic, $mGlu_5$	343618	hum	2	10 µM	3	
232810	Glutamate, NMDA, Agonism	343527	rat	2	10 µM	27	
232910	Glutamate, NMDA, Glycine	343614	rat	2	10 µM	-7	
233000	Glutamate, NMDA, Phencyclidine	343481	rat	2	10 µM	-4	
234000	Glutamate, NMDA, Polyamine	343474	rat	2	10 µM	9	
239000	Glycine, Strychnine-Sensitive	343475	rat	2	10 µM	-3	
239610	Histamine H_1	343552	hum	2	10 µM	4	
239710	Histamine H_2	343616	hum	2	10 µM	-8	
239820	Histamine H_3	343643	hum	2	10 µM	-1	
239900	Histamine H_4	343568	hum	2	10 µM	7	
250480	Leukotriene, Cysteinyl $CysLT_2$	343573	hum	2	10 µM	-21	
251010	Melanin-Concentrating Hormone MCH1 (SLC1)	343460	hum	2	10 µM	-6	
251100	Melanocortin MC_1	343482	hum	2	10 µM	6	
251300	Melanocortin MC_3	343483	hum	2	10 µM	4	
251350	Melanocortin MC_4	343484	hum	2	10 µM	3	

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
251400	Melanocortin MC_5	343485	hum	2	10 µM	11	
252610	Muscarinic M_1	343607	hum	2	10 µM	2	
252710	Muscarinic M_2	343608	hum	2	10 µM	-3	
252810	Muscarinic M_3	343609	hum	2	10 µM	-5	
252910	Muscarinic M_4	343610	hum	2	10 µM	10	
253010	Muscarinic M_5	343611	hum	2	10 µM	19	
257010	Neuropeptide Y Y_1	343476	hum	2	10 µM	-2	
257110	Neuropeptide Y Y_2	343621	hum	2	10 µM	2	
258010	Neurotensin NT_1	343577	hum	2	10 µM	9	
258590	Nicotinic Acetylcholine	343509	hum	2	10 µM	-4	
258700	Nicotinic Acetylcholine α1, Bungarotoxin	343510	hum	2	10 µM	-1	
260210	Opiate κ(OP2, KOP)	343622	hum	2	10 µM	-7	
260410	Opiate µ(OP3, MOP)	343623	hum	2	10 µM	-3	
260600	Orphanin ORL_1	343578	hum	2	10 µM	-1	
265010	Platelet Activating Factor (PAF)	343519	hum	2	10 µM	20	
265600	Potassium Channel [K_{ATP}]	343642	ham	2	10 µM	20	
267500	PPARγ	343641	hum	2	10 µM	39	
268020	Progesterone PR-B	343640	hum	2	10 µM	13	
268110	Prostanoid EP_1	343572	hum	2	10 µM	9	
268310	Prostanoid EP_3	343571	hum	2	10 µM	23	
268510	Prostanoid FP	343570	hum	2	10 µM	19	
268600	Prostanoid IP	343569	hum	2	10 µM	14	
268810	Purinergic P2Y	343639	rat	2	10 µM	20	
269500	Retinoid X Receptor RXRα	343638	hum	2	10 µM	7	
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	343486	hum	2	10 µM	27	
271200	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1B}$	343579	rat	2	10 µM	5	
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	343635	hum	2	10 µM	-7	
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	343636	hum	2	10 µM	4	
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	343637	hum	2	10 µM	4	
271910	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$	343565	hum	2	10 µM	0	
272200	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_6$	343545	hum	2	10 µM	1	
282700	Somatostatin sst2	343634	hum	2	10 µM	8	
255520	Tachykinin NK_1	343620	hum	2	10 µM	2	
255600	Tachykinin NK_2	343574	hum	2	10 µM	25	
286000	Thyrotropin Releasing Hormone (TRH)	343633	rat	2	10 µM	-19	
202000	Transporter, Adenosine	343560	gp	2	10 µM	-4	

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
219000	Transporter, Choline	343567	rat	2	10 µM	13	
220320	Transporter, Dopamine (DAT)	343549	hum	2	10 µM	-3	
226400	Transporter, GABA	343525	rat	2	10 µM	15	
204410	Transporter, Norepinephrine (NET)	343548	hum	2	10 µM	12	
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	343477	hum	2	10 µM	6	
287010	Vasoactive Intestinal Peptide VIP_1	343494	hum	2	10 µM	-12	
287530	Vasopressin V_{1A}	343542	hum	2	10 µM	-1	
287560	Vasopressin V_{1B}	343543	hum	2	10 µM	7	
287610	Vasopressin V_2	343544	hum	2	10 µM	2	

Representative procedure for rat and dog pharmacokinetics (PK) studies

The pharmacokinetics of compound **25** were studied in male Wistar Han rats and Dog Beagles after intravenous (IV) and oral administration (PO). For IV dosing at 0.5 mg/kg and 0.25 mg/kg, in rat and dog respectively, compound **25** was formulated as a solution in 30%captisol with 1 eq of NAOH, while for PO dosing at 1 mg/kg and 0.5 mg/kg in rat and dog respectively, the compound was formulated in 10%Tween 80. Plasma samples obtained from dosed animals were prepared for analysis by means of a single step protein precipitation technique by adding 200 μL of acetonitrile to 50 μL aliquots of individual subject samples. Samples were mixed by vortex for homogeneity and then subjected to centrifugation at 3500 rpm for 10 min. The supernatant (200 μL) was collected and injected into the LC-MS/MS for analysis. Pharmacokinetic parameters were calculated using established non-compartmental methods. The area under the plasma concentration versus time curve (AUC) was determined using the Watson software (version 7.3), with linear trapezoidal interpolation in the ascending slope and logarithmic trapezoidal interpolation in the descending slope. The portion of the AUC from the last measurable concentration to infinity was estimated from the equation, C_t/k_{el}, where C_t represents the last measurable concentration and k_{el} is the elimination rate constant. The latter was determined from the concentration versus time curve by linear regression at the terminal phase of the semi-logarithmic plot.

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