Supporting Information for:

VU6010608, a Novel $mGlu_7$ NAM from a Series of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides

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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. 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. 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. 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. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All final compounds were >98% pure by LCMS (254 nm, 214 nM and ELSD).

General Procedure 1: Synthesis of Esters (7)

To a suspension of methyl vanillate (100 mg, 0.549 mmol) and potassium carbonate (154 mg, 1.10 mmol) in DMF (1 mL) was added the appropriate alkyl halide (1.10 mmol) at room temperature. The resulting suspension was then heated to 100 °C and was monitored by LCMS. Once LCMS indicated complete consumption of starting material, the reaction was diluted with DCM and quenched with the addition of water. The layers were separated, and the aqueous layer was washed with DCM x 3. The combined organic material was passed through a phase separator and concentrated to afford the desired material, which was carried forward to the next step without any further purification.

General Procedure 2: Synthesis of Benzoic Acids (8)

To a solution of the appropriate methyl ester (1.0 eq) in THF: H2O (1:1; 0.2 M) was added lithium hydroxide (1.5 eq). The resulting reaction was heated to 60 °C and monitored by LCMS. After 2 hours, the reaction was allowed to cool to room temperature before the organic solvent was removed *in vacuo*. The resulting aqueous solution was acidified to pH = 2 using 2 M HCl, which resulted in a white precipitate crashing out of solution. This white solid was collected via

vacuum filtration, dried *in vacuo*, and carried forward to the next step without any further purification.

General Procedure 3: Synthesis of Benzamides (10)

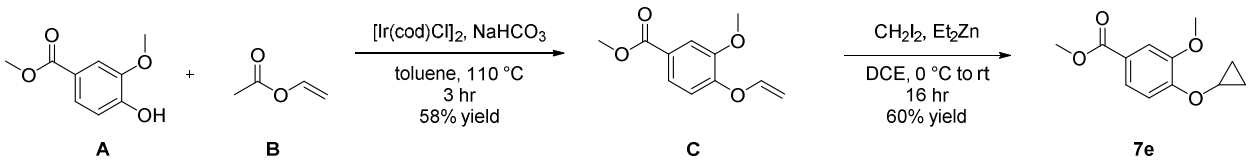
To a solution of the appropriate aniline (1.0 eq) in DCM (0.1 M) in a Biotage microwave vial was sequentially added N,N-diisopropylethylamine (2.0 eq), the appropriate benzoic acid (1.0 eq), and 1-(chloro-1-pyrrolidinylmethylene)pyrrolidiniumhexafluorophosphate (PyClU) (1.0 eq). The reaction vial was sealed and heated to 100 °C for 20 minutes using a Biotage microwave reactor. After cooling to room temperature, the reaction was diluted with DCM and quenched with the addition of saturated NH_4Cl. The layers were separated, and the aqueous layer was washed with DCM x 3. The combined organic layer was passed through a phase separator, concentrated, and purified using flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc).

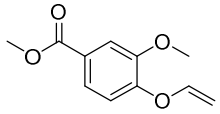
General Procedure 4: Synthesis of Triazoles (11)

To a suspension of the appropriate benzamide (1.0 eq), 1,2,4-triazole (1.0 eq), potassium phosphate tribasic (2.5 eq), and copper (I) iodide (0.05 eq) in DMF (0.1 M) was added trans-N,N'-dimethylcyclohexane-1,2-diamine (0.10 eq). The resulting suspension was degassed by vigorously bubbling argon through the mixture for one minute. The reaction was then heated to 100 °C for 16 hours, whereupon LCMS indicated complete consumption of starting material and formation of the desired product. The reaction was diluted with EtOAc and filtered over a pad of celite. The resulting organic material was concentrated and purified using a Gilson HPLC system (30 x 50 mm column; H_2O with 0.1% TFA:acetonitrile). Fractions containing the desired product

were quenched with saturated $NaHCO_3$, extracted with DCM, and concentrated to liberate the product as the free base.

Synthesis of 4-cyclopropoxy-3-methoxybenzoic acid (7e).

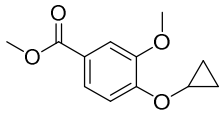




Synthesis of methyl 3-methoxy-4-(vinyloxy)benzoate (C)

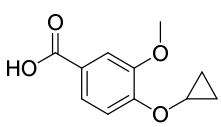
An oven dried vial was sequentially charged with methyl vanillate **A** (250 mg, 1.37 mmol), sodium carbonate (51.9 mg, 0.480 mmol), chloro(1,5-cyclooctadiene)iridium(I) dimer (9.21 mg, 0.0137 mmol), toluene (1.0 mL), and vinyl acetate **B** (0.253 mL, 2.74 mmol). The resulting solution was degassed by vigorously bubbling argon through the reaction mixture for two minutes. The vial was then heated to 110 °C, monitoring the reaction by LCMS. After 3 hours, the reaction was allowed to cool to room temperature and was filtered through a pad of celite. The resulting organic material was concentrated and purified using flash chromatography (Teledyne ISCO system; silica gel column; hexanes:EtOAc) to afford the desired material **C** as a yellow oil (165 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 7.61 (d, *J* = 1.9 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.62 (dd, *J* = 12.0 Hz, 6.0 Hz, 1H), 4.87 (dd, *J* = 12.0 Hz, 2.0 Hz, 1H), 4.54 (dd, *J* = 4.0 Hz, 2.0 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ = 166.7, 149.8, 149.6, 147.7, 125.7, 123.2, 116.5, 113.3, 96.8, 56.3, 52.3 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{12}O_4$, 208.0736 ; found, 208.0737.



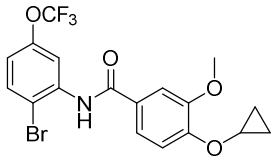
Synthesis of methyl 4-cyclopropoxy-3-methoxybenzoate (7e)

To a solution of methyl 3-methoxy-4-(vinyloxy)benzoate **C** (115 mg, 0.552 mmol) and diiodomethane (0.356 mL, 4.42 mmol) in DCE (2.0 mL) at 0 °C was added diethylzinc (4.42 mL, 4.42 mmol, 1.0 M solution in hexanes) dropwise. After the addition was complete, the flask was allowed to slowly warm to room temperature, stirring for an additional 16 hours. The reaction was diluted with DCM, quenched with the addition of saturated NH_4Cl, and filtered through a pad of celite. The layers were separated, and the aqueous layer was washed with DCM x 3. The combined organic material was passed through a phase separator, concentrated, and purified using flash chromatography (Teledyne ISCO system; silica gel column; hexanes:EtOAc) to afford the desired material **7e** as a slight yellow oil (74.1 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.80 (m, 1H), 0.90-0.83 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.0, 152.6, 148.6, 123.5, 123.1, 112.7, 112.2, 56.1, 52.1, 51.9, 6.5 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{14}O_4$, 222.0892 ; found, 222.0892.



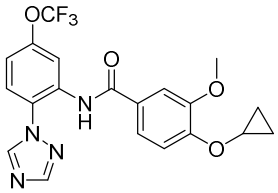
Synthesis of 4-cyclopropoxy-3-methoxybenzoic acid (8e)

This compound was synthesized according to general procedure 2. White solid (112.4 mg, quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (dd, *J* = 8.4 Hz, 1.9 Hz, 1H), 7.44 (d, *J* = 1.8 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 3.89 (m, 1H), 3.77 (s, 3H), 0.83-0.78 (m, 2H), 0.70-0.66 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 151.9, 148.2, 123.4, 123.0, 112.9, 112.0, 55.4, 51.4, 5.9 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{12}O_4$, 208.0736; found, 208.0735.



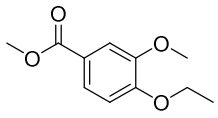
Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-4-cyclopropoxy-3-methoxybenzamide (10e)

This compound was synthesized according to general procedure 3. White solid (28.6 mg, 82% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.2 Hz, 1H), 8.49 (bs, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 7.47 (dd, *J* = 8.0 Hz, 2.1 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 6.89 (m, 1H), 3.94 (s, 3H), 3.83 (m, 1H), 0.92-0.86 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.0, 152.3, 149.5, 149.2, 137.3, 132.9, 127.0, 120.5 (q, $^{1}J_{CF}$ = 257 Hz), 119.5, 117.2, 114.2, 113.0, 111.0, 110.7, 56.2, 52.1, 6.5 ppm. HRMS (TOF, ES+) calc'd for $C_{18}H_{15}BrF_3NO_4$, 445.0137 ; found, 445.0140.



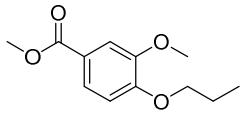
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-cyclopropoxy-3-methoxybenzamide (11e) (VU6012698)

This compound was synthesized according to general procedure 4. White solid (8.7 mg, 68% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 10.54 (bs, 1H), 8.77 (d, *J* = 2.1 Hz, 1H), 8.51 (s, 1H), 8.31 (s, 1H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.43 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.09 (dd, *J* = 8.8 Hz, 1.6 Hz, 1H), 3.93 (s, 3H), 3.82 (m, 1H), 0.90-0.84 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.2, 152.3, 149.8, 149.3, 143.9, 133.7, 126.8, 123.8, 123.6, 120.5 (q, $^{1}J_{CF}$ = 257 Hz), 119.9, 116.0, 115.6, 113.0, 110.9, 56.1, 52.0, 6.5 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{17}F_3N_4O_4$, 434.1202; found, 434.1204.



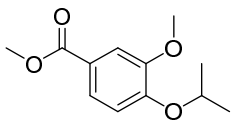
Synthesis of methyl 4-ethoxy-3-methoxybenzoate (7b)

This compound was synthesized according to general procedure 1 with ethyl iodide. White solid (113.1 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.15 (q, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 1.48 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 152.5, 148.9, 123.7, 122.6, 112.4, 111.4, 64.6, 56.2, 52.1, 14.8 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{14}O_4$, 210.0892; found, 210.0890.



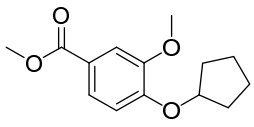
Synthesis of methyl 3-methoxy-4-propoxybenzoate (7c)

This compound was synthesized according to general procedure 1 with *n*-propyl iodide. Colorless oil (120.5 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.01 (t, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 1.87 (m, 2H), 1.03 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 152.8, 149.0, 123.7, 122.5, 112.5, 111.6, 70.6, 56.2, 52.1, 22.5, 10.5 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{16}O_4$, 224.1049; found, 224.1047.



Synthesis of methyl 4-isopropoxy-3-methoxybenzoate (7d)

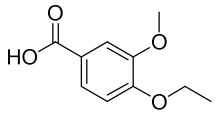
This compound was synthesized according to general procedure 1 with isopropyl iodide. Colorless oil (117.3 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.5 Hz, 2.0 Hz, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.62 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 1.38 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.0, 151.6, 149.6, 123.5, 113.4, 112.7, 71.3, 56.1, 52.0, 22.0 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{16}O_4$, 224.1049; found, 224.1046.



Synthesis of methyl 4-(cyclopentyloxy)-3-methoxybenzoate (7f)

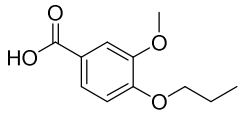
This compound was synthesized according to general procedure 1 with cyclopentyl iodide. Colorless oil (131.4 mg, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.51 (d, *J* = 1.9 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 4.81 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H),

1.98-1.77 (m, 6H), 1.64-1.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.0, 152.0, 149.4, 123.5, 122.2, 113.1, 112.6, 80.5, 56.1, 52.0, 32.9, 24.2 ppm. HRMS (TOF, ES+) calc'd for $C_{14}H_{18}O_4$, 250.1205; found, 250.1204.



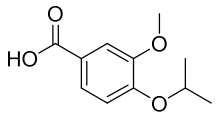
Synthesis of 4-ethoxy-3-methoxybenzoic acid (8b)

This compound was synthesized according to general procedure 2. White solid (88.8 mg, quantitative yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 4.08 (q, 2H), 3.80 (s, 3H), 1.34 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 151.9, 148.3, 123.1, 122.8, 112.0, 111.7, 63.8, 55.4, 14.6 ppm. HRMS (TOF, ES+) calc'd for $C_{10}H_{12}O_4$, 196.0736 ; found, 196.0734.



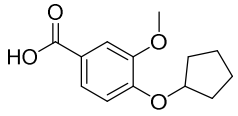
Synthesis of 3-methoxy-4-propoxybenzoic acid (8c)

This compound was synthesized according to general procedure 2. White solid (95.3 mg, quantitative yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.44 (d, *J* = 1.9 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 3.98 (t, 2H), 3.80 (s, 3H), 1.75 (m, 2H), 0.97 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.0, 148.4, 123.2, 122.8, 112.1, 111.8, 69.7, 55.5, 22.0, 10.4 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{14}O_4$, 210.0892 ; found, 210.0889.



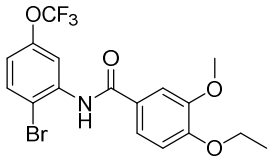
Synthesis of 4-isopropoxy-3-methoxybenzoic acid (8d)

This compound was synthesized according to general procedure 2. White solid (112.1 mg, quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 4.67 (m, 1H), 3.78 (s, 3H), 1.28 (d, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 150.8, 149.0, 123.1, 122.7, 113.3, 112.4, 70.2, 55.4, 21.8 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{14}O_4$, 210.0892 ; found, 210.0892.



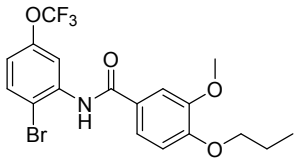
Synthesis of 4-(cyclopentyloxy)-3-methoxybenzoic acid (8f)

This compound was synthesized according to general procedure 2. White solid (122.0 mg, quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 4.86 (m, 1H), 3.78 (s, 3H), 1.96-1.90 (m, 2H), 1.73-1.68 (m, 4H), 1.61-1.54 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 151.0, 148.9, 123.1, 122.6, 113.1, 112.3, 79.6, 55.4, 32.3, 23.7 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{16}O_4$, 236.1049 ; found, 236.1050.



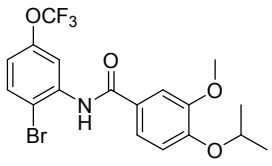
Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-4-ethoxy-3-methoxybenzamide (10b)

This compound was synthesized according to general procedure 3. White solid (37.5 mg, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.5 Hz, 1H), 8.48 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.44 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H), 4.18 (q, 2H), 3.96 (s, 3H), 1.51 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 152.3, 149.8, 149.2, 137.4, 132.9, 126.5, 120.6 (q, $^1J_{CF}$ = 256 Hz), 119.7, 117.2, 114.3, 111.7, 111.2, 110.7, 64.8, 56.3, 14.8 ppm. HRMS (TOF, ES+) calc'd for $C_{17}H_{15}BrF_3NO_4$, 433.0137 ; found, 433.0136.



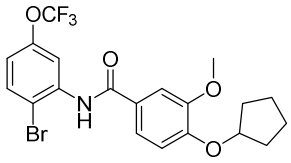
Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-3-methoxy-4-propoxybenzamide (10c)

This compound was synthesized according to general procedure 3. White solid (24.6 mg, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.4 Hz, 1H), 8.48 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.44 (dd, *J* = 8.0 Hz, 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 4.06 (t, 2H), 3.95 (s, 3H), 1.91 (m, 2H), 1.07 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 152.6, 149.9, 149.3, 149.2, 137.4, 132.9, 126.5, 120.6 (q, $^1J_{CF}$ = 257 Hz), 119.8, 117.2, 114.3, 111.9, 111.3, 110.7, 70.8, 56.4, 22.5, 10.6 ppm. HRMS (TOF, ES+) calc'd for $C_{18}H_{17}BrF_3NO_4$, 447.0293 ; found, 447.0288.



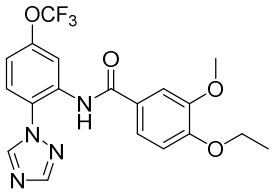
Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-4-isopropoxy-3-methoxybenzamide (10d)

This compound was synthesized according to general procedure 3. White solid (56.8 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.4 Hz, 1H), 8.48 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.43 (dd, *J* = 8.0 Hz, 2.2 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 4.66 (m, 1H), 3.94 (s, 3H), 1.43 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.0, 151.4, 150.5, 149.1, 137.4, 132.9, 126.5, 120.5 (q, $^{1}J_{CF}$ = 257 Hz), 119.6, 117.1, 114.2, 113.8, 111.5, 110.7, 71.6, 56.3, 22.1 ppm. HRMS (TOF, ES+) calc'd for $C_{18}H_{17}BrF_3NO_4$, 447.0293 ; found, 447.0285.



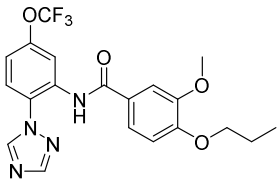
Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-4-(cyclopentyloxy)-3-methoxybenzamide (10f)

This compound was synthesized according to general procedure 3. White solid (65.7 mg, 71% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.4 Hz, 1H), 8.48 (bs, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.43 (dd, *J* = 8.0 Hz, 2.2 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 4.86 (m, 1H), 3.93 (s, 3H), 2.03-1.84 (m, 6H), 1.68-1.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 151.8, 150.3, 149.2, 137.4, 132.9, 126.2, 120.5 (q, $^{1}J_{CF}$ = 255 Hz), 119.6, 117.1, 114.2, 113.5, 111.4, 110.7, 80.8, 56.3, 33.0, 24.3 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{19}BrF_3NO_4$, 473.0450 ; found, 473.0450.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-ethoxy-3-methoxybenzamide (11b) (VU6011121)

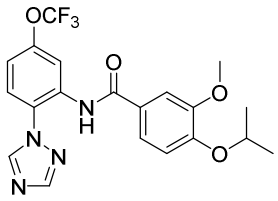
This compound was synthesized according to general procedure 4. White solid (15.6 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (bs, 1H), 8.76 (d, *J* = 2.0 Hz, 1H), 8.53 (s, 1H), 8.30 (s, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.41 (dd, *J* = 8.5 Hz, 2.2 Hz, 1H), 7.09 (m, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 4.18 (q, 2H), 3.95 (s, 3H), 1.51 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.2, 149.8, 149.5, 143.9, 133.7, 126.3, 123.8, 123.6, 120.5 (q, $^{1}J_{CF}$ = 257 Hz), 120.1, 116.0, 115.6, 111.7, 110.9, 64.7, 56.2, 14.8 ppm. HRMS (TOF, ES+) calc'd for $C_{19}H_{17}F_3N_4O_4$, 422.1202 ; found, 422.1204.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3-methoxy-4-propoxybenzamide (11c) (VU6010953)

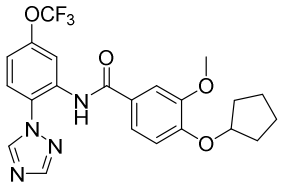
This compound was synthesized according to general procedure 4. Colorless oil (13.7 mg, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.76 (d, *J* = 2.1 Hz, 1H), 8.51 (s, 1H), 8.30 (s, 1H), 7.51 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.41 (dd, *J* = 8.5 Hz, 2.2 Hz, 1H), 7.09 (m, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 4.06 (t, 2H), 3.95 (s, 3H), 1.91 (m, 2H), 1.06 (t, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.4, 149.7, 149.6, 143.9, 133.7, 126.2, 123.8, 123.6, 120.5 (q, $^1J_{CF}$ = 257 Hz), 120.1, 116.0, 115.6, 111.9, 111.0, 70.7, 56.2, 22.5, 10.5 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{19}F_3N_4O_4$, 436.1358; found, 436.1358.



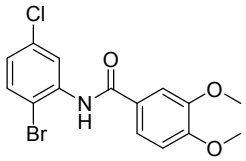
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-isopropoxy-3-methoxybenzamide (11d) (VU6010955)

This compound was synthesized according to general procedure 4. Colorless oil (11.0 mg, 56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (bs, 1H), 8.76 (d, *J* = 2.0 Hz, 1H), 8.51 (s, 1H), 8.30 (s, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.40 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H), 7.09 (m, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 4.66 (m, 1H), 3.94 (s, 3H), 1.42 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 151.3, 150.3, 149.8, 143.9, 133.8, 126.2, 123.8, 123.7, 120.5 (q, $^1J_{CF}$ = 255 Hz), 120.0, 116.0, 115.6, 113.7, 111.3, 71.5, 56.2, 22.1 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{19}F_3N_4O_4$, 436.1358 ; found, 436.1361.



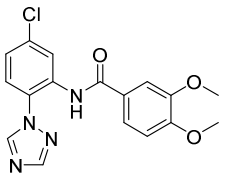
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopentyloxy)-3-methoxybenzamide (11f) (VU6011111)

This compound was synthesized according to general procedure 4. White solid (25.3 mg, 58% yield).[1]H NMR (400 MHz, $CDCl_3$) δ 10.50 (bs, 1H), 8.76 (d, *J* = 2.1 Hz, 1H), 8.51 (s, 1H), 8.30 (s, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.39 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 7.08 (dd, *J* = 8.7 Hz, 1.6 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 4.85 (m, 1H), 3.92 (s, 3H), 2.02-1.83 (m, 6H), 1.67-1.62 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.2, 153.1, 151.7, 150.1, 149.7, 143.9, 133.8, 125.9, 123.8, 123.6, 120.5 (q, $^1J_{CF}$ = 257 Hz), 120.0, 115.9, 115.6, 113.4, 111.2, 80.7, 56.2, 33.0, 24.3 ppm. HRMS (TOF, ES+) calc'd for $C_{22}H_{21}F_3N_4O_4$,462.1515 ; found, 462.1518.



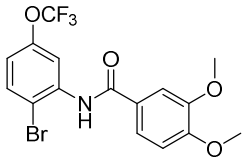
Synthesis of *N*-(2-bromo-5-chlorophenyl)-3,4-dimethoxybenzamide.

This compound was synthesized according to general procedure 3. White solid (102.1 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 2.5 Hz, 1H), 8.41 (bs, 1H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.46 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H), 6.99 (dd, *J* = 8.6 Hz, 2.5 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 3.97 (s, 3H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.9, 152.8, 149.6, 137.0, 134.7, 132.9, 126.9, 125.1, 121.5, 119.8, 111.2, 110.9, 110.7, 56.3, 56.2 ppm. HRMS (TOF, ES+) calc'd for $C_{15}H_{13}BrClNO_3$, 368.9767 ; found, 368.9768.



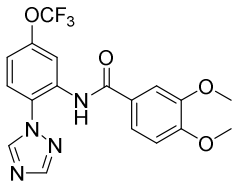
Synthesis of N-(5-chloro-2-(1H-1,2,4-triazol-1-yl)phenyl)-3,4-dimethoxybenzamide (5) (VU6009748)

This compound was synthesized according to general procedure 4. White solid (49.7 mg, 51% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (bs, 1H), 8.81 (d, *J* = 2.3 Hz, 1H), 8.50 (s, 1H), 8.28 (s, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.43 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.34 (d, *J* = 8.6 Hz,, 1H), 7.20 (dd, *J* = 8.5 Hz, 2.3 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.0, 153.0, 152.7, 149.3, 143.8, 135.8, 133.1, 126.6, 124.1, 123.4, 123.1, 120.1, 110.7, 110.6, 56.2, 56.1 ppm. HRMS (TOF, ES+) calc'd for $C_{17}H_{15}ClN_4O_3$,358.0833 ; found, 358.0835.



Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-3,4-dimethoxybenzamide (10a)

This compound was synthesized according to general procedure 3. White solid (32.6 mg, 81% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 2.6 Hz, 1H), 8.48 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.8 Hz, 1H), 7.46 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H), 3.97 (s, 3H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.9, 152.8, 149.6, 149.1, 137.3, 132.9, 126.7, 120.7 (q, $^{1}J_{CF}$ = 257 Hz), 119.2, 117.2, 114.2, 110.9, 110.7, 110.6, 56.3, 56.2 ppm. HRMS (TOF, ES+) calc'd for $C_{16}H_{13}BrF_3NO_4$, 418.9980 ; found, 418.9981.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3,4-dimethoxybenzamide (10b) (VU6010608)

This compound was synthesized according to general procedure 4. White solid (11.7 mg, 60% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (bs, 1H), 8.77 (d, *J* = 1.9 Hz, 1H), 8.51 (s, 1H), 8.30 (s, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.44 (m, 2H), 7.10 (m, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 3.97 (s, 3H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.8, 149.7, 149.4, 143.9, 133.7, 126.5, 123.8, 123.6, 120.5 (q, $^{1}J_{CF}$ = 257 Hz), 120.1, 116.0, 115.6, 110.7, 110.6, 56.2, 56.1 ppm. HRMS (TOF, ES+) calc'd for $C_{18}H_{15}F_3N_4O_4$, 408.1045 ; found, 408.1047.

Molecular Pharmacology Methods

Calcium assays. Human $mGlu_4/G_{qi5}$/CHO cells (30,000 cells/20 μL/well), rat $mGlu_7/G_{a15}$/HEK cells (15,000 cells/20 μL/well), and rat $mGlu_8/G_{a15}$/HEK cells (15,000 cells/20 μL/well) were plated in black-walled, clear-bottomed, TC treated, 384 well plates (Greiner Bio-One, Monroe, NC) in DMEM containing 10% dialyzed FBS, 20 mM HEPES, 100 units/mL penicillin/streptomycin, and 1 mM sodium pyruvate (Plating Medium) as described in Jalan-Sakrikar et al., 2014. The cells were grown overnight at 37 °C in the presence of 5% CO_2. The next day, the medium was removed and replaced with 20 μL of 1 μM Fluo-4, AM (Life Technologies, Thermo Fisher Scientific, Grand Island, NY) 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 (Sigma-Aldrich, St. Louis, MO)) for 45 minutes at 37 °C. Dye was removed and replaced with 20 µL of Assay Buffer. For concentration-response curve experiments, compounds were serially diluted 1:3 into 10 point concentration response curves in DMSO, transferred to daughter plates using an Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA) Echo, and diluted in Assay Buffer to a 2X final concentration. Calcium flux was measured using the Functional Drug Screening System 7000 (FDSS7000, Hamamatsu, Japan). After establishment of a fluorescence baseline for 4 seconds (4 images at 1 Hz; excitation, 470 ± 20 nm; emission, 540 ± 30 nm), 20 µL of test compounds were added to the cells, and the response was measured. 142 seconds later, 10 µL (5X) of an EC_{20} concentration of glutamate was added to the cells, and the response of the cells was measured; after an additional 120 seconds, 12 µL (5X) of an EC_{80} concentration of agonist was added and readings taken for an additional 40 seconds. Calcium fluorescence was recorded as fold over basal fluorescence and raw data were normalized to the maximal response to glutamate. Potency (EC_{50}) and maximum response (% Glu or L-AP4 Max) for compounds were determined using a four parameter logistical equation in GraphPad Prism (La Jolla, CA). For efficacy and selectivity experiments, a constant amount of compound was applied prior to the addition of a full glutamate concentration-response curve and the shift of the EC_{50} of the curves was calculated as "fold shift". mGlu receptor selectivity profiling, and GIRK assay were performed as previously reported[1,2,3].

DMPK Methods

In-Vitro DMPK Methods

Intrinsic clearance in human and rat liver microsomes (mouse, rat)

Murine or 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 that is species specific

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

where Q_h (hepatic blood flow) is species specific

Plasma Protein Binding (mouse, rat)

The protein binding of each compound was determined in rat or mouse 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 (mouse, rat)

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 mouse or rat brain tissue with one to three volumes (species specific) of phosphate buffer (25 mM, pH 7.4). The mixture was then subjected to mechanical homogenization 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:

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

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

Metabolite Identification in Rat Hepatic S9

Compound 11a was incubated with rat hepatic S9 fractions (5 mg/mL) +/- NADPH and +/- UDPGA and PAPs. Substrates (5 or 20 μM) were incubated at 37 °C in borosilicate glass test tubes under ambient oxygenation for 60 minutes in a potassium phosphate-buffered reaction (100 mM, pH 7.4) containing hepatic S9 from rat and $MgCl_2$ (3 mM). The total incubation volume was 0.5 mL. Reactions were initiated by the addition of substrate, terminated with the addition of 2 volumes of ice-cold acetonitrile, and subsequently centrifuged at 4000 rcf for 10 min. The resulting supernatants were dried under a stream of nitrogen and reconstituted in initial mobile phase in preparation for LC/MS analysis.

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

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).

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. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Time Course PK and Single Time Point Tissue Distribution Studies

IV cassette PK experiments in rats were carried out according to methods described previously (Bridges et al. *Pharmacol. Res. Perspect.* 2014; reference 49). 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). For time course PK studies, 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 and plasma samples prepared for bioanalysis. For single time point tissue distribution studies, compounds were formulated as described above (in cassette format) and dosed to male Sprague-Dawley rats for a final dose of 0.2-0.25 mg/kg per compound. Brain dissection and blood collections via the carotid artery were performed 0.25 hr post dose. The

brain samples were rinsed in PBS, snap frozen and stored at -80 °C. Prior to LC/MS/MS analysis, brain samples were thawed to room temperature and subjected to mechanical homogenation employing a Mini-Beadbeater™ and 1.0 mm Zirconia/Silica Beads (BioSpec Products).

Rodent (Mouse) Tissue Distribution Studies

Tissue distribution studies with compound 11a in mice were performed by formulating the compound in 20% BCD and dosing via intraperitoneal injection to 20 week old male C57/Bl6 mice (3-4 per time point). At 0.5 hours post dose, animals were euthanized and decapitated, blood was collected via cardiac puncture and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice.

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 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).

Extracellular field potential recordings

The 6-week-old male C57BL/6J mice (The Jackson Laboratory) were anesthetized with isofluorane, and the brains were removed and submerged in ice-cold cutting solution containing the following (in mM): 230 sucrose, 2.5 KCl, 8 $MgSO_4$, 0.5 CaCl2, 1.25 NaH_2PO_4, 10 D-glucose, 26 $NaHCO_3$. Coronal slices containing the hippocampus were cut at 400 m using a Compresstome (Precisionary Instruments). Slices were transferred to a holding chamber containing NMDG-HEPES recovery solution (in mM) as follows: 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 D-glucose, 5 sodium ascorbate, 2

thiourea, 3 sodium pyruvate, 10 $MgSO_4$, 0.5 $CaCl_2$, pH 7.3, 305 mOsm, for 15 min at 32 °C. Slices were then transferred to a room temperature holding chamber for at least 1 h containing aCSF (in mM) as follows: 126 NaCl, 1.25 NaH_2PO_4, 2.5 KCl, 10 D-glucose, 26 $NaHCO_3$, 2 CaCl2, 1 $MgSO_4$, supplemented with 600 M sodium ascorbate for slice viability. All buffers were continuously bubbled with 95% O_2/5% CO_2. Subsequently, slices were transferred to a 30 °C submersion recording chamber where they were perfused with aCSF at a rate of 2 mL/min. Borosilicate glass electrodes were pulled using a Flaming/Brown micropipette puller (Sutter Instruments) and had a resistance of 3–5 MΩ when filled with aCSF. Paired-pulse field EPSPs (fEPSPs) were recorded by from the stratum radiatum of CA1 and evoked by electrical stimulation (200 ms duration, every 20 s) delivered through a concentric bipolar stimulating electrode placed near the CA3-CA1 border. Input– output curves were generated for each slice, and the stimulation intensity was adjusted to 50% of the maximum response. VU6010608 was prepared in DMSO vehicle (0.05%). After a 10 min baseline recording, 10 μM VU6010608 was bath-applied for 10 min followed by 2 trains of high-frequency stimulation (HFS, 2 trains of 100 Hz, 20 sec inter-stimulus interval). All slopes calculated were normalized to the average slope calculated during the predrug period (percentage of baseline).

Eurofins Lead Profiling Data

A radioligand binding panel of 68 targets (GPCRs, ion channels, transporters, and nuclear hormones) with data reported as % inhibition of radioligand binding at a 10 μM concentration of **4** (ADX71743) and **11a** (VU6010608) from two independent determinations.

Supporting Table 1: Eurofins Profiling of **4** (ADX71743).

Target/Protein	Species	% Inhibition
Adenosine A_1	Human	-10
Adenosine A_{2A}	Human	-8
Adenosine A_3	Human	5
Adrenergic α_{1A}	Rat	9
Adrenergic α_{1B}	Rat	16
Adrenergic α_{1D}	Human	16
Adrenergic α_{2A}	Human	36
Adrenergic β_1	Human	3
Adrenergic β_2	Human	2
Androgen (Testosterone)	Human	8
Bradykinin B_1	Human	8
Bradykinin B_2	Human	-12
Calcium Channel L-Type, Benzothiazepine	Rat	19
Calcium Channel L-Type, Dihydropyridine	Rat	12
Calcium Channel N-Type	Rat	-3
Cannabinoid CB_1	Human	11

Dopamine D_1	Human	5
Dopamine D_{2S}	Human	-2
Dopamine D_3	Human	6
Dopamine $D_{4.2}$	Human	-5
Endothelin ET_A	Human	8
Endothelin ET_B	Human	-3
Epidermal Growth Factor (EGF)	Human	-3
Estrogen ERα	Human	4
$GABA_A$, Flunitrazepam, Central	Rat	-5
$GABA_A$, Muscimol, Central	Rat	-5
$GABA_{B1A}$	Human	-2
Glucocorticoid	Human	3
Glutamate, Kainate	Rat	-5
Glutamate, NMDA, Agonism	Rat	-5
Glutamate, NMDA, Glycine	Rat	20
Glutamate, NMDA, Phencyclidine	Rat	33
Histamine H_1	Human	14
Histamine H_2	Human	24
Histamine H_3	Human	-4
Imidazoline I_2, Central	Rat	-15
Interleukin IL-1	Mouse	2
Leukotriene, Cysteinyl $CysLT_1$	Human	10
Melatonin MT_1	Human	8
Muscarinic M_1	Human	5
Muscarinic M_2	Human	4
Muscarinic M_3	Human	-11

Neuropeptide Y Y_1	Human	5
Neuropeptide Y Y_2	Human	2
Nicotinic Acetylcholine	Human	-10
Nicotinic Acetylcholine α1, Bungarotoxin	Human	-1
Opiate δ_1 (OP1, DOP)	Human	1
Opiate κ (OP2, KOP)	Human	18
Opiate μ (OP3, MOP)	Human	26
Phorbol Ester	Mouse	-1
Platelet Activating Factor (PAF)	Human	7
Potassium Channel [K_{ATP}]	Hamster	1
Potassium Channel hERG	Human	12
Prostanoid EP_4	Human	4
Purinergic P2X	Rabbit	0
Purinergic P2Y	Rat	5
Rolipram	Rat	2
Serotonin (5-HT_{1A})	Human	5
Serotonin (5-HT_{2B})	Human	2
Serotonin (5-HT_3)	Human	5
Sigma σ_1	Human	30
Sodium Channel, Site 2	Rat	-7
Tachykinin NK_1	Human	31
Thyroid Hormone	Rat	-2
Transporter, Dopamine (DAT)	Human	13
Transporter, GABA	Rat	-2
Transporter, Norepinephrine (NET)	Human	14
Transporter, Serotonin (SERT)	Human	8

Supporting Table 2: Eurofins Profiling of **11a** (VU6010608).

Target/Protein	Species	% Inhibition
Adenosine A_1	Human	19
Adenosine A_{2A}	Human	-1
Adenosine A_3	Human	49
Adrenergic α_{1A}	Rat	35
Adrenergic α_{1B}	Rat	6
Adrenergic α_{1D}	Human	10
Adrenergic α_{2A}	Human	1
Adrenergic β_1	Human	1
Adrenergic β_2	Human	1
Androgen (Testosterone)	Human	9
Bradykinin B_1	Human	5
Bradykinin B_2	Human	-18
Calcium Channel L-Type, Benzothiazepine	Rat	15
Calcium Channel L-Type, Dihydropyridine	Rat	29
Calcium Channel N-Type	Rat	-3
Cannabinoid CB_1	Human	28
Dopamine D_1	Human	7
Dopamine D_{2S}	Human	1
Dopamine D_3	Human	4
Dopamine $D_{4.2}$	Human	4
Endothelin ET_A	Human	6

Endothelin ET_B	Human	-11
Epidermal Growth Factor (EGF)	Human	-5
Estrogen ERα	Human	4
$GABA_A$, Flunitrazepam, Central	Rat	-10
$GABA_A$, Muscimol, Central	Rat	-3
$GABA_{B1A}$	Human	17
Glucocorticoid	Human	-4
Glutamate, Kainate	Rat	8
Glutamate, NMDA, Agonism	Rat	16
Glutamate, NMDA, Glycine	Rat	4
Glutamate, NMDA, Phencyclidine	Rat	14
Histamine H_1	Human	11
Histamine H_2	Human	3
Histamine H_3	Human	-11
Imidazoline I_2, Central	Rat	12
Interleukin IL-1	Mouse	8
Leukotriene, Cysteinyl $CysLT_1$	Human	1
Melatonin MT_1	Human	10
Muscarinic M_1	Human	3
Muscarinic M_2	Human	2
Muscarinic M_3	Human	6
Neuropeptide Y Y_1	Human	4
Neuropeptide Y Y_2	Human	1
Nicotinic Acetylcholine	Human	-7
Nicotinic Acetylcholine α1, Bungarotoxin	Human	-5
Opiate δ_1 (OP1, DOP)	Human	3

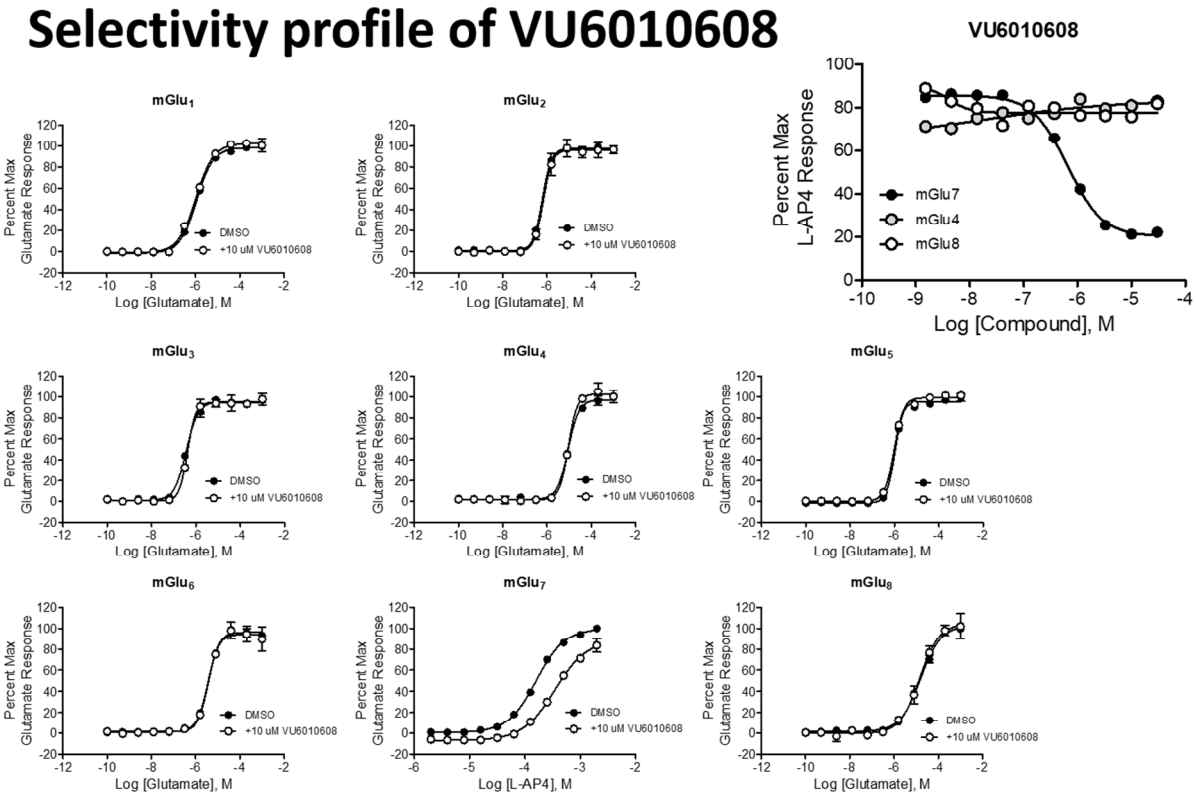
Opiate κ (OP2, KOP)	Human	12
Opiate μ (OP3, MOP)	Human	6
Phorbol Ester	Mouse	-1
Platelet Activating Factor (PAF)	Human	-7
Potassium Channel [K_{ATP}]	Hamster	8
Potassium Channel hERG	Human	33
Prostanoid EP_4	Human	11
Purinergic P2X	Rabbit	-1
Purinergic P2Y	Rat	4
Rolipram	Rat	45
Serotonin (5-HT_{1A})	Human	4
Serotonin (5-HT_{2B})	Human	14
Serotonin (5-HT_3)	Human	49
Sigma σ_1	Human	-4
Sodium Channel, Site 2	Rat	-1
Tachykinin NK_1	Human	-22
Thyroid Hormone	Rat	-11
Transporter, Dopamine (DAT)	Human	12
Transporter, GABA	Rat	-3
Transporter, Norepinephrine (NET)	Human	15
Transporter, Serotonin (SERT)	Human	7

References

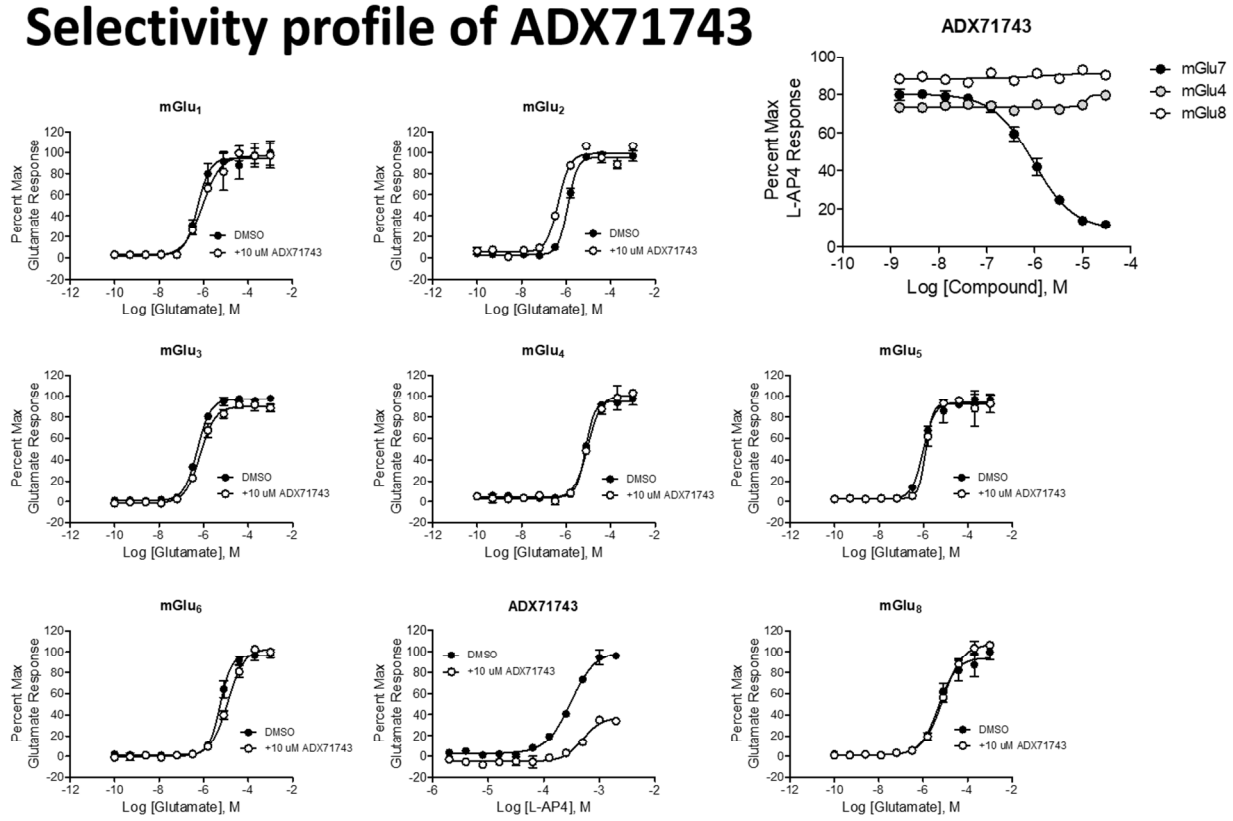
1. Niswender, C.M.; Johnson, K.A.; Luo, Q; Ayala, J.E.; Kim, C.; Conn, P.J.; Weaver, C.D. A novel assay of Gi/o-linked G protein-coupled receptor coupling to potassium channels provides new insight into the pharmacology of the group III metabotropic glutamate receptors. *Mol. Pharmacol.,* **2008**, *73*, 1213-1224.

2. Jones, C.K.; Bubser, M.; Thompson, A.D.; Dickerson, J.W.; Turle-Lorenzo, N.; Amalric, M.; Blobaum, A.L.; Bridges, T.M.; Morrison, R.D.; Jadhav, S.; Engers, D.W.; Italiano, K.; Bode, J.; Daniels, J.S.; Lindsley, C.W.; Hopkins, C.R.; Niswender, C.M. The metabotropic glutamare captor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine A2A antagonist in preclinical rodent models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 404-421.
3. Jalan-Sakrikar, N.; Field, J.R.; Klar, R.; Mattmann, M.E.; Gregory, K.J.; Zamorano, R.; Engers, D.W.; Bollinger, S.R.; Weaver, C.D.; Days, E.; Lewis, L.M.; Utley, T.J.; Hurtado, M.; Rigault, D.; Acher, F.; Walker, A.G.; Melancon, B.J.; Wood, M.R.; Lindsley, C.W.; Conn, P.J.; Xiang, Z.; Hopkins, C.R.; Niswender, C.M. Identification of positive allosteric modulators VU0155094 (ML397) and VU0422288 (ML396) reveals new insights into the biology of metabotropic glutamate receptor 7. *ACS Chem. Neurosci.* **2014**, *5*, 1221-1237.

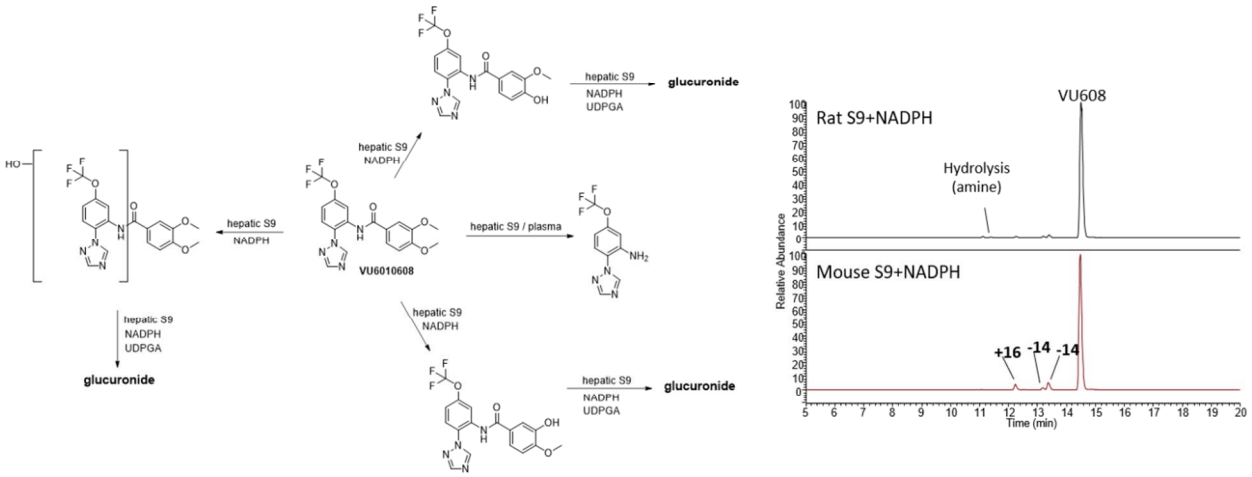
Supporting Figures.



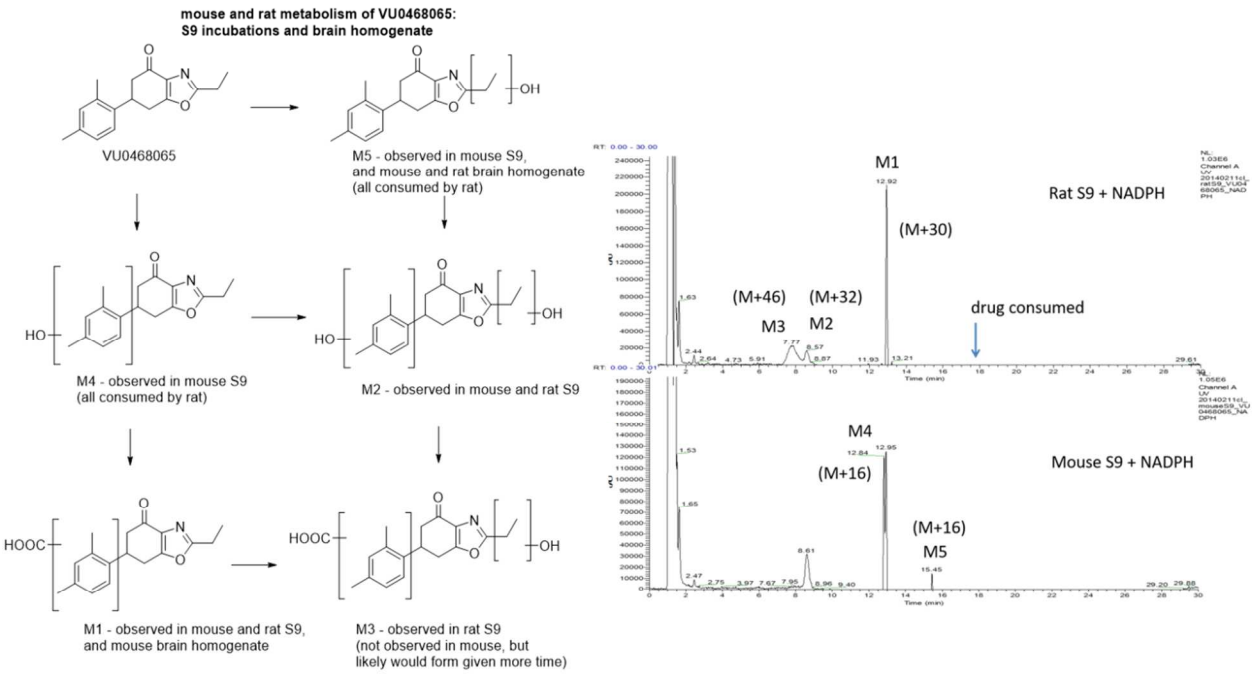
Supporting Figure 1. mGlu receptor selectivity for **11a** (VU6010608) in 10 µM, fold-shift assay using either glutamate or L-AP4 as the agonist. Either 10 µM VU6010608 or DMSO was added two minutes prior to the addition of increasing concentrations of agonist; this paradigm allows for the detection of PAMs (leftward shift) or antagonists/NAMs (shift to the right or decrease in max). At the upper right, a full concentration-response for VU06010608 is shown in the presence of EC_{80} agonist responses for $mGlu_4$, mGlu7, and $mGlu_8$.



Supporting Figure 2. mGlu receptor selectivity for **ADX71743** in 10 μM, fold-shift assay using either glutamate or L-AP4 as the agonist. Either 10 μM ADX71743 or DMSO was added two minutes prior to the addition of increasing concentrations of agonist; this paradigm allows for the detection of PAMs (leftward shift) or antagonists/NAMs (shift to the right or decrease in max). At the upper right, a full concentration-response for ADX71743 is shown in the presence of EC_{80} agonist responses for $mGlu_4$, mGlu7, and $mGlu_8$.



Supporting Figure 3. Metabolism of **11a** (VU6010608).



Supporting Figure 4. Metabolism of **4** (ADX71743).