Supporting Information

Discovery of a selective and CNS penetrant negative allosteric modulator of metabotropic glutamate receptor subtype 3 with antidepressant and anxiolytic activity in rodents

Julie L. Engers[†], Alice L. Rodriguez[†], Leah C. Konkol[†,‡], Ryan D. Morrison[†,‖], Analisa D. Thompson[†], Frank W. Byers[†], Anna L. Blobaum[†], Sichen Chang[†], Daryl F. Venable[†,⊥], Matthew T. Loch[†], Colleen M. Niswender[†], J. Scott Daniels[†,‖], Carrie K. Jones[†], P. Jeffrey Conn[†], Craig W. Lindsley[†,§] and Kyle A. Emmitte*[,†,§,∞]

[†]Vanderbilt Center for Neuroscience Drug Discovery, Department of Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee 37232

[§]Department of Chemistry, Vanderbilt University, Nashville, TN 37232

Present Addresses

[‡] Incyte Corporation, Experimental Station, E336/131A, Route 141 and Henry Clay Road, Wilmington, Delaware 19880

[‖] Sano Informed Prescribing, Cool Springs Life Sciences Center, 393 Nichol Mill Lane, Suite 34, Franklin, TN 37067

[⊥] Covance Inc., 671 South Meridian Road, Greenfield, IN 46140

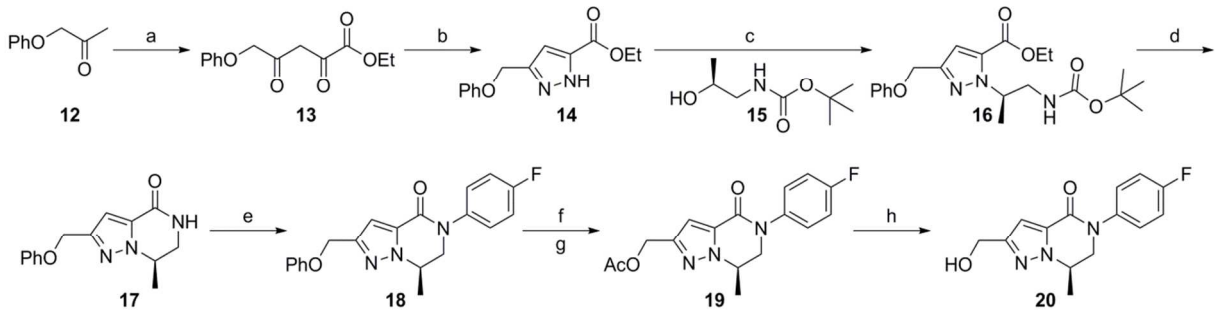
[∞] Department of Pharmaceutical Sciences, UNT System College of Pharmacy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107

Experimental Procedures and Spectroscopic Data

General. All NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 6120 or 6150 with ESI source. Method A: MS parameters were as follows: fragmentor: 70, capillary voltage: 3000 V, nebulizer pressure: 30 psig, drying gas flow: 13 L/min, drying gas temperature: 350 ºC. Samples were introduced via an Agilent 1290 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was generally observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Waters Acquity BEH C18, 1.0 x 50 mm, 1.7 um. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% TFA) over 1.4 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 55 ºC. Method B: MS parameters were as follows: fragmentor: 100, capillary voltage: 3000 V, nebulizer pressure: 40 psig, drying gas flow: 11 L/min, drying gas temperature: 350 ºC. Samples were introduced via an Agilent 1200 HPLC comprised of a degasser, G1312A binary pump, G1367B HP-ALS, G1316A TCC, G1315D DAD, and a Varian 380 ELSD (if applicable). UV absorption was generally observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Thermo Accucore C18, 2.1 x 30 mm, 2.6 um. Gradient conditions: 7% to 95% CH_3CN in H_2O (0.1% TFA) over 1.6 min, hold at 95% CH_3CN for 0.35 min, 1.5 mL/min, 45 ºC. High resolution mass spectra were obtained on an Agilent 6540 UHD Q-TOF with ESI source. MS parameters were as follows: fragmentor: 150, capillary voltage: 3500 V, nebulizer pressure: 60 psig, drying gas flow: 13 L/min, drying gas temperature: 275 ºC. Samples were introduced via an Agilent 1200 UHPLC comprised of a G4220A binary pump, G4226A

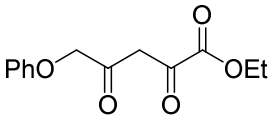
ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Agilent Zorbax Extend C18, 1.8 μm, 2.1 x 50 mm. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% formic acid) over 1 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 40 ºC. Optical specific rotations were obtained using JASCO P-2000 Digital Polarimeter equipped with Tungsten-Halogen lamp (WI), 589 nm wavelength, photomultiplier tube (1P28-01) detector and CG2-100 Cylindrical glass cell, 2.5ø x 100 mm. For compounds that were purified on a Gilson preparative reversed-phase HPLC, the system comprised of a 333 aqueous pump with solvent-selection valve, 334 organic pump, GX-271 or GX-281 liquid hander, two column switching valves, and a 155 UV detector. UV wavelength for fraction collection was user-defined, with absorbance at 254 nm always monitored. Method 1: Phenomenex Axia-packed Luna C18, 30 x 50 mm, 5 μm column. Mobile phase: CH_3CN in H_2O (0.1% TFA). Gradient conditions: 0.75 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.1% TFA) for 1 min, 50 mL/min, 23 ºC. Method 2: Phenomenex Axia-packed Gemini C18, 50 x 250 mm, 10 um column. Mobile phase: CH_3CN in H_2O (0.1% TFA). Gradient conditions: 7 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.1% TFA) for 7 min, 120 mL/min, 23 ºC. Chiral separation was performed on a Thar (Waters) Investigator SFC Column: Chiral Technologies CHIRALPAK IF, 4.6 x 250 mm, 5 μm column. Gradient conditions: 20% to 50% IPA in CO_2 over 7 min, hold at 50% CO_2 for 1 min. Flow rate: 3.5 mL/min. Column temperature: 40 ºC. System backpressure: 100 bar. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification.

Preparation of Intermediate 20. Compound **20** was prepared via the route pictured immediately below.



Reagents and conditions: (a) Na, EtOH, $(EtO_2C)_2$, 0 ºC to r.t., 27%; (b) $NH_2NH_2 \cdot H_2O$, EtOH, 80 ºC, 98%; (c) PPh_3, D^{t}BAD, THF, µwave, 120 ºC, 20 min.; (d) 4M HCl in dioxane, then saturated aq. $NaHCO_3$, 74%, 2 steps; (e) CuI, K_2CO_3, 4-fluorobromobenzene, *N,N′*-dimethylethylenediamine, PhMe, 120 ºC, 83%; (f) BBr_3, CH_2Cl_2, 0 ºC to r.t. (g) KOAc, DMF, 60 ºC, 100%, 2 steps; (h) 1M aq. LiOH, MeOH, THF, 94%.

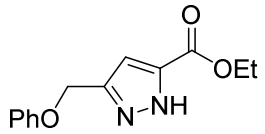
Ethyl 2,4-dioxo-5-phenoxypentanoate (13)



To a flame-dried round bottom flask was added ethanol (280 mL), which was then cooled to 0 ºC. To this was added portion-wise sodium (4.2 g, 183 mmol, 1.1 eq.). The mixture was gradually warmed to room temperature and stirred until the sodium was completely consumed. The mixture was cooled to 0 ºC and phenoxy acetone **12** (25.0 g, 166 mmol, 1.0 eq.) was added drop-wise. The mixture was stirred for 10 min at 0 ºC, and then diethyl oxalate (22.6 mL, 166 mmol, 1.0 eq.) was added. The reaction was allowed to warm to room temperature. Reaction progress was monitored by LCMS and was complete within 1 hour. The crude reaction mixture was concentrated *in vacuo* to remove the ethanol and the crude residue was taken back up in

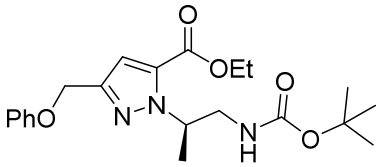
dichloromethane (100 mL). The solution was washed once with 1M HCl. The organic layer was washed once with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified via flash column chromatography yielding the title compound as an impure mixture (11.3 g, 45.1 mmol, 27% yield). This mixture was carried on without further purification. LCMS (Method B): R_T = 1.195 min, *m/z* = 251.1 $[M+H]^+$.

Ethyl 3-(phenoxymethyl)-1*H*-pyrazole-5-carboxylate (14)



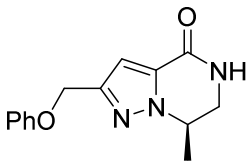
To a round bottom flask equipped with a reflux condenser was added impure **13** (11.3 g, 45.1 mmol, 1.0 eq.) in ethanol (50 mL). To this solution was added hydrazine hydrate (1.42 mL, 45.1 mmol, 1.0 eq.), and the reaction was heated to 80 ºC overnight after which time the reaction was deemed complete by LCMS. The solvent was removed *in vacuo*, and the residue was purified via flash column chromatography, yielding the title compound as a pale yellow solid (10.9 g, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (bs, 1H), 7.32–7.29 (m, 2H), 7.00–6.96 (m, 2H), 6.92 (s, 1H), 5.17 (s, 2H), 4.38 (q, *J* = 7.12 Hz, 2H), 1.39 (t, *J* = 7.12 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.5, 158.2, 146.9, 138.2, 129.6 (2C), 121.3, 114.8 (2C), 107.9, 62.8, 61.4, 14.2; LCMS (Method B): R_T = 0.957 min, *m/z* = 247.1 $[M+H]^+$; HRMS, calc'd for $C_{13}H_{14}N_2O_3$[M], 246.1004; found 246.1005.

Ethyl (*R*)-1-(1-((*tert*-butoxycarbonyl)amino)propan-2-yl)-3-(phenoxymethyl)-1*H*pyrazole-5-carboxylate (16)



Compound **14** (2.15 g, 8.7 mmol, 1.0 eq.) and *tert*-butyl (*S*)-(2-hydroxypropyl) carbamate **15** (3.05 g, 17.4 mmol, 2.0 eq.) were dissolved in THF (60 mL. 0.2 M) and triphenyl phosphine (4.11 g, 15.7 mmol, 1.8 eq.) were added. After stirring for 5 min, the mixture was cooled to 0 ºC and di-*tert*-butyl azodicarboxylate (3.61 g, 15.66 mmol, 1.8 eq.) was added. The reaction mixture was then split evenly among four microwave reaction vials and subjected to microwave irradiation at 120 ºC for 20 min. The solvent was removed *in vacuo*, and the residue was purified using flash column chromatography, yielding the title compound as a white solid (5.32 g, yield not determined due to contamination with D^{t}BAD byproduct, di-tert-butyl hydrazine-1,2-dicarboxylate). ^{1}H NMR (400 MHz, MeOD) δ 7.30 (dd, *J* = 8.7, 7.4 Hz, 2H), 7.01 (dd, *J* = 8.7, 1.0 Hz, 2H), 6.96 (t, *J* = 7.32 Hz, 1H), 6.91 (s, 1H), 5.56 (q, *J* = 6.67 Hz, 1H), 5.51 (s, 1H), 4.34 (q, *J* = 9.5 Hz, 2H), 3.44–3.43 (m, 2H), 1.48–1.36 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 158.5, 155.8, 147.6, 133.8 (2C), 129.5, 121.1, 114.8 (2C), 110.7, 79.3, 63.8, 61.1, 56.0, 45.3, 28.2 (3C), 18.5, 14.2; LCMS (Method B): R_T = 1.275 min, *m/z* = 404.2 $[M+H]^+$; HRMS, calc'd for $C_{21}H_{29}N_3O_5$ [M], 403.2107 found 403.2108.

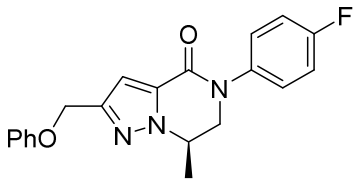
(*R*)-7-Methyl-2-(phenoxymethyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (17)



Compound **16** (5.32 g, 8.7 mmol, 1.0 eq.) was treated with a solution of 4N HCl in 1,4-dioxane 29 mL). Deprotection of the *tert*-butyl carbamate was monitored by LCMS. Once complete, the

reaction mixture was carefully basified with saturated aqueous $NaHCO_3$ (verified by pH paper) and was allowed to stir at room temperature overnight. The mixture was diluted with dichloromethane, and the aqueous layer was extracted with dichloromethane (3x). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide the title compound as a white solid (1.66 g, 74% yield over two steps) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (bs, 1H), 7.30–7.26 (m, 2H), 7.00–6.94 (m, 4H), 5.23 (s, 2H), 4.55–4.50 (m, 1H), 3.81–3.76 (m, 1H), 3.49–3.44 (m, 1H), 1.60 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8, 158.5, 148.8, 134.2, 129.5 (2C), 121.1, 115.7 (2C), 107.6, 63.7, 52.3, 46.3, 17.1; LCMS (Method B): R_T = 0.808 min, *m/z* = 258.1 $[M+H]^+$; HRMS, calc'd for $C_{14}H_{15}N_3O_2$ [M], 257.1164; found 257.1165; $[\alpha]^{25}_D = -36.3°$ (*c* 0.443, $CHCl_3$); Chiral HPLC analysis = 98.6% e.e.

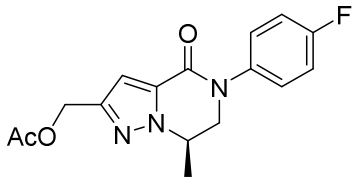
(*R*)-5-(4-Fluorophenyl)-7-methyl-2-(phenoxymethyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (18)



To a round bottom flask equipped with a condenser was added **17** (1.66 g, 6.45 mmol, 1.0 eq.), toluene (65 mL), 4-fluoro bromobenzene (1.50 mL, 13.2 mmol, 2.05 eq.), *N*,*N*′-dimethylethylenediamine (3.80 mL, 35.5 mmol, 5.5 eq.), and potassium carbonate (1.80 g, 13.2 mmol, 2.05 eq.). Lastly, copper (I) iodide (2.50 g, 13.2 mmol, 2.05 eq.) was added. The reaction was refluxed overnight after which time the reaction was deemed complete by LCMS. The reaction was cooled to room temperature and filtered through Celite, washing with EtOAc. The

filtrate was concentrated *in vacuo*, and the residue was purified using flash column chromatography, yielding the title compound as a white solid (1.88 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.28 (m, 4H), 7.15–7.11 (m, 2H), 7.04–6.96 (m, 4H), 5.13 (s, 2H), 4.72–4.67 (m, 1H), 4.16 (dd, J = 12.8, 4.2 Hz, 1H), 3.88 (dd, J = 12.8, 7.3 Hz, 1H), 1.69 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, $J_{C,F}$ = 247 Hz), 158.5, 157.1, 149.2, 137.1 (d, $J_{C,F}$ = 3 H), 134.7, 129.5 (2C), 127.2 (d, $J_{C,F}$ = 9 Hz, 2C), 121.1, 116.2 (d, $J_{C,F}$ = 23 Hz, 2C), 114.8 (2C), 108.5, 63.7, 55.3, 52.5, 17.1; LCMS (Method B): R_T = 1.085 min, *m/z* = 352.1 $[M+H]^+$; HRMS, calc'd for $C_{20}H_{18}FN_3O_2$ [M], 351.1383; found 351.1382; $[\alpha]^{25}_D$ = –18.7º (*c* 0.203, $CHCl_3$).

(*R*)-(5-(4-Fluorophenyl)-7-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazin-2-yl)methyl acetate (19)

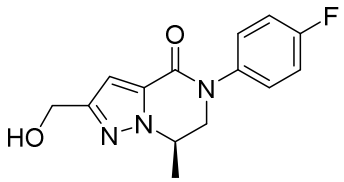


To a solution of **18** (1.88 g, 5.35 mmol, 1.0 eq.) in dichloromethane (30 mL) at 0 ºC was added a solution of 1M BBr_3 in dichloromethane (21.4 mL, 21.4 mmol, 4.0 eq.). The reaction was warmed to room temperature and stirred for 2 h. The reaction was diluted with dichloromethane (30 mL). The organic layers were washed sequentially with 1M aqueous HCl (40 mL), saturated aqueous $NaHCO_3$ (40 mL), 1M aqueous NaOH (40 mL), and brine (40 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* providing the title compound as a light brown solid (1.62 g, 90% yield) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.29 (m, 2H), 7.15–7.11 (m, 2H), 6.98 (s, 1H), 4.70–4.65 (m, 1H), 4.54, 4.50

(ABq, *J*AB = 11.0 Hz, 2H), 4.15 (dd, *J* = 15.1, 4.3 Hz, 1H), 3.88 (dd, *J* = 12.8, 7.2 Hz, 1H), 1.68 (d, *J* = 6.7 Hz); HRMS, calc'd for $C_{14}H_{13}BrN_3O$ [M], 337.0226; found 337.0224.

Potassium acetate (1.66 g, 16.9 mmol, 3.5 eq.) was added to a solution of bromo intermediate (1.62 g, 4.82 mmol, 1.0 eq.) in DMF (24 mL). The mixture was stirred at 60 ºC for 2 h and then diluted with EtOAc (60 mL), washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded the title compound (1.53 g, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (m, 2H), 7.17–7.12 (m, 2H), 6.97 (s, 1H), 5.17 (s, 2H), 4.74–4.66 (m, 1H), 4.15 (dd, *J* = 12.8, 4.2 Hz, 1H), 3.89 (dd, *J* = 12.8, 7.3 Hz, 1H), 2.12 (s, 3H), 1.68 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 161.1 (d, $J_{C,F}$ = 246 Hz), 157.1, 148.2, 137.1 (d, $J_{C,F}$ = 3 Hz), 134.7, 127.1 (d, $J_{C,F}$ = 9 Hz, 2C), 116.2 (d, $J_{C,F}$ = 23 Hz, 2C), 108.8, 59.8, 55.3, 52.3, 20.9, 17.1; LCMS (Method B): R_T = 0.854 min, *m/z* = 318.1 $[M+H]^+$; HRMS, calc'd for $C_{16}H_{16}FN_3O_3$ [M], 317.1176; found 317.1175.

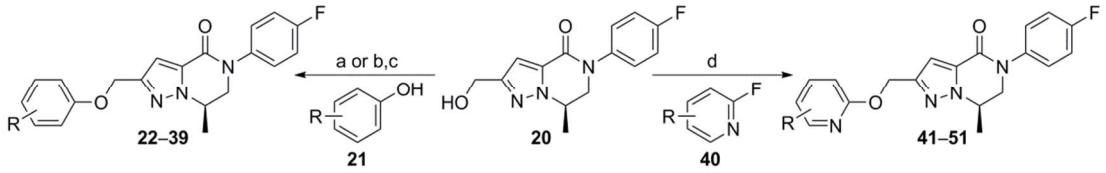
(*R*)-5-(4-Fluorophenyl)-2-(hydroxymethyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (20)



Compound **19** (1.52 g, 4.8 mmol, 1.0 eq.) was dissolved in MeOH (5.0 mL) and THF (5.0 mL). To this solution, 1N aqueous LiOH (24.0 mL, 24 mmol, 5.0 eq.) was added. The mixture was stirred for 30 min, then diluted with water and extracted with EtOAc (3x). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography on silica gel gave the title compound (1.25 g, 94% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.31 (m, 2H), 7.16-7.11 (m, 2H), 6.93 (s, 1H), 4.75 (d, *J* =

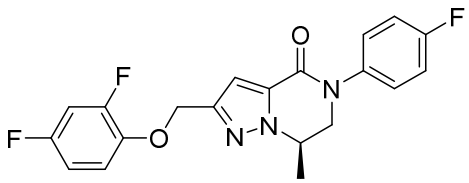
5.6 Hz, 2H), 4.71-4.63 (m, 1H), 4.16 (dd, J = 12.8, 4.3 Hz, 1H), 3.88 (dd, J = 12.8, 7.3 Hz, 1H), 2.3 (t, 1H), 1.68 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, $J_{C,F}$ = 247 Hz), 157.2, 152.8, 137.1 (d, $J_{C,F}$ = 3 Hz), 134.6, 127.2 (d, $J_{C,F}$ = 9 Hz, 2C), 116.2 (d, $J_{C,F}$ = 23 Hz, 2C), 107.1, 58.9, 55.3, 52.4, 17.1; LCMS (Method B): R_T = 0.654 min, *m/z* = 276.1 $[M+H]^+$; HRMS, calc'd for $C_{14}H_{14}FN_3O_2$ [M], 275.1070; found 275.1073; $[\alpha]^{25}_D$ = –22.9º (*c* 0.333, MeOH).

General Experimental Procedure I. Compounds **22**–**39** and **41**–**51** were prepared via one of three methods from intermediate **20** as pictured immediately below. The synthesis of compounds **25**, **38**, and **44** are described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) PS-PPh_3, THF, DIAD, 7–37%; (b) NEt_3, MsCl, CH_2Cl_2, 63%; (c) Cs_2CO_3, DMF, 90 ºC, 35-52%; (d) NaH, DMF, 8–37%.

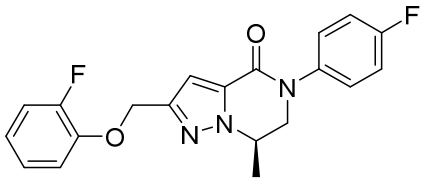
(7*R*)-2-[(2,4-Difluorophenoxy)methyl]-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one (25)



To a solution of compound **20** (450 mg, 1.63 mmol, 1.0 eq.) in dichloromethane (5.5 mL) was added Et_3N (0.273 mL, 1.96 mmol, 1.2 eq.) and methanesulfonyl chloride (0.152 mL, 1.96 mmol, 1.2 eq.). The reaction mixture was stirred at room temperature for 60 min. 1M HCl (5 mL) was added. The mixture was extracted with dichloromethane (3x). The combined extracts were washed with sat. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using by flash chromatography gave the mesylate intermediate which was used immediately in subsequence reactions. To a solution of 2,4-difluorophenol (10.5 □L, 0.11 mmol, 2.5 eq.) in DMF (0.3 mL, 0.1 M) was added Cs_2CO_3 (35 mg, 0.11 mmol, 2.5 eq.). The mixture was stirred for 5 min and a solution of mesylate intermediate (15 mg, 0.042 mmol, 1.0 eq.) in DMF (0.2 mL) was added. The resulting mixture was stirred at 90 ºC for 30 min. Upon completion by LCMS, the reaction mixture was filtered. Purification using reverse phase HPLC Method 1 with 26% to 55% CH_3CN in H_2O (0.1% TFA) over 4 min provided the title compound (5.7 mg, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.29 (m, 2H), 7.14-7.09 (m, 2H), 7.06-7.00 (m, 2H), 6.88-6.82 (m, 1H), 6.79-6.74 (m, 1H), 5.14 (s, 2H), 4.71-4.4 (m, 1H), 4.15 (dd, J = 12.8, 4.3 Hz, 1H), 3.87 (dd, J = 12.8, 7.3 Hz, 1H), 1.67 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, $J_{C,F}$ = 247 Hz), 156.9 (dd, $J_{C,F}$ = 240, 10 Hz), 157.1, 154.9 (dd, $J_{C,F}$ = 248, 12 Hz), 148.6, 142.9 (dd, $J_{C,F}$ = 11, 4 Hz), 137.1 (d, $J_{C,F}$ = 3 Hz), 134.8, 127.1 (d, $J_{C,F}$ = 9 Hz, 2C), 116.9 (dd, $J_{C,F}$ = 9, 3 Hz), 116.2 (d, $J_{C,F}$ = 23 Hz, 2C), 110.4 (dd, $J_{C,F}$ = 23, 4 Hz), 108.6, 105.0 (dd, $J_{C,F}$ = 26, 22 Hz), 65.9, 55.2, 52.5, 17.1; LCMS (Method A): R_T = 0.809 min, *m/z* = 388.3 $[M+H]^+$; HRMS, calc'd for $C_{20}H_{16}F_3N_3O_2$ [M], 387.1195; found 387.1195; $[\alpha]^{25}_D$ = −19.0º (*c* 0.246, $CHCl_3$).

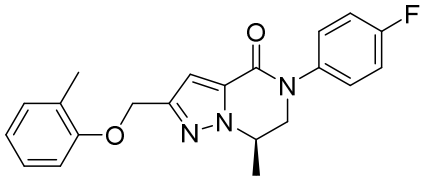
The following compounds were prepared analogous to compound ***25***

(*R*)-2-((2-Fluorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (22)



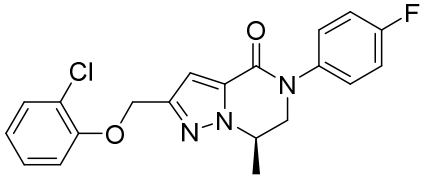
LCMS (Method B): R_T = 1.105 min, *m/z* = 369.7 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-((*m*-tolyloxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (27)



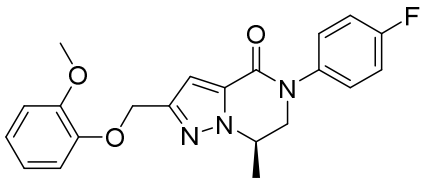
LCMS (Method B): R_T = 1.150 min, *m/z* = 366.2 $[M+H]^+$.

(*R*)-2-((2-Chlorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (29)



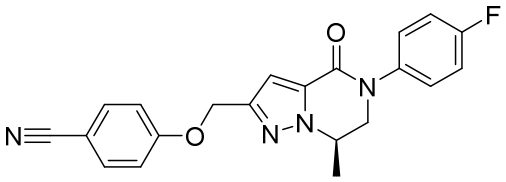
LCMS (Method B): R_T = 1.150 min, *m/z* = 385.7 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-((2-methoxyphenoxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (32)



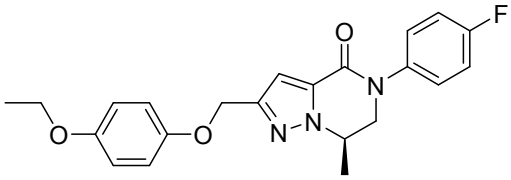
LCMS (Method B): R_T = 1.057 min, *m/z* = 381.7 $[M+H]^+$.

(*R*)-4-((5-(4-Fluorophenyl)-7-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazin-2-yl)methoxy)benzonitrile (37)



LCMS (Method B): R_T = 1.046 min, *m/z* = 367.7 $[M+H]^+$.

(7*R*)-2-[(4-Ethoxyphenoxy)methyl]-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one (38)

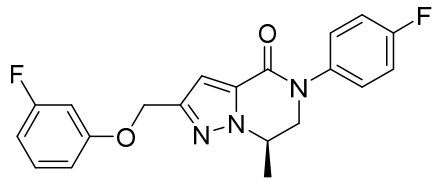


To a solution of compound **20** (15 mg, 0.054 mmol, 1.0 eq.) in THF (0.5 mL) was added 4-ethoxyphenol (9.0 mg, 0.063 mmol, 1.2 eq.), PS-PPh_3 (3 mmol/g, 21 mg, 0.063 mmol, 1.2 eq.) and DIAD (12 µL, 0.063 mmol, 1.2 eq.). The reaction mixture was stirred at room temperature and monitored by LCMS. Upon completion, the mixture was filtered through a Celite pad which was washed with EtOAc. Solvents were removed. Purification using reverse phase HPLC Method 1 with: 56% to 83% CH_3CN in H_2O (0.1% TFA) over 4 min provided the title compound (3.7 mg, 18% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.29 (m, 2H), 7.15-7.13 (m,

2H), 7.01 (s, 1H), 6.94-6.91 (m, 2H), 6.85-6.82 (m, 2H), 5.07 (s, 2 H), 4.73-4.65 (m, 1H), 4.16 (dd, J = 12.8, 4.3 Hz, 1H), 3.98 (q, J = 7.0 Hz, 2H), 3.87 (dd, J = 12.8, 7.3 Hz, 1H), 1.69 (d, J = 6.5 Hz, 3H), 1.39 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6 (d, $J_{C,F}$ = 247 Hz), 157.2, 153.4, 152.5, 149.5, 137.2 (d, $J_{C,F}$ = 3 Hz), 134.6, 127.1 (d, $J_{C,F}$ = 9 Hz, 2C), 116.2 (d, $J_{C,F}$ = 23 Hz, 2C), 115.9 (2C), 115.4 (2C), 108.5, 64.4, 64.0, 55.3, 52.5, 17.2, 15.0; LCMS (Method A): R_T = 1.106 min, *m/z* = 396.4 $[M+H]^+$; HRMS, calc'd for $C_{22}H_{22}FN_3O_3$ [M], 395.1645; found 395.1648; $[\alpha]^{25}_D = -11.7°$ (*c* 0.14, $CHCl_3$).

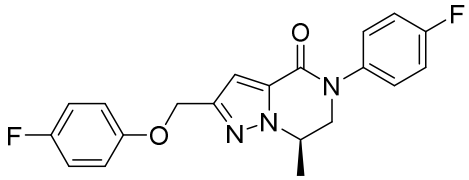
The following compounds were prepared analogous to compound ***38***

(*R*)-2-((3-Fluorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (22)



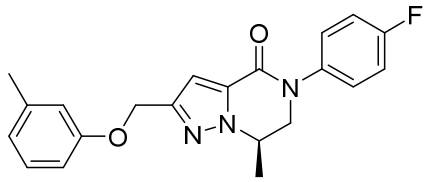
LCMS (Method B): R_T = 1.117 min, *m/z* = 370.1 $[M+H]^+$.

(*R*)-2-((4-Fluorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (24)



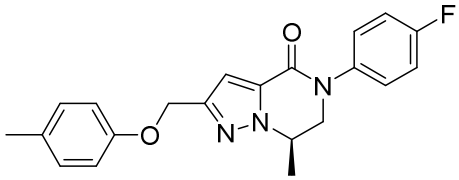
LCMS (Method B): R_T = 1.095 min, *m/z* = 370.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-((*m*-tolyloxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (27)



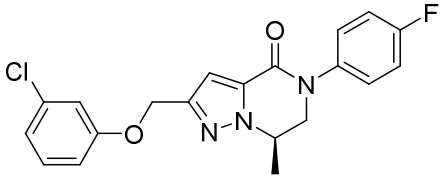
LCMS (Method B): R_T = 1.137 min, *m/z* = 366.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-((*p*-tolyloxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (28)



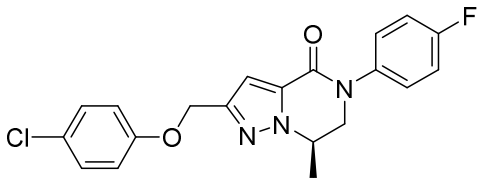
LCMS (Method B): R_T = 1.138 min, *m/z* = 366.1 $[M+H]^+$.

(*R*)-2-((3-Chlorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (30)



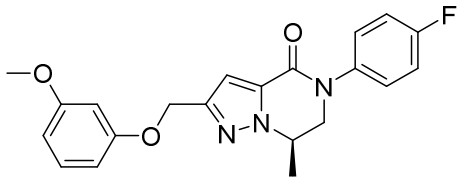
LCMS (Method B): R_T = 1.166 min, *m/z* = 386.0 $[M+H]^+$.

(*R*)-2-((4-Chlorophenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (31)



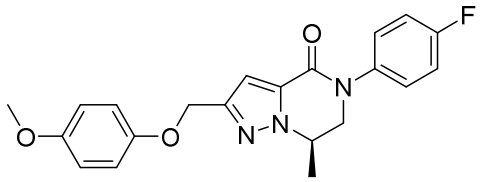
LCMS (Method B): R_T = 1.159 min, *m/z* = 386.0 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-((3-methoxyphenoxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (33)



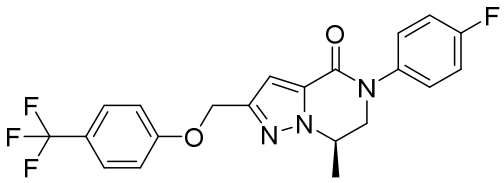
LCMS (Method B): R_T = 1.085 min, *m/z* = 382.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-((4-methoxyphenoxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (34)



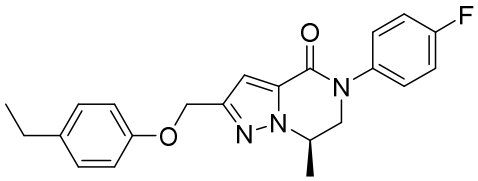
LCMS (Method A): R_T = 0.994 min, *m/z* = 382.3 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-((4-(trifluoromethyl)phenoxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (35)



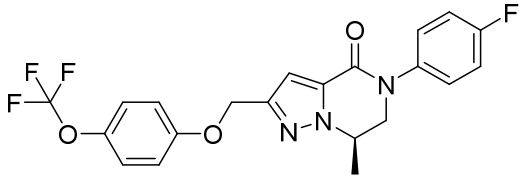
LCMS (Method B): R_T = 1.196 min, *m/z* = 420.1 $[M+H]^+$.

(*R*)-2-((4-Ethylphenoxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (36)



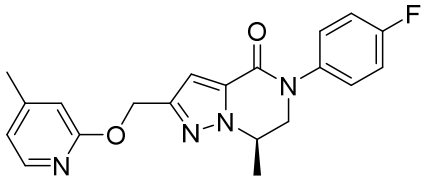
LCMS (Method B): R_T = 1.196 min, *m/z* = 380.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-((4-(trifluoromethoxy)phenoxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (39)



LCMS (Method B): R_T = 1.213 min, *m/z* = 436.0 $[M+H]^+$.

(7*R*)-5-(4-Fluorophenyl)-7-methyl-2-[(4-methyl-2-pyridyl)oxymethyl]-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one (44)

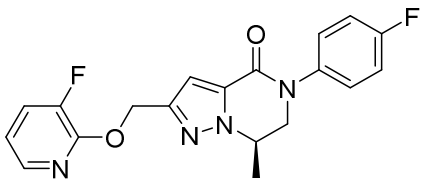


To a solution of compound **20** (15 mg, 0.054 mmol, 1.0 eq.) in DMF (0.5 mL, 0.11 M) at room temperature was added NaH (60% in mineral oil, 3.0 mg, 0.07 mmol, 1.3 eq.). The resulting mixture was stirred for 15 min and 2-fluoro-4-methylpyridine (12 µL, 0.11 mmol, 2.0 eq.) was added. The reaction mixture was stirred overnight. Water was added. The mixture was extracted

with EtOAc (3x). The combined extracts were concentrated. Purification using reverse phase HPLC Method 1, gradient condition: 23% to 55% CH_3CN in H_2O (0.1% TFA) over 4 min provided the title compound (1.7 mg, 9 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 5.2 Hz, 1H), 7.32-7.29 (m, 2H), 7.14-7.09 (m, 2H), 7.01 (s, 1H), 6.72 (d, *J* = 5.1 Hz, 1H), 6.61 (s, 1H), 5.42 (s, 2H), 4.73-4.65 (m, 1H), 4.16 (dd, *J* = 12.8, 4.3 Hz, 1H), 3.86 (dd, *J* = 12.8, 7.2 Hz, 1H), 2.29 (s, 3H), 1.69 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 160.0 (d, $J_{C,F}$ = 246 Hz), 152.2, 148.9, 148.7, 145.3, 136.2 (d, $J_{C,F}$ = 3 Hz), 133.4, 126.1 (d, $J_{C,F}$ = 8 Hz, 2C), 117.7, 115.1 (d, $J_{C,F}$ = 22 Hz, 2C), 110.3, 107.8, 59.9, 54.3, 51.4, 19.9, 16.2; LCMS (Method A): R_T = 0.795 min, *m/z* = 367.2 $[M+H]^+$; HRMS, calc'd for $C_{20}H_{19}FN_4O_2$ [M], 366.1492; found 366.1491.

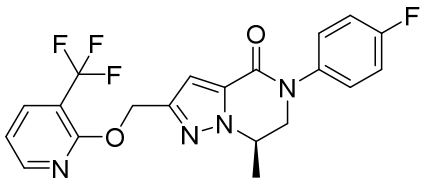
*The following compounds were prepared analogous to compound **44***

(*R*)-5-(4-Fluorophenyl)-2-(((3-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (41)



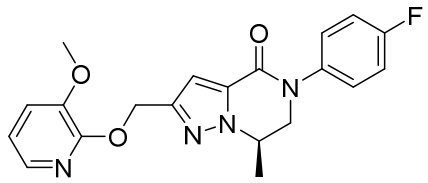
LCMS (Method B): R_T = 1.037 min, *m/z* = 371.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-(((3-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (42)



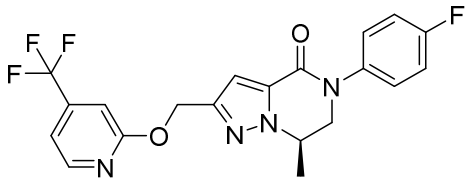
LCMS (Method B): R_T = 1.127 min, *m/z* = 421.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-(((3-methoxypyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (43)



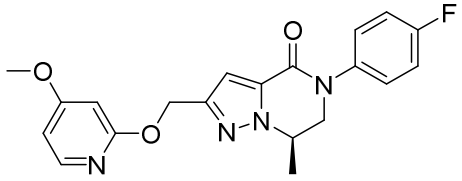
LCMS (Method B): R_T = 0.981 min, *m/z* = 383.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-(((4-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (45)



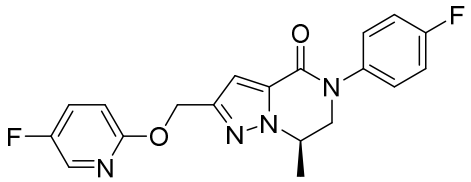
LCMS (Method B): R_T = 1.166 min, *m/z* = 421.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-(((4-methoxypyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (46)



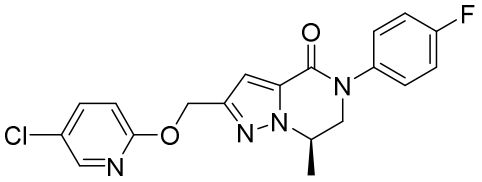
LCMS (Method A): R_T = 0.760 min, *m/z* = 383.3 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (47)



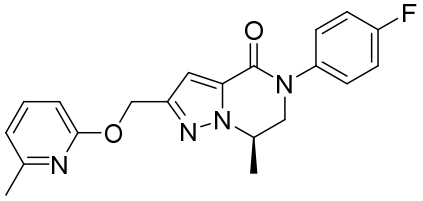
LCMS (Method B): R_T = 1.034 min, *m/z* = 371.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(4-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (48)



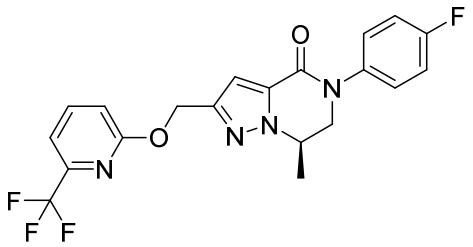
LCMS (Method B): R_T = 1.083 min, *m/z* = 387.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-(((6-methylpyridin-2-yl)oxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (49)



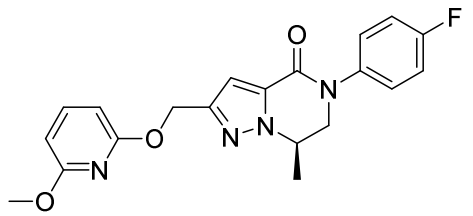
LCMS (Method A): R_T = 0.843 min, *m/z* = 367.4 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-7-methyl-2-(((6-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (50)



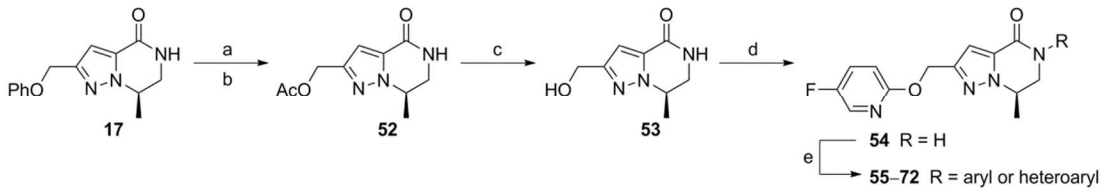
LCMS (Method B): R_T = 1.165 min, *m/z* = 421.1 $[M+H]^+$.

(*R*)-5-(4-Fluorophenyl)-2-(((6-methoxypyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (51)



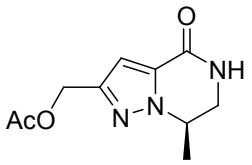
LCMS (Method B): R_T = 1.084 min, *m/z* = 383.1 $[M+H]^+$.

General Experimental Procedure II. Compounds **55–72** were prepared from intermediate **17** via the method pictured immediately below. The synthesis of compound **68** is described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0 ºC to r.t.; (b) KOAc, DMF, 60 ºC, 54%, 2 steps; (c) 1M aq. LiOH, MeOH, THF, 37%; (d) NaH, DMF, 2,5-difluoropyridine, 76%; (e) CuI, K_2CO_3, aryl or heteroaryl halide, *N,N'*-dimethylethylenediamine, PhMe, 120 ºC, 17–70%.

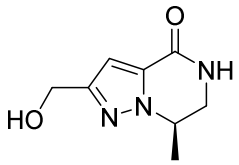
***(R)*-(7-Methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazin-2-yl)methyl acetate (52)**



To a solution of **17** (1.02 g, 3.98 mmol, 1.0 eq.) in dichloromethane (20 mL) at 0 ºC was added a solution of 1M BBr_3 in dichloromethane (7.96 mL, 7.96 mmol, 2.0 eq.). The reaction was warmed to room temperature and stirred for 2 h. The reaction was diluted with dichloromethane (30 mL). The organic layers were washed sequentially with 1M HCl (40 mL), saturated aqueous $NaHCO_3$ (40 mL), 1M aqueous NaOH (40 mL), and brine (40 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* providing the title compound as a light brown solid (656 mg) which was used without further purification. LCMS (Method B): R_T = 0.654 min, *m/z* = 276.1 $[M+H]^+$; HRMS, calc'd for $C_8H_{10}BrN_3O$ [M], 243.0007; found 243.0008.

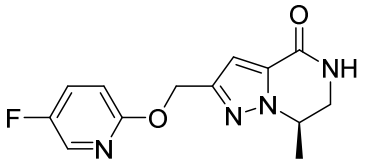
Potassium acetate (924 mg, 9.42 mmol, 3.50 eq.) was added to a solution of bromo intermediate above (656 mg, 2.69 mmol, 1.00 eq.) in DMF (13.5 mL, 0.2 M). The mixture was stirred at 60 ºC for 2 h, then diluted with EtOAc (60 mL), washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded the title compound (478 mg, 80% yield) as a pale yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (bs, 1H), 6.87 (s, 1H), 5.13 (s, 2H), 4.56-4.48 (m, 1H), 3.79 (ddd, *J* = 12.8, 7.4, 7.4 Hz, 1H), 3.46 (ddd, *J* = 9.8, 4.8, 2.6 Hz, 1H), 2.08 (s, 3H), 1.60 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 159.9, 147.7, 134.2, 107.8, 59.7, 52.4, 46.2, 20.9, 17.1; LCMS (Method A): R_T = 0.555 min, *m/z* = 224.4 $[M+H]^+$; HRMS, calc'd for $C_{10}H_{13}N_3O_3$ [M], 223.0957; found 223.0957; $[\alpha]^{25}_D = -44.0^o$ (*c* 0.175, $CHCl_3$).

(*R*)-2-(Hydroxymethyl)-7-methyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (53)



Compound **52** (478 mg, 2.14 mmol, 1.0 eq.) was dissolved in MeOH (2.1 mL) and THF (2.1 mL). To this solution, 1N aqueous LiOH (10.7 mL, 10.7 mmol, 5.0 eq.) was added. The mixture was stirred for 30 min, then diluted with water and extracted with EtOAc (3X). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography on silica gel gave the title compound (144 mg, 37% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (bs, 1H), 6.63 (s, 1H), 5.31 (bs, 2H), 4.43-4.36 (m, 1H), 3.64 (ddd, J = 13.0, 7.7, 3.8 Hz, 1H), 3.29 (ddd, J = 13.0, 7.7, 2.4 Hz, 1H), 1.41 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.9, 153.2, 134.7, 105.8, 57.4, 52.0, 45.6, 17.2; LCMS (Method B): R_T = 0.186 min, *m/z* = 182.3 $[M+H]^+$; HRMS, calc'd for $C_8H_{11}N_3O_2$ [M], 181.0851; found 181.0850; $[\alpha]^{25}_D = -18.0^o$ (*c* 0.502, MeOH).

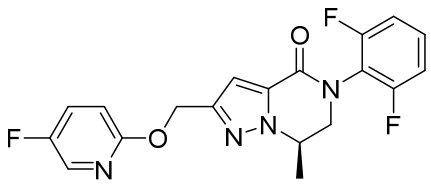
(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (54).



To a solution of compound **53** (144 mg, 0.79 mmol, 1.0 eq.) in DMF (4 mL, 0.2 M) at room temperature was added sodium hydride (48 mg, 1.98 mmol, 2.5 eq.). The resulting mixture was stirred for 15 min and 2,5-difluoropyridine (0.214 mL, 2.37 mmol, 3.0 eq.) was added. The reaction mixture was stirred overnight. Water was added. The mixture was extracted with EtOAc

(3x). The combined extracts were concentrated. Purification using reverse phase HPLC Method 2 with 56% to 83% CH_3CN in H_2O (0.1% TFA) over 23 min to provide the title compound (165 mg, 76% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (bs, 1H), 8.19 (d, J = 3.1 Hz, 1H), 7.71 (ddd, J = 3.1, 3.1, 8.8 Hz, 1H), 6.91 (dd, J = 9.1, 3.6 Hz, 1H), 6.76 (s, 1H), 5.28 (s, 2H), 4.51-4.46 (m, 1H), 3.66 (ddd, J = 13.1, 3.8, 3.8 Hz, 1H), 3.37-3.91 (m, 1H), 1.46 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 158.7, 155.7 (d, $J_{C,F}$ = 243 Hz), 147.9, 135.0, 133.4 (d, $J_{C,F}$ = 26 Hz), 128.0 (d, $J_{C,F}$ = 21 Hz), 112.4 (d, $J_{C,F}$ = 5 Hz), 107.3, 61.7, 52.3, 45.6, 17.1; LCMS (Method B): R_T = 0.763 min, *m/z* = 277.0 $[M+H]^+$; HRMS, calc'd for $C_{13}H_{13}FN_4O_2$ [M], 276.1023; found 276.1026; $[\alpha]^{25}_D = -39.9^o$ (*c* 0.511, $CHCl_3$).

(*R*)-5-(2,6-Difluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (68)

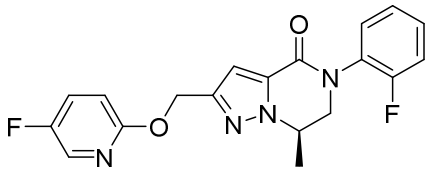


Copper (I) iodide (19 mg, 0.082 mmol, 2.0 eq.) was added to a suspension of compound **54** (15 mg, 0.05 mmol, 1.0 eq.), 2-bromo-1,3-difluorobenzene (2.0 eq.), potassium carbonate (14 mg, 0.01 mmol, 2.0 eq.) and *N,N'*-dimethylethylenediamine (30 uL, 0.28 mmol, 5.5 eq.) in toluene (0.500 mL) in a sealed tube. The reaction mixture was stirred at 120 ºC for 16 h. The mixture was diluted with EtOAc (2.0 mL), filtered through a Celite pad which was rinsed with EtOAc (2x) and concentrated *in vacuo*. Purification of the residue using reserve phase HPLC Method 1 with 47% to 76% CH_3CN in H_2O (0.1% TFA) over 4 min provided the title compound as a white powder (5.4 mg, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 8.00 (d, J = 3.0 Hz, 1H), 7.36-7.29

(m, 2H), 7.03-6.99 (m, 3H), 6.77 (dd, J = 9.0, 3.6 Hz, 1H), 5.39 (s, 2H), 4.77-4.69 (m, 1H), 4.13 (dd, J = 12.8, 4.2 Hz, 1H), 3.79 (dd, J = 12.8, 6.7 Hz, 1H), 1.69 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9 (dd, $J_{C,F}$ = 252, 5 Hz, 2C), 155.6 (d, $J_{C,F}$ = 246 Hz), 159.3, 156.5, 149.2, 133.9, 133.1 (d, $J_{C,F}$ = 26 Hz), 133.4, 129.5 (t, $J_{C,F}$ = 10 Hz), 126.7 (d, $J_{C,F}$ = 21 Hz), 117.7 (t, $J_{C,F}$ = 16 Hz), 112.4 (dd, $J_{C,F}$ = 6, 6 Hz), 112.2 (dd, $J_{C,F}$ = 3, 3 Hz, 2C), 111.9 (d, $J_{C,F}$ = 5 Hz), 108.9, 61.5, 54.5, 52.7, 29.7, 17.2; LCMS (Method A): R_T = 1.038 min, *m/z* = 389.2 $[M+H]^+$; HRMS, calc'd for $C_{19}H_{15}F_3N_4O_2$ [M], 388.1147; found 388.1147.

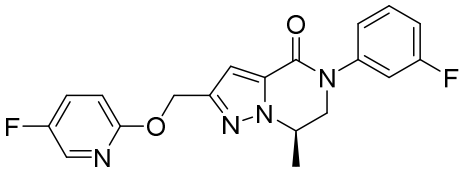
The following compounds were prepared analogous to compound ***68***

(*R*)-5-(2-Fluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (55)



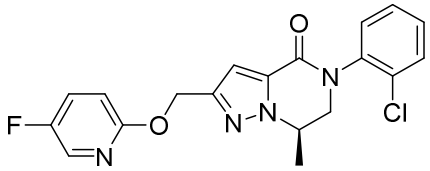
LCMS (Method B): R_T = 1.041 min, *m/z* = 370.9 $[M+H]^+$.

(*R*)-5-(3-Fluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (56)



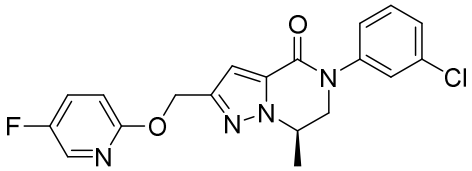
LCMS (Method B): R_T = 1.059 min, *m/z* = 370.9 $[M+H]^+$.

(*R*)-5-(2-Chlorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (57)



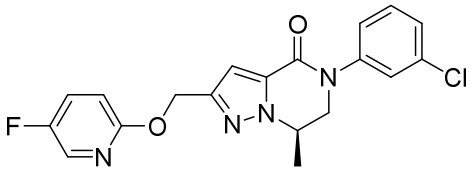
LCMS (Method B): R_T = 1.068 min, *m/z* = 386.8 $[M+H]^+$.

(*R*)-5-(3-Chlorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (58)



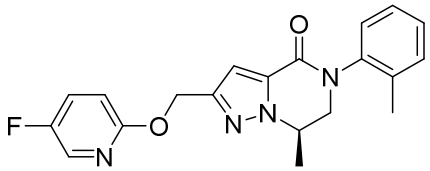
LCMS (Method B): R_T = 1.113 min, *m/z* = 386.8 $[M+H]^+$.

(*R*)-5-(4-Chlorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (59)



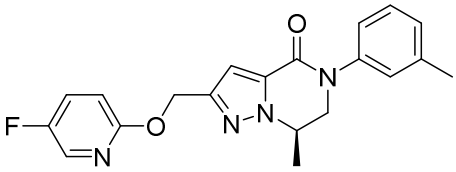
LCMS (Method B): R_T = 1.107 min, *m/z* = 386.8 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(*o*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (60)



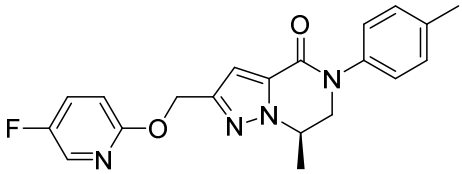
LCMS (Method B): R_T = 1.054 min, *m/z* = 366.9 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(*m*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (61)



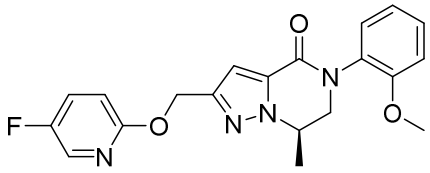
LCMS (Method B): R_T = 1.079 min, *m/z* = 366.9 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(*p*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (62)



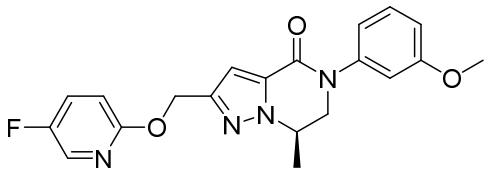
LCMS (Method A): R_T = 1.007 min, *m/z* = 367.2 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-5-(2-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (63)



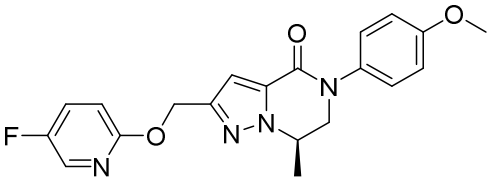
LCMS (Method A): R_T = 0.955 min, *m/z* = 383.2 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-5-(3-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (64)



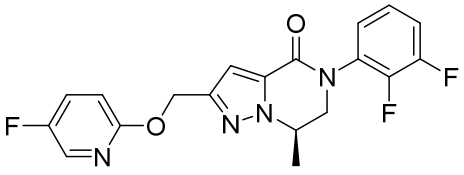
LCMS (Method A): R_T = 0.962 min, *m/z* = 383.2 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-5-(4-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (65)



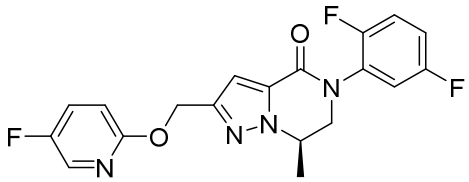
LCMS (Method A): R_T = 0.946 min, *m/z* = 383.2 $[M+H]^+$.

(*R*)-5-(2,3-Difluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (66)



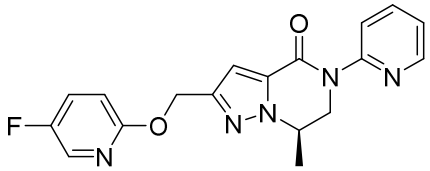
LCMS (Method B): R_T = 1.071 min, *m/z* = 388.8 $[M+H]^+$.

(*R*)-5-(2,5-Difluorophenyl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (67)



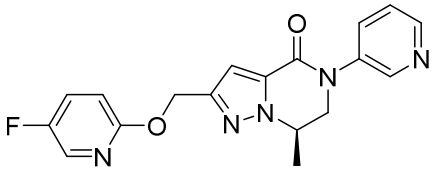
LCMS (Method B): R_T = 1.066 min, *m/z* = 388.8 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(pyridin-2-yl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (69)



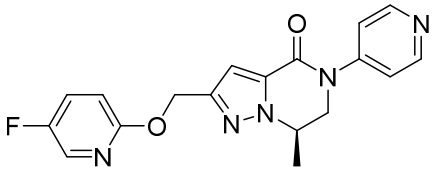
LCMS (Method A): R_T = 0.895 min, *m/z* = 354.1 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(pyridin-3-yl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (70)



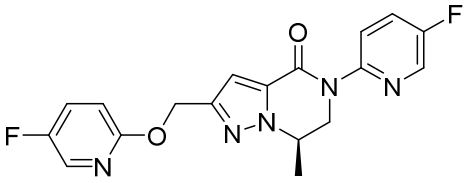
LCMS (Method A): R_T = 0.670 min, *m/z* = 354.1 $[M+H]^+$.

(*R*)-2-(((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-5-(pyridin-4-yl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (71)



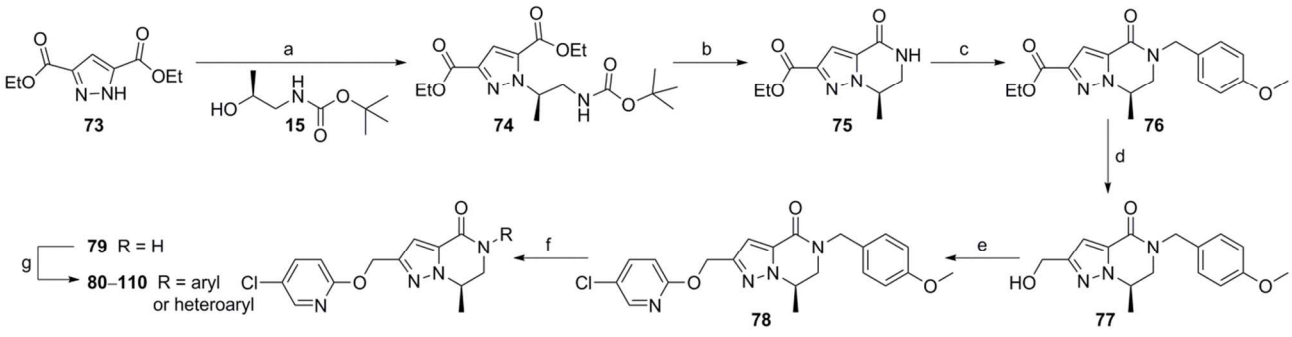
LCMS (Method A): R_T = 0.676 min, *m/z* = 354.1 $[M+H]^+$.

(*R*)-5-(5-Fluoropyridin-2-yl)-2-(((5-fluoropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (72)



LCMS (Method A): R_T = 0.963 min, *m/z* = 372.1 $[M+H]^+$.

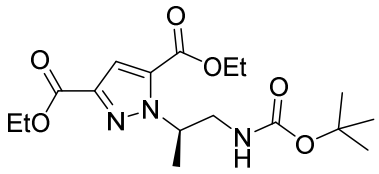
General Experimental Procedure III. Compounds **80–110** were prepared from commercially available **73** via the method pictured immediately below. The synthesis of compound **106** is described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) PPh_3, D^{t}BAD, THF, µwave, 120 ºC, 20 min.; (b) 4M HCl in dioxane, then saturated aq. $NaHCO_3$, 89%, 2 steps; (c) NaH, 4-methoxybenzyl chloride, DMF, 0 ºC to r.t., 73%; (d) $NaBH_4$, THF, MeOH, 0 to

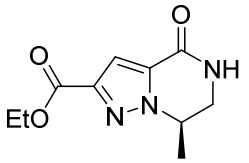
60 ºC, 84%; (e) NaH, DMF, 5-chloro-2-fluoropyridine, 0 ºC to r.t., 84%; (f) $(NH_4)_2Ce(NO_3)_6$, MeCN, H_2O, 66%; (g) CuI, K_2CO_3, aryl or heteroaryl halide, *N,N'*-dimethylethylenediamine, PhMe, 120 ºC,16–99%.

Diethyl (*R*)-1-(1-((tert-butoxycarbonyl)amino)propan-2-yl)-1*H*-pyrazole-3,5-dicarboxylate (74)



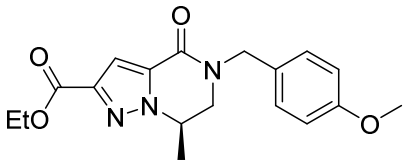
Diethyl 3,5-pyrazoledicarboxylate **73** (4.24 g, 20 mmol, 1.0 eq) and tert-butyl (*S*)-(2-hydroxypropyl) carbamate **15** (7.01 g, 40 mmol, 2.0 eq.) were dissolved in THF (100 mL. 0.2 M) and triphenyl phosphine (9.44 g, 36 mmol, 1.8 eq.) was added. After 5 min, the mixture was cooled to 0 ºC and di-*tert*-butyl azodicarboxylate (8.29 g, 36 mmol, 1.8 eq.) was added. The reaction mixture was then subjected to microwave irradiation for 25 min at 120 ºC. The mixture was cooled to room temperature and the solvent was removed *in vacuo*. Purification via flash chromatography on silica gel provided the title compound as a semi-solid (8.2 g, yield was not determined due to contamination of D′BAD byproduct, di-tert-butyl hydrazine-1,2-dicarboxylate). ^{1}H NMR (400 MHz, MeOD) δ 7.29 (s, 1H), 5.69-5.01 (m, 1H), 4.41-4.35 (m, 4H), 3.51-3.39 (m, 2H), 1.51 (d, *J* = 6.8 Hz, 3H), 1.41-1.38 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 159.0, 155.8, 142.6, 134.2, 114.0, 79.4, 61.4, 61.1, 53.4, 45.1, 28.2 (3C), 18.5, 14.3, 14.2; LCMS (Method A): R_T = 1.052 min, *m/z* = 314.2 $[M+H]^+$. HRMS, calc'd for $C_{17}H_{27}N_3O_6$ [M], 369.1900; found 369.1899.

Ethyl (*R*)-7-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazine-2-carboxylate (75)



Compound **74** (8.2 g, 22.2 mmol, 1.0 eq.) was treated with a solution of 4N HCl in 1,4-dioxane (78 mL). Deprotection of the *tert*-butyl carbamate protecting group was monitored by LCMS. Once deprotection was complete, the reaction mixture was carefully basified with saturated aqueous $NaHCO_3$ (verified by pH paper) and was allowed to stir at room temperature overnight. The mixture was diluted with dichloromethane, and the aqueous layer was extracted with dichloromethane (3x). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide the title compound as a white solid (4.4 g, 89% yield over two steps), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.13 (bs, 1H), 4.68–4.62 (m, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 3.86 (ddd, *J* = 15.8, 10.2, 1.4 Hz, 1H), 3.51 (ddd, *J* = 9.4, 6.4, 3.0 Hz, 1H), 1.65 (d, *J* = 6.6 Hz, 3H), 1.39 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 159.3, 143.8, 134.4, 110.7, 61.3, 53.2, 46.0, 17.4, 14.3; LCMS (Method A): R_T = 0.546 min, *m/z* = 224.2 [M+H]; HRMS, calc'd for $C_{10}H_{13}N_3O_3$ [M], 223.0957; found 223.0957; $[\alpha]^{25}_D$ = –29.9º (*c* 0.500, $CHCl_3$).

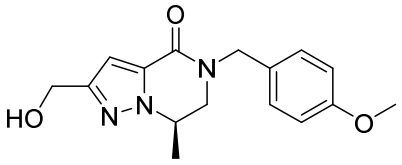
Ethyl (*R*)-5-(4-methoxybenzyl)-7-methyl-4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazine-2-carboxylate (76)



Compound **75** (2.23 g, 10 mmol, 1.0 eq.) was dissolved in DMF (50 mL, 0.2 M), cooled to 0 ºC and treated with 60% sodium hydride in mineral oil (480 mg, 12 mmol, 1.2 eq.) in five portions.

The reaction mixture was stirred for 15 min and 4-methoxybenzyl chloride (1.63 mL, 12 mmol, 1.2 eq.) was added. After 16 h, the reaction mixture was diluted with water and extracted with EtOAc (3x). The combined extracts were washed with water, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel to provide the title compound (2.51 g, 73% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.23 (d, *J* = 14.1, 2H), 6.87 (d, *J* = 14.1, 2H), 4.76 (d, *J* = 14.5, 1H), 4.59-4.50 (m, 2H), 4.44-4.36 (m, 2H), 3.79 (s, 3H), 3.69 (dd, *J* = 13.1, 4.6 Hz, 1H), 3.35 (dd, *J* = 13.1, 6.3 Hz, 1H), 1.47 (d, *J* = 6.6 Hz, 3H), 1.38 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 159.5, 137.1, 143.9, 134.8, 129.9 (2C), 127.9, 114.3, 110.9 (2C), 61.2, 55.3, 52.9, 50.4, 48.8, 17.5, 14.3; LCMS (Method A): R_T = 0.955 min, *m/z* = 344.2 $[M+H]^+$; HRMS, calc'd for $C_{18}H_{21}N_3O_4$ [M], 343.1532; found 343.1533; $[\alpha]^{25}_D = -8.1°$ (*c* 0.157, $CHCl_3$).

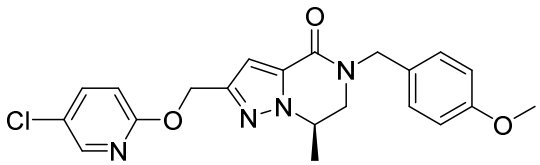
(*R*)-2-(Hydroxymethyl)-5-(4-methoxybenzyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (77)



Sodium borohydride (1.16 g, 30.6 mmol, 5.0 eq.) was added slowly to a solution of compound **76** (2.1 g, 6.11 mmol, 1.0 eq.) in THF (20 mL) and MeOH (5.0 mL) at 0 ºC. The reaction was heated to 60 ºC, and after 30 min at that temperature, the reaction mixture was diluted with water and extracted with dichloromethane. The aqueous layer was acidified with a 1M aqueous HCl solution and extracted with dichloromethane (2x). The combined extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography on silica gel provided the title compound as a viscous oil (1.55 g, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.21

(m, 2H), 6.87-6.84 (m, 3H), 4.72 (d, J = 14.5 Hz, 1H), 4.68 (s, 2H), 4.59 (d, J = 14.4 Hz, 1H), 4.43-4.35 (m, 1H), 3.78 (s, 3H), 3.60 (dd, J = 13.0, 4.6 Hz, 1H), 3.31 (dd, J = 13.0, 7.4 Hz, 1H), 1.42 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 157.8, 152.6, 134.5, 129.8 (2C), 128.2, 114.3 (2C), 106.5, 58.7, 55.3, 52.0, 50.8, 48.7, 17.2; LCMS (Method A): LCMS: R_T = 0.680 min, *m/z* = 302.2 $[M+H]^+$; HRMS, calc'd for $C_{16}H_{19}N_3O_3$ [M], 301.1426; found 301.1428; $[\alpha]^{25}_D = -5.3°$ (*c* 0.98, $CHCl_3$).

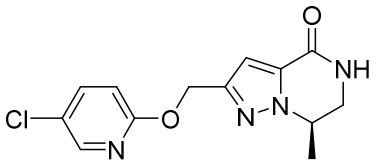
(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(4-methoxybenzyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (78)



To a solution of compound **77** (1.5 g, 4.98 mmol, 1.0 eq.) in DMF (25 mL, 0.2 M) at 0 ºC was added NaH (300 mg, 12.44 mmol, 2.5 eq.). The resulting mixture was stirred for 15 min and 5-chloro-2-fluoropyridine (1.25 mL, 12.44 mmol, 2.5 eq.) was added. The mixture was stirred overnight and extracted with EtOAc (3x). The combined extracts were concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded the title compound (1.72 g, 84% yield) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 2.6 Hz, 1H), 7.54 (dd, J = 8.8, 2.6 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 6.98 (s, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.8 Hz, 1H), 5.39 (s, 2H), 4.75 (d, J = 14.5 Hz, 1H), 4.63 (d, J = 14.5 Hz, 1H), 4.50-4.42 (m, 1H), 3.81 (s, 3H), 3.64 (dd, J = 13.0, 4.6 Hz, 1H), 3.35 (dd, J = 13.0, 7.4 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 159.4, 157.7, 148.9, 145.1, 138.6, 134.5, 129.8 (2C), 128.2, 124.4, 114.2 (2C), 112.3, 108.2, 61.6, 55.3, 52.1, 50.8, 48.8, 17.2; LCMS (Method A): R_T

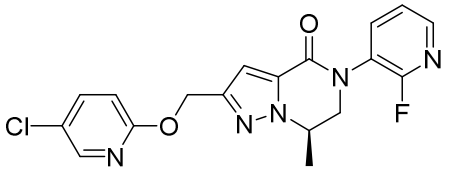
= 1.080 min, *m/z* = 413.2 $[M+H]^+$; HRMS, calc'd for $C_{21}H_{21}ClN_4O_3$ [M], 412.1302; found 412.1305; $[\alpha]^{25}_D = -10.3°$ (*c* 1.512, $CHCl_3$).

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (79)



Compound **78** (1.65 mg, 4.0 mmol, 1.0 eq.) was dissolved in MeCN (40 mL, 0.1 M) and a solution of ceric ammonium nitrate (6.57 g, 12 mmol, 4.0 eq.) in water (12 mL) was added. After 30 min at room temperature, solvents were removed *in vacuo*. Purification using flash chromatography on silica gel provided the title compound (764 mg, 65% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, *J* = 2.7 Hz, 1H), 8.21 (s, 1H), 7.83 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.92 (dd, *J* = 8.8, 0.5 Hz, 1H), 6.77 (s, 1H), 5.29 (s, 2H), 4.51-4.46 (m, 1H), 3.66 (ddd, *J* = 13.0, 8.7, 8.7 Hz, 1H), 3.34 (ddd, *J* = 13.1, 7.9, 2.2 Hz, 1H), 1.45 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 158.7, 147.7, 145.3, 139.7, 135.1, 124.1, 112.9, 107.4, 61.7, 52.3, 45.6, 17.0; LCMS (Method A): LCMS: R_T = 0.804 min, *m/z* = 293.2 $[M+H]^+$; HRMS, calc'd for $C_{13}H_{13}ClN_4O_2$ [M], 292.0727; found 292.0727; $[\alpha]^{25}_D = -38.3°$ (*c* 0.442, $CHCl_3$).

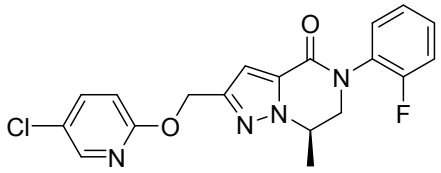
(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2-fluoropyridin-3-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (106)



Copper (I) iodide (13.7 mg, 0.072 mmol, 2.1 eq.) was added to a suspension of compound **79** (10 mg, 0.035 mmol, 1.0 eq.), 3-bromo-2-fluoropyridine (7.40 μL, 0.072 mmol, 2.1 eq.), potassium carbonate (10 mg, 0.072 mmol, 2.1 eq.) and *N,N'*-dimethylethylenediamine (20.7 μL, 0.19 mmol, 5.5 eq.) in toluene (0.44 mL) in a sealed reaction vial. The reaction mixture was stirred at 120 ºC. After 16 h, the mixture was diluted with EtOAc, filtered through a Celite pad which was rinsed with EtOAc (2x) and concentrated *in vacuo*. Purification using reserve phase HPLC Method 1 with 39% to 71% CH_3CN in H_2O (0.1% TFA) over 4 min provided the title compound (7.2 mg, 53% yield) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (dd, J = 2.7, 0.5 Hz, 1H), 8.23 (ddd, J = 4.8, 2.7, 2.7 Hz, 1H), 8.09 (ddd, J = 9.6, 7.7, 1.8 Hz, 1H), 7.84 (dd, J = 8.8, 2.7 Hz, 1H), 7.50 (ddd, J = 9.0, 4.9, 1.3 Hz, 1H) 6.95 (s, 1H), 6.92 (d, J = 0.5 Hz, 1H), 5.35 (s, 2H), 4.82-4.75 (m, 1H), 4.27 (dd, J = 12.8, 4.3 Hz, 1H), 3.98 (dd, J = 12.8, 7.2 Hz, 1H) 1.55 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 158.2 (d, $J_{C,F}$ = 239 Hz), 156.6, 148.4, 146.4 (d, $J_{C,F}$ = 14 Hz), 145.3, 133.9 (d, $J_{C,F}$ = 13 Hz), 139.8, 134.0, 124.4 (d, $J_{C,F}$ = 28 Hz), 124.2, 123.2 (d, $J_{C,F}$ = 4 Hz), 112.9, 108.7, 61.6, 54.0, 52.7, 17.1; LCMS (Method A): LCMS: R_T = 0.944 min, *m/z* = 388.2 $[M+H]^+$; HRMS, calc'd for $C_{18}H_{15}ClFN_5O_2$ [M], 387.0898; found 387.0899; $[\alpha]^{25}_D = -23.6$º (*c* 0.100, DMSO).

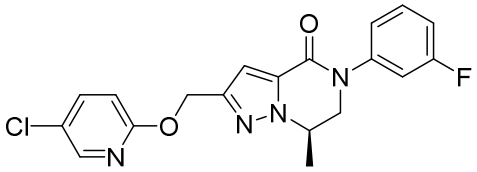
*The following compounds were prepared analogous to compound **106***

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (80)



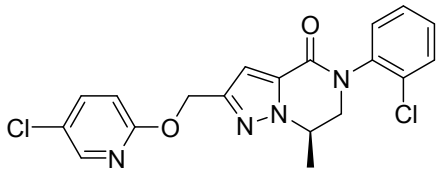
LCMS (Method A): LCMS: R_T = 1.073 min, *m/z* = 387.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(3-fluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (81)



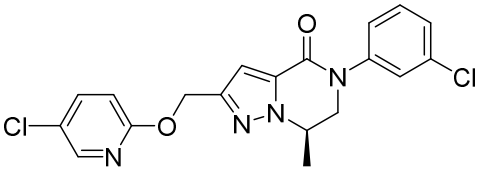
LCMS (Method A): LCMS: R_T = 1.080 min, *m/z* = 387.1 $[M+H]^+$.

(*R*)-5-(2-Chlorophenyl)-2-(((5-chloropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (82)



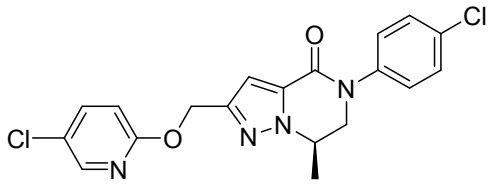
LCMS (Method A): LCMS: R_T = 1.088 min, *m/z* = 403.1 $[M+H]^+$.

(*R*)-5-(3-Chlorophenyl)-2-(((5-chloropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (83)



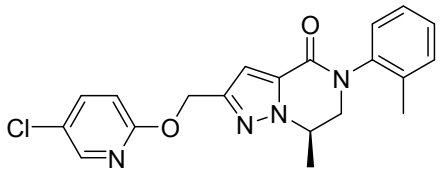
LCMS (Method A): LCMS: R_T = 1.127 min, *m/z* = 403.1 $[M+H]^+$.

(*R*)-5-(4-Chlorophenyl)-2-(((5-chloropyridin-2-yl)oxy)methyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (84)



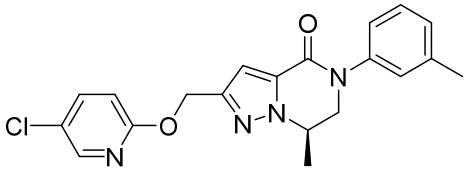
LCMS (Method A): LCMS: R_T = 1.129 min, *m/z* = 403.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-5-(*o*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (85)



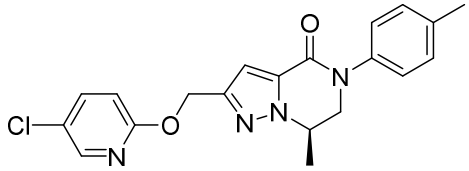
LCMS (Method A): LCMS: R_T = 1.078 min, *m/z* = 383.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-5-(*m*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (86)



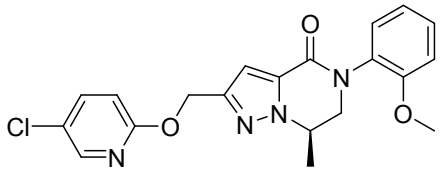
LCMS (Method A): LCMS: R_T = 1.115 min, *m/z* = 383.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-5-(*p*-tolyl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (87)



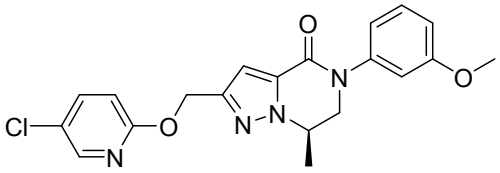
LCMS (Method A): LCMS: R_T = 1.107 min, *m/z* = 383.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (88)



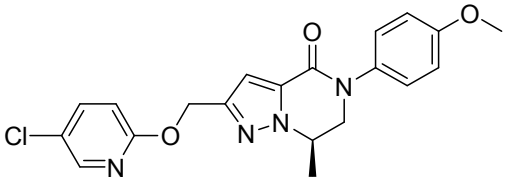
LCMS (Method A): LCMS: R_T = 1.051 min, *m/z* = 399.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(3-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (89)



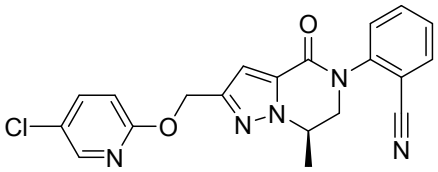
LCMS (Method A): LCMS: R_T = 1.064 min, *m/z* = 399.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(4-methoxyphenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (90)



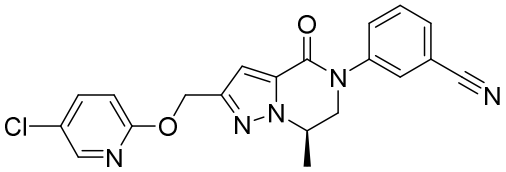
LCMS (Method A): LCMS: R_T = 1.050 min, *m/z* = 399.1 $[M+H]^+$.

(*R*)-2-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)benzonitrile (91)



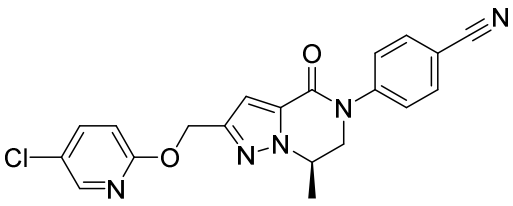
LCMS (Method A): LCMS: R_T = 1.011 min, *m/z* = 394.2 $[M+H]^+$.

(*R*)-3-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)benzonitrile (92)



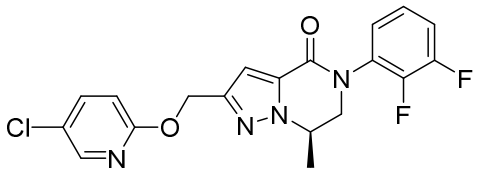
LCMS (Method A): LCMS: R_T = 1.037 min, *m/z* = 394.2 $[M+H]^+$.

(*R*)-4-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)benzonitrile (93)



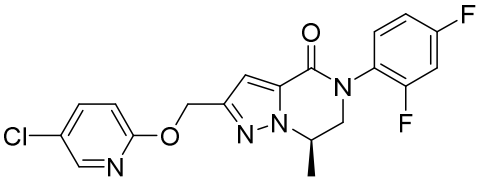
LCMS (Method A): LCMS: R_T = 1.042 min, *m/z* = 394.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2,3-difluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (94)



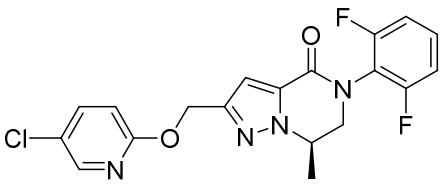
LCMS (Method A): LCMS: R_T = 1.088 min, *m/z* = 405.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2,4-difluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (95)



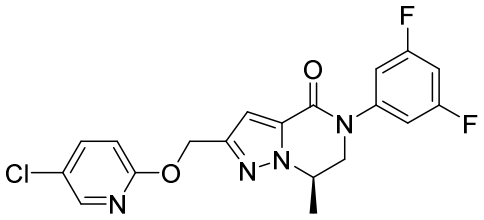
LCMS (Method A): LCMS: R_T = 1.060 min, *m/z* = 405.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(2,6-difluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (96)



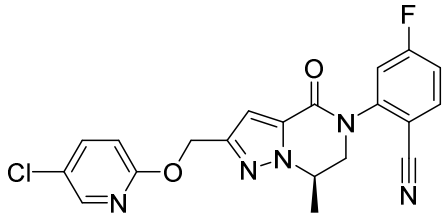
LCMS (Method A): LCMS: R_T = 1.099 min, *m/z* = 405.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(3,5-difluorophenyl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (97)



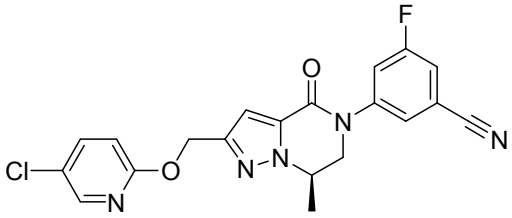
LCMS (Method A): LCMS: R_T = 1.072 min, *m/z* = 405.2 $[M+H]^+$.

(*R*)-2-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)-4-fluorobenzonitrile (98)



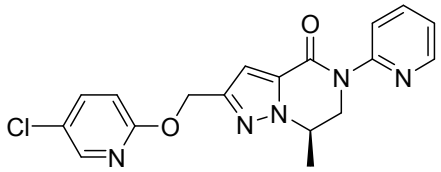
LCMS (Method A): LCMS: R_T = 1.017 min, *m/z* = 412.1 $[M+H]^+$.

(*R*)-3-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)-5-fluorobenzonitrile (99)



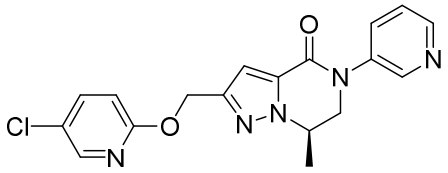
LCMS (Method A): LCMS: R_T = 1.065 min, *m/z* = 412.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-5-(pyridin-2-yl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (100)



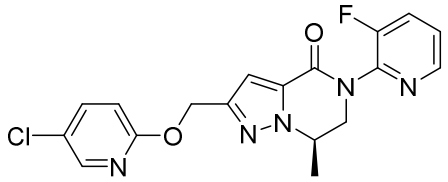
LCMS (Method A): LCMS: R_T = 1.008 min, *m/z* = 370.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-5-(pyridin-3-yl)-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (101)



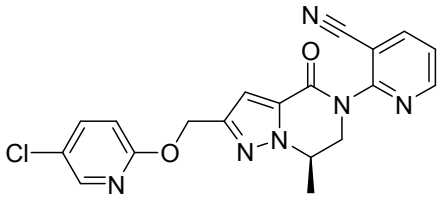
LCMS (Method A): LCMS: R_T = 0.782 min, *m/z* = 370.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(3-fluoropyridin-2-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (102)



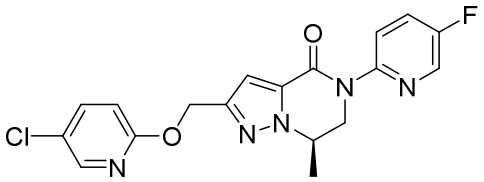
LCMS (Method A): LCMS: R_T = 0.973 min, *m/z* = 388.2 $[M+H]^+$.

(*R*)-2-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)nicotinonitrile (103)



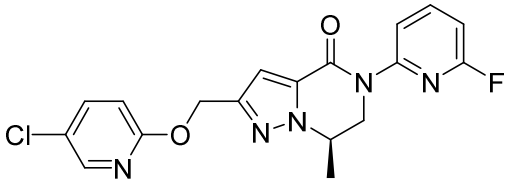
LCMS (Method A): LCMS: R_T = 0.968 min, *m/z* = 395.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(5-fluoropyridin-2-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (104)



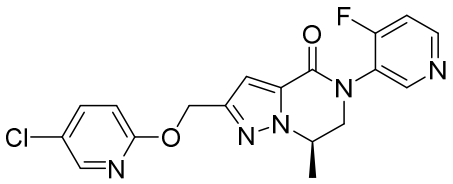
LCMS (Method A): LCMS: R_T = 1.029 min, *m/z* = 388.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(6-fluoropyridin-2-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (105)



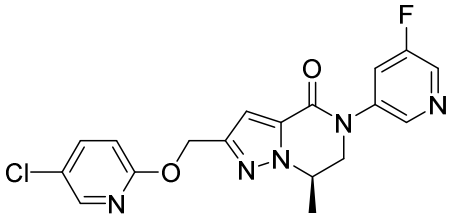
LCMS (Method A): LCMS: R_T = 1.078 min, *m/z* = 388.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(4-fluoropyridin-3-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (107)



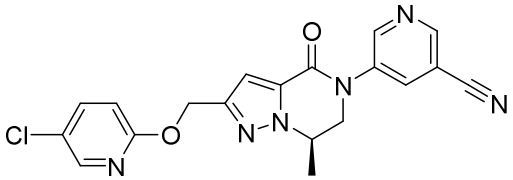
LCMS (Method A): LCMS: R_T = 0.884 min, *m/z* = 388.1 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(5-fluoropyridin-3-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (108)



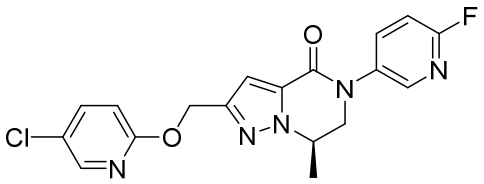
LCMS (Method A): LCMS: R_T = 0.945 min, *m/z* = 388.1 $[M+H]^+$.

(*R*)-5-(2-(((5-Chloropyridin-2-yl)oxy)methyl)-7-methyl-4-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrazin-5(4*H*)-yl)nicotinonitrile (109)



LCMS (Method A): LCMS: R_T = 0.971 min, *m/z* = 395.2 $[M+H]^+$.

(*R*)-2-(((5-Chloropyridin-2-yl)oxy)methyl)-5-(6-fluoropyridin-3-yl)-7-methyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (110)



LCMS (Method A): LCMS: R_T = 0.956 min, *m/z* = 388.2 $[M+H]^+$.

Molecular Pharmacology Methods

$mGlu_3$ and $mGlu_2$ Ca^{2+} flux assays (CRC format)

$G_{\alpha 15}$/TREx cells stably expressing rat $mGlu_3$ or $G_{\alpha 15}$ HEK293 cells stably expressing rat $mGlu_2$ were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates in 20 µL of assay medium (For $mGlu_3$ assay: DMEM containing 10% dialyzed FBS, 20 mM HEPES, 25 ng/mL tetracycline, and 1 mM sodium pyruvate; For $mGlu_2$ assay: DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) at a density of 20K cells/well ($mGlu_3$) or 12K cells/well ($mGlu_2$). The cells were grown overnight at 37 ºC in the presence of 5% CO_2. The next day, medium was removed and the cells incubated with 20 µL of 2.3 µM Fluo-4 AM prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in assay buffer (Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid) for 60 minutes at room temperature ($mGlu_3$) or 45 minutes at 37 ºC ($mGlu_2$). Dye was removed, 20 µL of assay buffer was added, and the plate was incubated for 10 ($mGlu_3$) or 5 ($mGlu_2$) minutes at room temperature.

Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS7000, Hamamatsu, Japan). After establishment of a fluorescence baseline for about 3 seconds, the test compounds were added to the cells, and the response in cells was measured. 2.3 minutes later an EC_{20} concentration of the $mGlu_{2/3}$ receptor agonist glutamate was added to the cells, and the response of the cells was measured for 1.9 minutes; an EC_{80} concentration of agonist was added

and readings taken for an additional 1.7 minutes. All test compounds were dissolved and diluted to a concentration of 10 mM in 100% DMSO. Compounds were then serially diluted 1:3 in DMSO into 10 point concentration response curves, transferred to daughter plates, and further diluted into assay buffer to a 2x stock. Calcium fluorescence measures were recorded as fold over basal fluorescence; raw data was then normalized to the maximal response to glutamate. Antagonism of the agonist response of the $mGlu_3$ or $mGlu_2$ receptor was observed as a decrease in response to nearly maximal concentrations of glutamate in the presence of compound compared to the response to glutamate in the absence of compound.

The raw data file containing all time points was used as the data source in the analysis template. This was saved by the FDSS as a tab-delimited text file. Data were normalized using a static ratio function (F/F_0) for each measurement of the total 360 values per well divided by each well's initial value. Data were then reduced to peak amplitudes (Max – Initial Min) using a time range that starts approximately 3 seconds prior to the glutamate EC_{80} addition and continues for approximately 90 seconds. This is sufficient time to capture the peak amplitude of the cellular calcium response. Individual amplitudes were expressed as % EC_{Max} by multiplying each amplitude by 100 and then dividing the product by the mean of the amplitudes derived from the glutamate EC_{Max}-treated wells. IC_{50} values for test compounds were generated by fitting the normalized values versus the log of the test compound concentration (in mol/L) using a 4 parameter logistic equation where none of the parameters were fixed. Each of the three values collected at each concentration of test compound were weighted evenly.

A compound was designated as a negative allosteric modulator (NAM) if the compound showed a concentration-dependent decrease in the glutamate EC_{80} addition. For NAMs, potency (IC_{50}) and maximum response (% Glu Max), i.e. the amplitude of response in the presence of 30

μM test compound as a percentage of the maximal response to glutamate, are reported. For NAMs that show a decrease in the EC_{80} response, but do not hit a plateau, the average of the maximum response at a single concentration (30 μM) was determined (% Glu Max) and potencies were reported as ">10,000 nM". Compounds with no measurable activity are designated as ">30,000 nM" since the top concentration of compound tested in the assay is 30 μM.

$mGlu_3$ Ca^{2+} flux assay (fold-shift format)

$G_{\alpha 15}$/TREx cells stably expressing rat $mGlu_3$ were prepared as described above. Compounds were diluted by half-log in DMSO and further diluted into assay buffer to a 2x stock which was applied to cells at t = 3 s. Cells were incubated with the test compounds for 2.3 minutes and then stimulated with varying concentrations of glutamate, and readings taken for an additional 2.6 minutes. Data were collected at 1 Hz. Concentration response curves were generated using a four point logistical equation with XLfit curve fitting software for Excel (IDBS, Guildford, U.K.) or GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

$mGlu_5$ Ca^{2+} flux assay (CRC format)

HEK 293A cells stably expressing rat $mGlu_5$ were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates in 20 μL of assay medium (DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) at a density of 20K cells/well. The cells were grown overnight at 37 ºC in the presence of 5% CO_2. The next day, medium was removed and the cells incubated with 20 μL of 2.3 μM Fluo-4 AM prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in assay buffer

(Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid) for 45 minutes at 37 ºC. Dye was removed, 20 µL of assay buffer was added, and the plate was incubated for 5 minutes at room temperature.

Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS7000, Hamamatsu, Japan). After establishment of a fluorescence baseline for about 3 seconds, the test compounds were added to the cells, and the response in cells was measured. 2.3 minutes later an EC_{20} concentration of the $mGlu_5$ receptor agonist glutamate was added to the cells, and the response of the cells was measured for 1.9 minutes; an EC_{80} concentration of agonist was added and readings taken for an additional 1.7 minutes. All test compounds were dissolved and diluted to a concentration of 10 mM in 100% DMSO. Compounds were then serially diluted 1:3 in DMSO into 10 point concentration response curves, transferred to daughter plates, and further diluted into assay buffer to a 2x stock. Calcium fluorescence measures were recorded as fold over basal fluorescence; raw data was then normalized to the maximal response to glutamate. Potentiation of the agonist response of the $mGlu_5$ receptor was observed as an increase in response to submaximal concentrations of glutamate in the presence of compound compared to the response to glutamate in the absence of compound. Antagonism of the agonist response of the $mGlu_5$ receptor was observed as a decrease in response to nearly maximal concentrations of glutamate in the presence of compound compared to the response to glutamate in the absence of compound.

The raw data file containing all time points was used as the data source in the analysis template. This was saved by the FDSS as a tab-delimited text file. Data were normalized using a static ratio function (F/F_0) for each measurement of the total 360 values per well divided by each well's initial value. Data were then reduced to peak amplitudes (Max – Initial Min) using a time

range that starts approximately 3 seconds prior to the glutamate EC_{20}/EC_{80} addition and continues for approximately 90-120 seconds. This is sufficient time to capture the peak amplitude of the cellular calcium response. Individual amplitudes were expressed as % E_{Max} by multiplying each amplitude by 100 and then dividing the product by the mean of the amplitudes derived from the glutamate EC_{Max}-treated wells. EC_{50} values for test compounds were generated by fitting the normalized values versus the log of the test compound concentration (in mol/L) using a 4 parameter logistic equation where none of the parameters were fixed. Each of the three values collected at each concentration of test compound were weighted evenly.

A compound was designated as a positive allosteric modulator (PAM) if the compound showed a concentration-dependent increase in the glutamate EC_{20} addition. For PAMs, potency (EC_{50}) and maximum response (% Glu Max), i.e. the amplitude of response in the presence of 30 μM test compound as a percentage of the maximal response to glutamate, are reported. For PAMs that show an increase in the EC_{20} response, but do not hit a plateau, the average of the maximum response at a single concentration (30 μM) was determined (% Glu Max) and potencies were reported as ">10,000 nM". A compound was designated as a negative allosteric modulator (NAM) if the compound showed a concentration-dependent decrease in the glutamate EC_{80} addition. For NAMs, potency (IC_{50}) and maximum response (% Glu Max), i.e. the amplitude of response in the presence of 30 μM test compound as a percentage of the maximal response to glutamate, are reported. For NAMs that show a decrease in the EC_{80} response, but do not hit a plateau, the average of the maximum response at a single concentration (30 μM) was determined (% Glu Max) and potencies were reported as ">10,000 nM". Compounds with no measurable activity are designated as ">30,000 nM" since the top concentration of compound tested in the assay is 30 μM.

$mGlu_1$ fold-shift selectivity assay

Human $mGlu_1$ TREx293 cells were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates (BD Biosciences, San Jose, CA) at a density of 20,000 cells/well in 20 µL of assay medium (DMEM supplemented with 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) containing tetracycline (TET) to induce the $mGlu_1$ expression; 50ng/mL TET was used. The cells were grown overnight at 37 ºC in the presence of 5% CO_2. The next day, cells were washed with assay buffer (Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid (Sigma-Aldrich, St. Louis, MO)) using an ELX405 microplate washer (BioTek) leaving 20 µL/well. Immediately cells were incubated with 20 µl/well of Fluo-4 AM (Invitrogen) calcium indicator dye solution (1.15 µM final concentration) for 45 m at 37 ºC. The Fluo-4 dye prepared as a DMSO stock, was mixed in a 1:1 ratio with 10% pluronic acid F-127 and then diluted in assay buffer. The dye was then removed and washed with assay buffer using an ELX405, leaving 20 µL/well. Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS7000, Hamamatsu, Japan). Compounds were diluted by half-log in DMSO and further diluted into assay buffer to a 2x stock which was applied to cells at t = 3 s. Cells were incubated with the test compounds for 2.3 minutes and then stimulated with varying concentrations of glutamate, and readings taken for an additional 2.6 minutes. Data were collected at 1 Hz. Concentration response curves were generated using a four point logistical equation with XLfit curve fitting software for Excel (IDBS, Guildford, U.K.) or GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

$mGlu_{4/6/7/8}$ fold-shift selectivity assay

Compound **106** activity at the group III mGlu receptors was assessed using thallium flux through G-protein-coupled inwardly rectifying potassium (GIRK) channels, a method that has been described in detail (Niswender et al. *Mol. Pharmacol.* 2008, reference 45). These cell lines were grown in growth media containing 45% DMEM, 45% F-12, 10% FBS, 20 mM HEPES, 2 mM L-glutamine, antibiotic/antimycotic, nonessential amino acids, 700 μg/mL G418, and 0.6 μg/mL puromycin at 37 ºC in the presence of 5% CO_2. Briefly, HEK/GIRK cells expressing rat $mGlu_4$, human $mGlu_6$, rat $mGlu_7$, or rat $mGlu_8$ were plated into 384 well, black-walled, clear-bottom poly-D-lysine coated plates at a density of 15,000 cells/20 μL/well in assay medium and incubated overnight at 37 ºC in the presence of 5% CO_2. The following day, the medium from the cells and 20 μL/well of 1.7 μM concentration of the indicator dye BTC-AM (Invitrogen, Carlsbad, CA) in assay buffer was added. Cells were incubated for 1 hour at room temperature and the dye was replaced with 20 μL/well of assay buffer. After establishment of a fluorescence baseline for about 3 seconds, test compound was added to the cells at 2x final concentration, and the response in cells was measured. 2.3 min later the appropriate concentration of agonist (L-AP4 for $mGlu_7$, glutamate for all other mGlu receptors) was added and readings taken for an additional 2.6 minutes. Agonists were diluted in thallium buffer (125 mM sodium bicarbonate, 1 mM magnesium sulfate, 1.8 mM calcium sulfate, 5 mM glucose, 12 mM thallium sulfate, 10 mM HEPES) at 5x the final concentration to be assayed. Data were analyzed as described in Niswender et al. *Mol. Pharmacol.* 2008 (reference 45).

$mGlu_5$ radioligand binding assay

Membranes were prepared from rat $mGlu_5$ HEK293A cells. Compounds were diluted in assay buffer (50 mM Tris/0.9% NaCl, pH 7.4) to a 5x stock and 100 μL test compound was

added to each well of a 96 deep-well assay plate. 300 µL aliquots of membranes diluted in assay buffer (40 µg/well) were added to each well. 100 µL [^{3}H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine (2 nM final concentration) was added and the reaction was incubated at room temperature for 1 h with shaking. After the incubation period, the membrane-bound ligand was separated from free ligand by filtration through glass-fiber 96 well filter plates (Unifilter-96, GF/B, PerkinElmer Life and Analytical Sciences, Boston, MA). The contents of each well were transferred simultaneously to the filter plate and washed 3-4 times with assay buffer using a cell harvester (Brandel Cell Harvester, Brandel Inc., Gaithersburg, MD). 40 µL scintillation fluid was added to each well and the membrane-bound radioactivity determined by scintillation counting (TopCount, PerkinElmer Life and Analytical Sciences). Non-specific binding was estimated using 5 µM MPEP. Concentration response curves were generated using a four parameter logistical equation in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

In-Vitro **DMPK Methods**

Intrinsic clearance in rat liver microsomes

Rat liver microsomes (0.5 mg/mL) and 1 µM test compound were incubated in 100 mM potassium phosphate pH 7.4 buffer with 3 mM $MgCl_2$ at 37 ºC with constant shaking. After a 5 min preincubation, the reaction was initiated by addition of NADPH (1 mM). At selected time intervals (0, 3, 7, 15, 25, and 45 min), 50 µL aliquots were taken and subsequently placed into a 96-well plate containing 150 µL of cold acetonitrile with internal standard (50 ng/mL carbamazepine). Plates were then centrifuged at 3000 rcf (4 ºC) for 10 min, and the supernatant was transferred to a separate 96-well plate and diluted 1:1 with water for LC/MS/MS analysis. The *in vitro* half-life ($T_{1/2}$, min, Eq. 1), intrinsic clearance (CL_{int}, mL/min/kg, Eq. 2) and

subsequent predicted hepatic clearance (CL_{hep}, mL/min/kg, Eq. 3) were determined employing the following equations:

(1) $$T_{1/2} = \frac{Ln(2)}{k}$$

where k represents the slope from linear regression analysis of the natural log percent remaining of test compound as a function of incubation time

(2) $$CL_{\text{int}} = \frac{0.693}{in\,vitro\,T_{1/2}} x \frac{mL\ incubation}{mg\ microsomes} x \frac{45\,mg\,microsomes}{gram\ liver} x \frac{45^{a}\ gram\ liver}{kg\ body\ wt}$$

[a] scale-up factor of 45 for rat

(3) $$CL_{hep} = \frac{Q_h \cdot CL\,\text{int}}{Q_h + CL\,\text{int}}$$

where Q_h (hepatic blood flow, mL/min/kg) is 70 for the rat.

Plasma Protein Binding

The protein binding of each compound was determined in rat plasma via equilibrium dialysis employing HTDialysis Teflon dialysis chamber and cellulose membranes (MWCO 12-14 K) (HTDialysis LLC, Gales Ferry, CT). Plasma was added to the 96 well plate containing test compound and mixed thoroughly for a final concentration of 5 µM. Subsequently, 150 µL of the plasma-compound mixture was transferred to the dialysis chamber, with an accompanying 150 µL of phosphate buffer (25 mM, pH 7.4) on the other side of the membrane. The device plate was sealed and incubated for 4 hours at 37 ºC with shaking. At completion, aliquots from each chamber were diluted 1:1 with either plasma (for the buffer sample) or buffer (for the plasma sample) and transferred to a new 96 well plate, at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) (2 volumes) was added to extract the matrices. The

plate was centrifuged (3000 rcf, 10 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$F_u = \frac{Conc_{buffer}}{Conc_{plasma}}$$

Brain Homogenate Binding

The brain homogenate binding of each compound was determined in brain homogenate via equilibrium dialysis employing HTDialysis Teflon dialysis chamber and cellulose membranes (MWCO 12-14 K) (HTDialysis LLC, Gales Ferry, CT). Brain tissue homogenate was prepared by diluting one volume whole rat brain tissue with three volumes of phosphate buffer (25 mM, pH 7.4). The mixture was then subjected to mechanical homogenation employing a Mini-Beadbeater™ and 1.0 mm Zirconia/Silica Beads (BioSpec Products). Brain homogenate spiked with test compound and mixed thoroughly for a final concentration of 5 µM. Subsequently, 150 µL of the brain homogenate-compound mixture was transferred to the dialysis chamber with an accompanying 150 µL of phosphate buffer (25 mM, pH 7.4) on the other side of the membrane. The block was sealed and incubated for 6 hours at 37 ºC with shaking. At completion, aliquots from each side of the chamber were diluted 1:1 with either brain homogenate (to the buffer side) or buffer (to the brain homogenate side) in a new 96 well plate, at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for

LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$F_{u,tissue} = \frac{1/D_f}{(1/F_{u,\text{hom}} - 1) + 1/D_f}$$

Where $F_{u,hom}$ represent the measured fraction unbound in the diluted homogenate and D_f represents dilution factor

LC/MS/MS Bioanalysis of Samples from *In Vitro* Assays

Samples were analyzed on a Thermo Electron TSQ Quantum Ultra triple quad mass spectrometer (San Jose, CA) with electrospray ionization (ESI), Shimadzu LC-10ADvp pumps (Columbia, MD), and a Leap Technologies CTC PAL autosampler (Carrboro, NC). Analytes were separated by gradient elution using Fortis C18 (3.0 x 50 mm, 3 μm) columns (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 ºC. HPLC mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The gradient started at 30% B after a 0.2 min hold and was linearly increased to 95% B over 0.8 min; hold at 95% B for 0.2 min; returned to 30% B in 0.1 min. The total run time was 1.3 min and the HPLC flow rate was 0.5 mL/min. Compound optimization, data collection and processing was performed using Thermo Electron's QuickQuan software (v2.3) and Xcalibur (v2.0.7 SP1).

Cytochrome P450 Cocktail Inhibition Assay in Human Liver Microsomes

A cocktail of substrates for cytochrome P450 enzymes (1A2: Phenacetin, 10 μM; 2C9: Diclofenac, 5 μM; 2D6: Dextromethorphan, 5 μM; 3A4: Midazolam, 2 μM) were mixed for cocktail analysis. The positive control for pan-P450 inhibition (miconazole) was included

alongside test compound in analysis. A reaction mixture of 100 mM Kpi, pH 7.4, 0.1 mg/mL human liver microsomes (HLM) and Substrate Mix is prepared and aliquoted into a 96-deepwell block. Test compound and positive control (in duplicate) were then added such that the final concentration of test compound ranged from 0.1 – 30 μM. The plate was vortexed briefly and then pre-incubated at 37 ºC while shaking for 15 minutes. The reaction was initiated with the addition of NADPH (1 mM final concentration). The incubation continued for 8 min and the reaction quenched by 2x volume of cold acetonitrile containing internal standard (50 nM carbamazepine). The plate was centrifuged for 10 minutes (4000 rcf, 4 ºC) and the resulting supernatant diluted 1:1 with water for LC/MS/MS analysis. A 12 point standard curve of substrate metabolites over the range of 0.98 nM to 2000 nM.

Samples were analyzed via electrospray ionization (ESI) on an AB Sciex API-4000 (Foster City, CA) triple-quadrupole instrument that was coupled with Shimadzu LC-10AD pumps (Columbia, MD) and a Leap Technologies CTC PAL auto-sampler (Carrboro, NC). Analytes were separated by gradient elution using a Fortis C18 3.0 x 50 mm, 3 μm column (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 ºC. HPLC mobile phase A was 0.1% formic acid in water (pH unadjusted), mobile phase B was 0.1% formic acid in acetonitrile (pH unadjusted). The gradient started at 10% B after a 0.2 min hold and was linearly increased to 90% B over 1.2 min; held at 90% B for 0.1 min and returned to 10% B in 0.1 min followed by a re-equilibration (0.9 min). The total run time was 2.5 min and the HPLC flow rate was 0.5 mL/min. The source temperature was set at 500 ºC and mass spectral analyses were performed using multiple reaction monitoring (MRM), with transitions specific for each compound utilizing a Turbo-Ionspray® source in positive ionization mode (5.0 kV spray voltage).

The IC_{50} values for each compound were obtained for the individual CYP enzymes by quantitating the inhibition of metabolite formation for each probe substrate. A 0 µM compound condition (or control) was set to 100% enzymatic activity and the effect of increasing test compound concentrations on enzymatic activity could then be calculated from the % of control activity. Curves were fitted using XLfit 5.2.2 (four-parameter logistic model, equation 201) to determine the concentration that produces half-maximal inhibition (IC_{50}).

P-gp Substrate Assessment Using MDR1-MDCK Cell Monolayers

This study was run by Absorptions Systems®, 436 Creamery Way, Suite 600, Exton, PA 19341 (http://www.absorption.com/). Briefly, MDR1-MDCK cell monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. Details of the plates and their certification are shown below. The permeability assay buffer was Hanks Balanced Salt Solution (HBSS) containing 10 mM HEPES and 15 mM glucose at a pH of 7.4. The buffer in the receiver chamber also contained 1% bovine serum albumin. The dosing solution concentration was 5 µM test article in the assay buffer +/- 1 µM valspodar. Cells were first pre-incubated for 30 minutes with HBSS +/- 1 µM valspodar. Cell monolayers were then dosed on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37 ºC with 5% CO_2 in a humidified incubator. Samples were taken from the donor and receiver chambers at 120 minutes. Each determination was performed in duplicate. The flux of co-dosed lucifer yellow was also measured for each monolayer to ensure no damage was inflicted to the cell monolayers during the flux period. All samples were assayed by LC-MS/MS using electrospray ionization. Liquid chromatography was carried out on a Waters ACQUITY UPLC BEH Phenyl 30 x 2.1 mm, 1.7 µm column using an M.P. buffer of 25 mM ammonium

formate (pH 3.5), an aqueous reservoir (A) of 90% water and 10% buffer, and an organic reservoir (B) of 90% acetonitrile and 10% buffer with a flow rate of 0.7 mL/min. The gradient program was run from 100% (A) to 100% (B) with a total run time of 1.0 min. Injection volume was 0.2 µL. The mass spectrometer was a PE SCIEX API 4000 with a turbospray interface and was run in multiple reaction monitoring mode. The apparent permeability (Papp) and percent recovery were calculated as follows:

(1) $P_{app} = (dC_r/dt) \times V_r/(A \times C_A)$

Percent recovery = $100 \times ((V_r \times C_r^{final}) + (V_d \times C_d^{final}))/(V_d \times C_N)$

Where,

dC_r/dt is the slope of the cumulative concentration in the receiver compartment versus time in $\mu M \cdot s^{-1}$;

V_r is the volume of the receiver compartment in cm^3;

V_d is the volume of the donor compartment in cm^3;

A is the area of the insert (1.13 cm^2 for 12-well Transwell);

C_A is the average of the nominal dosing concentration and the measured 120 minute donor concentration in µM;

C_N is the nominal concentration of the dosing solution in µM;

C_r^{final} is the cumulative receiver concentration in µM at the end of the incubation period;

C_d^{final} is the concentration of the donor in µM at the end of the incubation period.

Efflux ratio (ER) is defined as P_{app} (B-to-A) / P_{app} (A-to-B)

***In-Vivo* PK Methods**

All rodent PK experiments were conducted in accordance with the National Institute of Health regulations of animal care covered in Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee.

Rodent Time Course PK Studies

IV cassette PK experiments in rats were carried out according to methods described previously (Bridges et al. *Pharmacol. Res. Perspect.* 2014; reference 52). Briefly, A cassette of compounds (n = 4–5/cassette) were formulated from 10 mM solutions of compounds in DMSO. In order to reduce the absolute volume of DMSO that was administered, the compounds were combined and diluted with ethanol and PEG 400 to achieve a final concentration of 0.4–0.5 mg/mL for each compound (2 mg/mL total) administered in each cassette. The final dosing solutions consisted of approximately 10% ethanol, 40% PEG400, and 50% DMSO (v/v). Each cassette dose was administered IV via the jugular vein to two dual-cannulated (carotid artery and jugular vein) adult male Sprague–Dawley rats, each weighing between 250 and 350 g (Harlan, Indianapolis, IN) for a final dose of 0.2–0.25 mg/kg per compound. Whole blood collections via the carotid artery were performed at 0.033, 0.117, 0.25, 0.5, 1, 2, 4, 7, and 24 hours post dose.

The discrete IV PK experiment in rats (n=2) was carried out analogously using a 1.0 mg/kg solution of **106** at 1 mg/mL in 10% EtOH, 50% PEG 400, 40% saline. The discrete IP PK experiment in rats (n=3) was carried out analogously using a 10 mg/kg fine microsuspension of **106** at 4 mg/mL in 0.1% Tween 80 and 0.5% methyl cellulose in H_2O. Whole blood collections via the carotid artery were performed at 0.117, 0.25, 0.5, 1, 2, 4, 7, and 24 hours post dose. The discrete PO PK experiment in rats (n=2) was carried out analogously using a 3.0 mg/kg fine

microsuspension of **106** at 0.3 mg/mL in 0.1% Tween 80 and 0.5% methyl cellulose in H_2O. Whole blood collections via the carotid artery were performed at 0.25, 0.5, 1, 2, 4, 7, and 24 hours post dose. The discrete IP PK experiment in mice (n=21, 3 mice per time point) was carried out analogously using a 10 mg/kg fine microsuspension of **106** at 1.0 mg/mL in 0.1% Tween 80 and 0.5% methyl cellulose in H_2O. Mice were euthanized and whole blood collections via cardiac puncture were performed at 0.25, 0.5, 1, 2, 4, 7, and 24 hours post dose.

Rodent Tissue Distribution Studies

Single time point IP tissue distribution experiments in rodents were carried out according to methods described previously (Bridges et al. *Drug Metab. Dispos.* 2014; reference 56). Briefly, male Sprague–Dawley rats, each weighing between 250 and 350 g, or male CD-1 mice, each weighing between 20-30 g (Harlan, Indianapolis, IN) were dosed with test compound (IP). Formulations were a fine homogeneous suspension at 4.0 mg/mL in 10% EtOH and 90% PEG400 for **106** or a fine homogeneous suspension at 4.0 mg/mL in 0.1% Tween 80 and 0.5% methyl cellulose in H_2O for **99** and **109**. Animals were euthanized and decapitated, and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice. The blood (cardiac puncture) and brain were collected at 0.25 (rats) or 0.5 (mice) hours post dose.

Plasma and Brain Sample Preparation

Plasma was separated by centrifugation (4000 rcf, 4 ºC) and stored at –80 ºC until analysis. On the day of analysis, frozen whole-rat brains were weighed and diluted with 1:3 (w/w) parts of 70:30 isopropanol:water. The mixture was then subjected to mechanical homogenation

employing a Mini-Beadbeater™ and 1.0 mm Zirconia/Silica Beads (BioSpec Products) followed by centrifugation. The sample extraction of plasma (20 μL) or brain homogenate (20 μL) was performed by a method based on protein precipitation using three volumes of ice-cold acetonitrile containing an internal standard (50 ng/mL carbamazepine). The samples were centrifuged (3000 rcf, 5 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis.

LC/MS/MS Bioanalysis of Samples from *In Vivo* Assays

In vivo samples were analyzed via electrospray ionization (ESI) on an AB Sciex API-4000 (Foster City, CA) triple-quadrupole instrument that was coupled with Shimadzu LC-10AD pumps (Columbia, MD) and a Leap Technologies CTC PAL auto-sampler (Carrboro, NC). Analytes were separated by gradient elution using a Fortis C18 3.0 x 50 mm, 3 μm column (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 ºC. HPLC mobile phase A was 0.1% formic acid in water (pH unadjusted), mobile phase B was 0.1% formic acid in acetonitrile (pH unadjusted). The source temperature was set at 500 ºC and mass spectral analyses were performed using multiple reaction monitoring (MRM), with transitions specific for each compound utilizing a Turbo-Ionspray® source in positive ionization mode (5.0 kV spray voltage). The calibration curves were constructed, and linear response was obtained by spiking known amounts of test compound in blank brain homogenate or plasma. All data were analyzed using AB Sciex Analyst software v1.5.1. The final PK parameters were calculated by noncompartmental analysis using Phoenix (version 6.2) (Pharsight Inc., Mountain View, CA).

Behavioral Pharmacology Methods

Compounds. The $mGlu_3$ NAM **106** and the $mGlu_5$ NAM MTEP were prepared in house according to methods disclosed herein and in the literature (Cosford et al. *J. Med. Chem.* 2003; reference 70) respectively. Ketamine was purchased from Patterson Veterinary Supply Inc. Doses of **106** and MTEP were dissolved in 10% Tween 80 in H_2O, vortexed vigorously, heated gently with a Master Heat Gun (Master Appliance Corp., Racine, WI), and sonicated at 37 ºC for 30 min. The pH was checked using 0-14 EMD strips and adjusted to approximately 7. Compound **106** and MTEP were administered IP. Ketamine was dissolved in sterile saline. Ketamine was administered SC.

Marble burying dose groups. vehicle, 15 mg/kg MTEP (positive control), 10 mg/kg **106**, 30 mg/kg **106**, and 56.6 mg/kg **106**.

Marble burying subjects. This study was conducted using male Harlan CD-1 mice (Indianapolis, IN), weighing 30 to 35 grams. Subjects were housed in a large colony room under a 12-h light/dark cycle (lights on at 6:00 a.m.) with food and water provided *ad libitum*. Test sessions were performed between 10:00 a.m. and 4:00 p.m. All dose groups consisted of 8-10 mice. All experiments were conducted in accordance with the National Institute of Health regulations of animal care covered in Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee.

Marble burying procedure. Eight small Plexiglass cages (32 x 17 x 14 cm) were arranged in two rows of four cages on top of a large, round table. Mice were transported from the colony

room to the testing room and allowed to habituate for 30 minutes. Mice were pretreated with vehicle, a dose of **106**, or a dose of MTEP for 15 min and individually placed in the cages in which 12 black glass marbles (14 mm diameter) had been evenly distributed (spaced 6.4 cm vertically and 4.25 cm horizontally from each other and the walls of the cage) on top of 2.5 cm Diamond Soft Bedding (Harlan Teklad, Madison, WI). The compound and comparator were evaluated in a counterbalanced design, in which all doses of compounds were tested in each session. Mice receiving the same dose were placed in cages on opposite sides of the table to control for effects of lighting and context. Clear, perforated plastic lids were set on top of each cage and the amount of marble burying was recorded over a 30 min interval. The mice were then removed from the cages and the number of buried marbles was counted using the criteria of greater than two-thirds covered by bedding. Each session was videotaped with a Sony MiniDV camcorder equipped with a Sony wide-angle lens mounted on a 1.5 m tripod. Effects that could be characterized as overtly sedating or impairing motor function were monitored visually and were not observed with compound **106**.

Forced swim test (FST) dose groups. vehicle, 10 mg/kg ketamine (positive control), 30 mg/kg **106**, 56.6 mg/kg **106**

FST subjects. This study was conducted using male Harlan Sprague-Dawley rats (Indianapolis, IN), weighing 275 to 325 grams. Subjects were housed in a large colony room under a 12-h light/dark cycle (lights on at 6:00 a.m.) with food and water provided *ad libitum*. Test sessions were performed between 10:00 a.m. and 4:00 p.m. All dose groups consisted of 8-10 rats. All experiments were conducted in accordance with the National Institute of Health regulations of

animal care covered in Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee.

FST procedure. Rats were exposed to 2 swim sessions, a habituation session and 24 hours later a 6 minute testing session. In the habituation session rats were placed in plexiglass cylinders (45 x 20cm) containing 23-25 ºC water approximately 30 cm deep for 15 minutes. On the testing day compound or vehicle was administered thirty minutes prior to the test. After the pre-treatment period the animals were placed in swim tanks as described above and then underwent a 6 minute testing session which was recorded. Following both swim sessions, rats were removed from the cylinder, dried with a paper towel, then transferred to a drying environment for 15 minutes or until the rat is sufficiently dry to return to its home cage. Each session was videotaped with a Sony MiniDV camcorder equipped with a Sony wide-angle lens mounted on a 1.5 m tripod. Videos were manually scored for the time the animal exhibited immobility.

Data Analysis. The data for the dose-response studies were analyzed by a between-group analysis of variance. If there was a main effect of dose, then each dose group was compared with the vehicle control group using a Dunnett's comparison. The calculations were performed using JMP IN 8 (SAS Institute, Cary, NC) statistical software and graphed using SigmaPlot9 (Sasgua, MA).

Ancillary Pharmacology Profile of Compound 106

LeadProfilingScreen®, Eurofins Panlabs, Inc. (http://www.eurofinspanlabs.com)

Compound tested at 10 μM

Target	Sp	% Inh
Adenosine A_1	hum	21
Adenosine A_{2A}	hum	8
Adenosine A_3	hum	6
Adrenergic α_{1A}	rat	1
Adrenergic α_{1B}	rat	9
Adrenergic α_{1D}	hum	-3
Adrenergic α_{2A}	hum	7
Adrenergic β_1	hum	1
Adrenergic β_2	hum	6
Androgen AR	rat	8
Bradykinin B_1	hum	4
Bradykinin B_2	hum	18
Ca^{2+} channel L-type, benzothiazepine	rat	-14
Ca^{2+} channel L-type, dihydropyridine	rat	12
Ca^{2+} channel, N-type	rat	-2
Cannabinoid CB_1	hum	19
Dopamine D_1	hum	-3
Dopamine D_{2S}	hum	-11
Dopamine D_3	hum	19
Dopamine $D_{4.2}$	hum	6

Target	Sp	% Inh
Endothelin ET_A	hum	8
Endothelin ET_B	hum	2
Epidermal Growth Factor (EGF)	hum	9
Estrogen ERα	hum	8
$GABA_A$, flunitrazepam, central	rat	2
$GABA_A$, muscimol, central	rat	12
$GABA_{B1A}$	hum	-5
Glucocorticoid	hum	16
Glutamate, kainate	rat	15
Glutamate, NMDA, agonism	rat	13
Glutamate, NMDA, glycine	rat	15
Glutamate, NMDA, phencyclidine	rat	0
Histamine H_1	hum	8
Histamine H_2	hum	-1

Target	Sp	% Inh
Histamine H_3	hum	1
Imidazoline I_2, central	rat	-15
Interleukin IL-1	mouse	-1
Leukotriene, cysteinyl $CysLT_1$	hum	12
Melatonin MT_1	hum	5
Muscarinic M_1	hum	11
Muscarinic M_2	hum	1
Muscarinic M_3	hum	4
Neuropeptide Y Y_1	hum	-3
Neuropeptide Y Y_2	hum	3
Nicotinic acetylcholine	hum	4
Nicotinic acetylcholine α1, bungarotoxin	hum	1
Opiate δ_1 (OP1, DOP)	hum	5
Opiate κ(OP2, KOP)	hum	16
Opiate μ(OP3, MOP)	hum	26
Phorbol ester	mouse	3
Platelet activating factor (PAF)	hum	37
Potassium Channel [K_{ATP}]	ham	-8
Potassium Channel hERG	hum	10
Prostanoid EP_4	hum	8

Target	Sp	% Inh
Purinergic P_{2X}	rabbit	16
Purinergic P_{2Y}	rat	-7
Rolipram	rat	7
Serotonin 5-HT_{1A}	hum	14
Serotonin 5-HT_{2B}	hum	65
Serotonin 5-HT_3	hum	6
Sigma σ_1	hum	17
Sodium channel, site 2	rat	19
Tachykinin NK_1	hum	9
Thyroid hormone	rat	17
Transporter, dopamine (DAT)	hum	16
Transporter, GABA	rat	-4
Transporter, norepinephrine (NET)	hum	9
Transporter, serotonin (SERT)	hum	9