Supporting Information

Discovery of an orally bioavailable and Central Nervous System (CNS) penetrant $mGlu_7$ Negative Allosteric Modulator (NAM) *in vivo* tool compound: *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (VU6012962)

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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% CH3CN in H2O (0.1% TFA) over 1.4 min, hold at 95% CH3CN 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% CH3CN in H2O (0.1% formic acid) over 1 min, hold at 95% CH3CN 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 Axiapacked Luna C18, 30 x 50 mm, 5 µm column. Mobile phase: CH3CN in H2O (0.1% TFA). Gradient conditions: 0.75 min equilibration, followed by user defined gradient (starting organic percentage, ending S3 organic percentage, duration), hold at 95% CH3CN in H2O (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: CH3CN in H2O (0.1% TFA). Gradient conditions: 7 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH3CN in H2O (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 > 95% pure by LCMS (254 nm, 214 nM and ELSD) as well as ^{1}H and ^{13}C NMR.

General Procedure 1: Synthesis of Anilines (6a-d, or 9)

To a solution of $LiAlH_4$ (3.5 eq, 1.0 M stock solution in THF) cooled to 0 °C was added a solution of the appropriate triazole (**5**, **8**, or **14b-d**) in THF (1.0 mL) dropwise under an inert atmosphere of argon. Upon completion of addition, the reaction was stirred at room temperature for 3 hr, whereupon LCMS indicated complete consumption of starting material and formation of the desired product. The reaction was cooled to 0 °C and quenched with the sequential addition of EtOAc and saturated aqueous Rochelle salt solution. The resulting mixture was stirred at room temperature for 2 hr before the layers were separated. The aqueous layer was washed with EtOAc x 3. The combined organic material was passed through a phase separator, 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.

General Procedure 2: Synthesis of Triazoles (7d, 22, or 23)

To a suspension of the appropriate bromo nitro benzene (**20** or **21**) (1.0 eq) or benzamide (**19**) (1.0 eq), 1*H*-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 3-5 min. 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 combined organic material was washed with sat. NH_4Cl x 2, brine, dried over $MgSO_4$, filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to afford the desired products.

General Procedure 3: Synthesis of Anilines (13, 24)

To a solution of triazole (**22** or **23**) (1.0 eq) in EtOH : H_2O (5:1) (0.3M) was sequentially added Fe powder (10.0 eq) and NH_4Cl (10.0 eq). The resulting suspension was heated to 75 °C for 4 hr, whereupon LCMS indicated complete consumption of starting material and formation of the desired product. Upon cooling to room temperature, the crude reaction mixture was filtered over a pad of celite and concentrated. The residue was dissolved in EtOAc and water. The layers were separated, and the aqueous layer was washed with EtOAc x 3. The combined organic material was washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to afford the desired products.

General Procedure 4: Synthesis of Triazoles (7a-g, 14a-d)

To a solution of aniline (**13**) (1.0 eq) in CH_2Cl_2 (0.1 M) in a Biotage microwave vial was added the appropriate benzoic acid (**12**, **17a-g**, or **27**) (1.0 eq), *N,N*-diisopropylethylamine (3.0 eq), and chlorodipyrrolidinocarbenium hexafluorophosphate (PyClU) (1.0 eq) at room temperature. The vial was sealed and heated to 100 °C using a Biotage microwave reactor for 30 min, whereupon LCMS showed formation of the desired product. The reaction mixture 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 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.

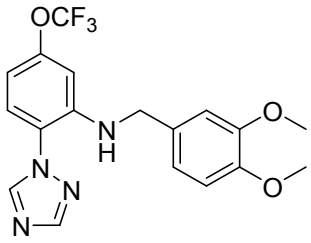
General Procedure 5: Synthesis of Esters (16a-g)

To a suspension of methyl vanillate (1.0 eq) and potassium carbonate (2.0 eq) in MeCN (0.4 M) in a Biotage microwave vial was added the appropriate alkyl halide (1.5 eq) at room temperature. The vial was then sealed and heated to 170 °C using a Biotage microwave reactor for one hour. Once LCMS indicated complete consumption of starting material, the reaction was filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to afford the desired products.

General Procedure 6: Synthesis of Benzoic Acids (17a-g)

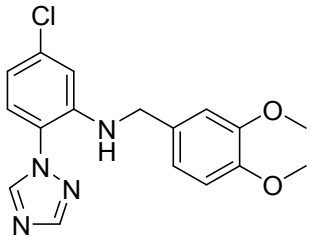
To a solution of the appropriate ester (**16a-g**) (1.0 eq) in THF : H_2O (1:1) (0.2 M) was added LiOH (5.0 eq) in one portion. The resulting suspension was stirred at the appropriate temperature, and reaction progression was monitored by LCMS. When LCMS indicated reaction completion, the reaction was diluted with EtOAc and acidified to pH ~ 1 with the addition of 2.0 M HCl. The

layers were separated, and the aqueous layer was washed with EtOAc x 3. The combined organic material was dried over $MgSO_4$, filtered, and concentrated to afford the desired products which were carried forward to the next step without any further purification.



Synthesis of *N*-(3,4-dimethoxybenzyl)-2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)aniline (6a)

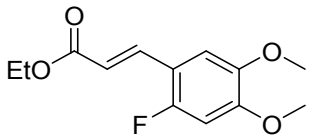
This compound was synthesized according to general procedure 1. White solid (26.7 mg, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.13 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.87-6.81 (m, 3H), 6.62-6.58 (m, 2H), 5.87 (t, 1H), 4.28 (d, 2H), 3.86 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 152.8, 150.8 (d, J_{CF} = 1.3 Hz), 149.4, 148.6, 143.8, 143.7, 130.1, 125.4, 120.8, 120.5 (q, J_{CF} = 256.3 Hz), 119.6, 111.4, 110.6, 108.2, 105.3, 56.00, 55.96, 47.5 ppm. HRMS (TOF, ES+) calc'd for $C_{18}H_{17}F_3N_4O_3$, 394.1253; found, 394.1257.



Synthesis of 5-chloro-*N*-(3,4-dimethoxybenzyl)-2-(1*H*-1,2,4-triazol-1-yl)aniline (9)

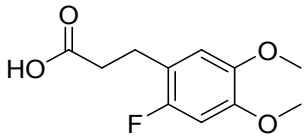
This compound was synthesized according to general procedure 1. Beige solid (32.1 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.12 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 6.87-6.81 (m, 3H), 6.77 (d, J = 2.1 Hz, 1H) , 6.71 (dd, J = 8.3, 2.2 Hz, 1H), 5.81 (bs, 1H), 4.28 (d, 2H),

3.86 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 152.8, 149.4, 148.6, 143.8, 143.1, 136.3, 130.3, 125.2, 121.1, 119.5, 116.5, 112.8, 111.5, 110.5, 56.02, 56.0, 47.4 ppm. HRMS (TOF, ES+) calc'd for $C_{17}H_{17}ClN_4O_2$, 344.1040; found, 344.1043.



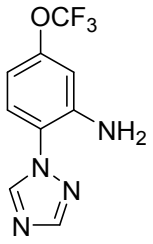
Synthesis of ethyl (*E*)-3-(2-fluoro-4,5-dimethoxyphenyl)acrylate (11)

To a solution of 6-fluoroveratraldehyde (300 mg, 1.63 mmol) in DCM (6.52 mL) was added (carbethoxymethylene)triphenylphosphorane (681 mg, 1.95 mmol) at room temperature. The resulting solution was stirred for 16 hr at room temperature whereupon LCMS indicated complete consumption of starting material and formation of the desired product. The crude reaction mixture was concentrated and purified via flash chromatography (Teledyne ISCO system; silica gel column; hexanes:EtOAc; 0-30% EtOAc gradient) to afford the desired material as a white solid (372 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 16.1 Hz, 1H), 6.95 (d, J_{HF} = 6.8 Hz, 1H), 6.63 (d, J_{HF} = 11.6 Hz, 1H), 6.37 (d, J = 16.1 Hz, 1H), 4.26 (q, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 1.33 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 156.5 (d, J_{CF} = 247.0 Hz), 152.0 (d, J_{CF} = 10.1 Hz), 145.7, 137.0 (d, J_{CF} = 2.1 Hz), 118.0 (d, J_{CF} = 5.8 Hz), 113.5 (d, J_{CF} = 12.7 Hz), 109.4 (d, J_{CF} = 4.4 Hz), 100.2 (d, J_{CF} = 28.1 Hz), 60.5, 56.4, 56.3, 14.4 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{15}FO_4$, 254.0954; found, 254.0954.



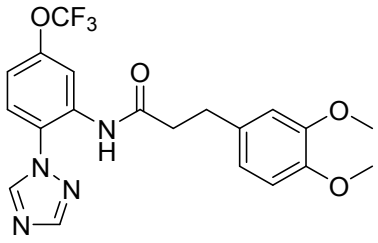
Synthesis of 3-(2-fluoro-4,5-dimethoxyphenyl)propanoic acid (12)

To a suspension of ethyl (*E*)-3-(2-fluoro-4,5-dimethoxyphenyl)acrylate (**11**) (340 mg, 1.34 mmol) and Pd/C (285 mg, 0.134 mmol, 10% w/w Pd) in toluene (10.6 mL) was sequentially added acetic acid (153 uL, 2.67 mmol) and sodium borohydride (203 mg, 5.35 mmol) portionwise. The reaction was sealed and stirred at room temperature for 1 hr whereupon LCMS showed complete reduction of the α,β-unsaturated olefin. The reaction was diluted with EtOAc and filtered over a pad of celite. The crude organic material was concentrated to yield a colorless oil. This oil was redissolved in THF : H_2O (1:1) (16 mL), and lithium hydroxide (100 mg, 4.01 mmol) was added. After stirring at room temperature for 3 hr, the reaction was diluted with EtOAc and acidified to pH ~ 1 using 2 M HCl. The layers were separated, and the aqueous layer was washed with EtOAc x 3. The combined organic material was passed through a phase separator and concentrated to afford the desired material as a colorless oil (262 mg, 86% yield) which was carried forward to the next step without any further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (d, J_{HF} = 7.5 Hz, 1H), 6.82 (d, J_{HF} = 11.6 Hz, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 2.75 (t, 2H), 2.48 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 173.6, 154.3 (d, J_{CF} = 234.0 Hz), 148.0 (d, J_{CF} = 10.0 Hz), 144.9 (d, J_{CF} = 2.0 Hz), 117.6 (d, J_{CF} = 17.0 Hz), 113.4 (d, J_{CF} = 7.0 Hz), 100.5 (d, J_{CF} = 28.0 Hz), 56.0, 55.8, 34.3, 23.4 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{13}FO_4$, 228.0798; found, 228.0798.



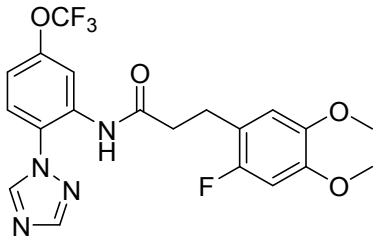
Synthesis of 2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)aniline (13)

This compound was synthesized according to general procedure 3. White solid (1.1g, 53% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.16 (s, 1H), 7.19 (d, J = 8.6 Hz, 1H), 6.70-6.69 (m, 1H), 6.67-6.64 (m, 1H), 4.64 (bs, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 152.8, 150.3 (d, J_{CF} = 2.0 Hz), 143.6, 142.7, 125.6, 121.1, 120.5 (q, J_{CF} = 256.0 Hz), 110.1, 109.4 ppm. HRMS (TOF, ES+) calc'd for $C_9H_7F_3N_4O$, 244.0572; found, 244.0574.



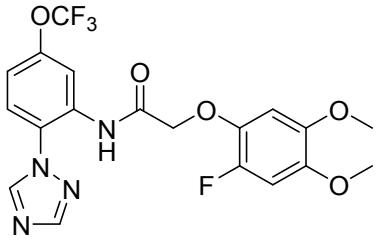
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propanamide (14b)

This compound was synthesized according to general procedure 4. White solid (91.1 mg, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.52 (bs, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.40 (s, 1H), 8.18 (s, 1H), 7.34 (d, J = 8.8 Hz, 1H), 7.05 (dd, J = 8.7, 1.4 Hz, 1H), 6.75-6.69 (m, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 2.96 (t, 2H), 2.64 (t, 2H) ;^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.9, 153.1, 149.6, 149.0, 147.7, 143.7, 133.1, 132.8, 123.8, 123.6, 120.5 (q, J_{CF} = 257.2 Hz), 120.2, 116.1, 115.7, 111.8, 111.4, 56.0, 55.9, 40.4, 31.0 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{19}F_3N_4O_4$, 436.1358; found, 436.1363.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3-(2-fluoro-4,5-dimethoxyphenyl)propanamide (14c)

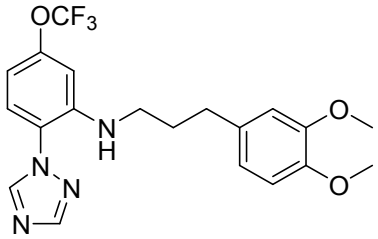
This compound was synthesized according to general procedure 4. White solid (107 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (bs, 1H), 8.55 (d, J = 2.1 Hz, 1H), 8.40 (s, 1H), 8.19 (s, 1H), 7.34 (d, J = 8.8 Hz, 1H),7.07-7.04 (m, 1H), 6.64 (d, J = 7.2 Hz, 1H), 6.54 (d, J = 11.2 Hz, 1H), 3.83 (s, 3H), 3.75 (s, 3H), 2.96 (t, 2H), 2.63 (t, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.8, 155.0 (d, J_{CF} = 236.4 Hz), 153.1, 149.6, 148.5 (d, J_{CF} = 9.8 Hz), 145.1 (d, J_{CF} = 2.0 Hz), 143.7, 133.1, 123.7, 123.6, 120.5 (q, J_{CF} = 257.1 Hz), 117.3 (d, J_{CF} = 17.2 Hz), 116.1, 115.7, 113.0 (d, J_{CF} = 6.1 Hz), 100.2 (d, J_{CF} = 27.9 Hz), 56.4, 56.2, 38.8, 24.9 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{18}F_4N_4O_4$, 454.1264; found, 454.1264.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-2-(2-fluoro-4,5-dimethoxyphenoxy)acetamide (14d)

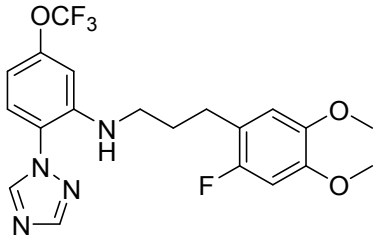
This compound was synthesized according to general procedure 4. White solid (91.6 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.72 (bs, 1H), 8.65 (d, J = 2.4 Hz, 1H), 8.44 (s, 1H), 8.19 (s, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.14-7.11 (m, 1H), 6.73 (d, J = 12.1 Hz, 1H), 6.58 (d, J = 7.9 Hz, 1H), 4.60 (s, 2H), 3.84 (s, 6H) ;^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 153.4 (d, J_{CF} = 2.5 Hz), 149.7, 146.8 (d, J_{CF} = 238.0 Hz), 145.3 (d, J_{CF} = 2.6 Hz), 144.9 (d, J_{CF} = 7.9 Hz), 143.6, 137.9 (d, J_{CF} = 12.0 Hz), 132.7, 124.3, 124.2, 120.5 (q, J_{CF} = 257.4 Hz), 116.7, 115.7, 102.3 (d, J_{CF} = 2.0

Hz), 101.8 (d, J_{CF} = 23.4 Hz), 70.3, 56.8, 56.7 ppm. HRMS (TOF, ES+) calc'd for $C_{19}H_{16}F_4N_4O_5$, 456.1057; found, 456.1063.



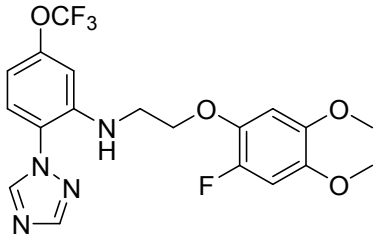
Synthesis of *N*-(3-(3,4-dimethoxyphenyl)propyl)-2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)aniline (6b) (VU6015848)

This compound was synthesized according to general procedure 1. Colorless oil (22.7 mg, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.16 (s, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 7.8 Hz, 1H), 6.71-6.69 (m, 2H), 6.58-6.55 (m, 2H), 5.47 (t, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.13 (q, 2H), 2.65 (t, 2H), 1.93 (m, 2H);^{13}C NMR (100 MHz, $CDCl_3$) δ = 152.8, 151.0, 149.1, 147.5, 143.9, 143.8, 133.7, 125.4, 120.6, 120.5 (q, J_{CF} = 256.0 Hz), 120.3, 111.7, 111.4, 107.7, 104.7, 56.0, 55.9, 42.6, 32.8, 30.5 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{21}F_3N_4O_3$, 422.1566; found, 422.1568.



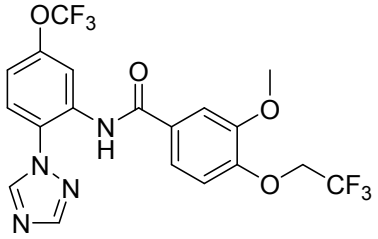
Synthesis of *N*-(3-(2-fluoro-4,5-dimethoxyphenyl)propyl)-2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)aniline (6c) (VU6016771)

This compound was synthesized according to general procedure 1. Colorless oil (21.8 mg, 45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.16 (s, 1H), 7.16 (d, J = 8.4 Hz, 1H), 6.63-6.55 (m, 4H), 5.49 (t, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.14 (q, 2H), 2.66 (t, 2H), 1.91 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 155.1 (d, J_{CF} = 236.0 Hz), 152.9, 151.0, 148.3 (d, J_{CF} = 9.8 Hz), 145.3 (d, J_{CF} = 2.4 Hz), 143.9, 143.8, 125.5, 120.7, 120.5 (q, J_{CF} = 256.0 Hz), 118.3 (d, J_{CF} = 17.2 Hz), 112.9 (d, J_{CF} = 6.4 Hz), 107.7, 104.7, 100.3 (d, J_{CF} = 28.4 Hz), 56.6, 56.3, 42.6, 29.4, 26.1 ppm. HRMS (TOF, ES+) calc'd for $C_{20}H_{20}F_4N_4O_3$, 440.1472; found, 440.1472.



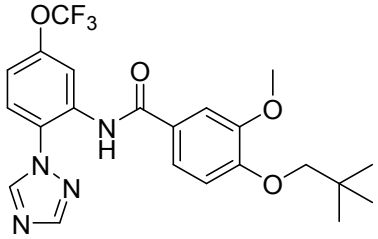
Synthesis of *N*-(2-(2-fluoro-4,5-dimethoxyphenoxy)ethyl)-2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)aniline (6d) (VU6016769)

This compound was synthesized according to general procedure 1. White solid (20.8 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.17 (s, 1H), 7.20 (d, J = 8.6 Hz, 1H), 6.69-6.59 (m, 4H), 5.92 (t, 1H), 4.20 (t, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.50 (q, 2H);^{13}C NMR (100 MHz, $CDCl_3$) δ = 153.0, 151.0, 147.6 (d, J_{CF} = 237.4 Hz), 145.2 (d, J_{CF} = 2.4 Hz), 144.6 (d, J_{CF} = 8.2 Hz), 143.9, 143.8, 138.8 (d, J_{CF} = 11.9 Hz), 125.7, 121.2, 120.6 (q, J_{CF} = 256.3 Hz), 108.4, 105.0, 104.0 (d, J_{CF} = 2.5 Hz), 101.6 (d, J_{CF} = 23.9 Hz), 70.1, 56.7, 56.6, 43.0 ppm. HRMS (TOF, ES+) calc'd for $C_{19}H_{18}F_4N_4O_4$, 442.1264; found, 442.1265.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3-methoxy-4-(2,2,2-trifluoroethoxy)benzamide (7a) (VU6011327)

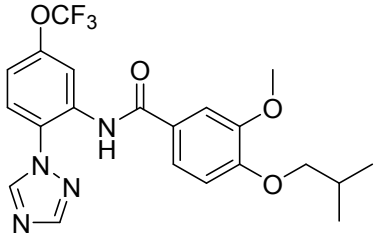
This compound was synthesized according to general procedure 4. White solid (16.0 mg, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.60 (bs, 1H), 8.74 (d, J = 2.1 Hz, 1H), 8.52 (s, 1H), 8.29 (s, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.39 (dd, J = 8.3, 2.0 Hz, 1H), 7.10 (dd, J = 8.7, 1.6 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 4.46 (q, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.7, 153.1, 150.6, 150.3, 149.7 (d, J_{CF} = 1.7 Hz), 143.9, 133.4, 129.5, 123.9, 123.5, 123.4 (q, J_{CF} = 277.0 Hz), 120.5 (q, J_{CF} = 257.0 Hz), 119.6, 116.24, 116.16, 115.6, 112.1, 67.5 (q, J_{CF} = 35.5 Hz), 56.2 ppm. HRMS (TOF, ES+) calc'd for $C_{19}H_{14}F_6N_4O_4$, 476.0919; found, 476.0923.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-3-methoxy-4-(neopentyloxy)benzamide (7b) (VU6013572)

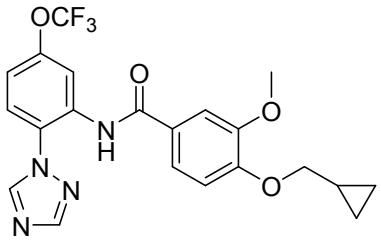
This compound was synthesized according to general procedure 4. Colorless oil (14.4 mg, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (bs, 1H), 8.77 (d, J = 2.1 Hz, 1H), 8.51 (s, 1H), 8.29

(s, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.41 (dd, J = 8.4, 2.2 Hz, 1H), 7.09 (dd, J = 8.8, 1.7 Hz, 1H) 6.92 (d, J = 8.4 Hz, 1H), 3.93 (s, 3H), 3.70 (s, 2H), 1.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.2, 153.3, 153.1, 150.0, 149.7 (d, J_{CF} = 2.0 Hz),, 143.9, 133.8, 126.1, 123.8, 123.6, 120.5 (q, J_{CF} = 257.3 Hz), 120.3, 116.0, 115.6, 112.4, 111.6, 79.1, 56.5, 32.3, 26.8 ppm. HRMS (TOF, ES+) calc'd for $C_{22}H_{23}F_3N_4O_4$, 464.1671; found, 464.1675.



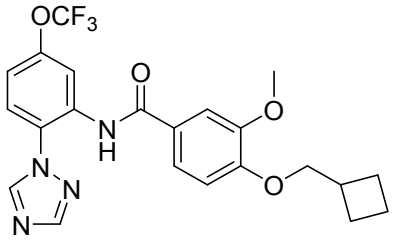
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-isobutoxy-3-methoxybenzamide (7c) (VU6013571)

This compound was synthesized according to general procedure 4. White solid (14.8 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.75 (d, J = 2.2 Hz, 1H), 8.51 (s, 1H), 8.29 (s, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.40 (dd, J = 8.4, 2.1 Hz, 1H), 7.07 (dd, J = 8.8, 1.6 Hz, 1H) 6.92 (d, J = 8.4 Hz, 1H), 3.93 (s, 3H), 3.83 (d, 2H), 2.19 (m, 1H), 1.05 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.7, 149.7, 143.9, 133.7, 126.2, 123.8, 123.6, 120.5 (q, J_{CF} = 257.0 Hz), 120.1, 115.9, 115.6, 112.1, 111.2, 75.6, 56.3, 28.2, 19.4 ppm. HRMS (TOF, ES+) calc'd for $C_{21}H_{21}F_3N_4O_4$, 450.1515; found, 450.1520.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (7d) (VU6012962)

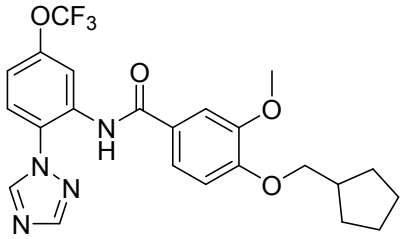
This compound was synthesized according to general procedure 4. Beige solid (16.2 mg, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.75 (d, J = 2.2 Hz, 1H), 8.51 (s, 1H), 8.28 (s, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.39 (dd, J = 8.4, 2.1 Hz, 1H), 7.07 (dd, J = 8.7, 1.7 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 3.95 (s, 3H), 3.92 (d, 2H), 1.39-1.32 (m, 1H), 0.69-0.65 (m, 2H), 0.40-0.36 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.3, 149.7 (d, J_{CF} = 2.0 Hz), 149.6, 143.9, 133.7, 126.3, 123.8, 123.6, 120.5 (q, J_{CF} = 257.1 Hz), 120.0, 115.9, 115.5, 112.1, 111.0, 74.1, 56.2, 10.2, 3.6 ppm. HRMS (TOF, ES+) calc'd for $C_{21}H_{19}F_3N_4O_4$, 448.1358; found, 448.1365.



Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclobutylmethoxy)-3-methoxybenzamide (7e) (VU6013215)

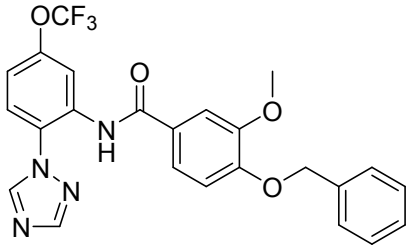
This compound was synthesized according to general procedure 4. White solid (14.8 mg, 39% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.76 (d, J = 2.1 Hz, 1H), 8.51 (s, 1H), 8.29 (s, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.40 (dd, J = 8.5, 2.0 Hz, 1H), 7.08 (dd, J = 8.7, 1.6 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 4.06 (d, 2H), 3.93 (s, 3H), 2.87 (m, 1H), 2.21-2.14 (m, 2H), 2.04-1.83 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.7, 149.8, 143.9,

133.8, 126.3, 123.8, 123.6, 120.5 (q, J_{CF} = 257.2 Hz), 120.1, 116.0, 115.6, 112.2, 111.2, 73.5, 56.3, 34.5, 25.2, 18.8 ppm. HRMS (TOF, ES+) calc'd for $C_{22}H_{21}F_3N_4O_4$, 462.1515; found, 462.1515.



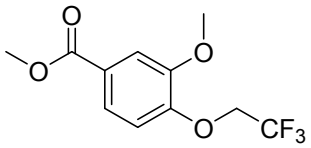
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopentylmethoxy)-3-methoxybenzamide (7f) (VU6014099)

This compound was synthesized according to general procedure 4. Colorless oil (18.7 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.76 (d, *J* = 2.0 Hz, 1H), 8.51 (s, 1H), 8.29 (s, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.40 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.08 (dd, *J* = 8.8, 1.6 Hz, 1H) 6.93 (d, *J* = 8.4 Hz, 1H), 3.95 (d, 2H), 3.93 (s, 3H), 2.46 (m, 1H), 1.90-1.83 (m, 2H), 1.68-1.56 (m, 4H), 1.40-1.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.1, 153.1, 152.7, 149.7, 143.9, 133.7, 126.1, 123.8, 123.6, 120.5 (q, J_{CF} = 257.0 Hz), 120.1, 115.9, 115.6, 112.1, 111.2, 73.5, 56.3, 39.0, 29.7, 25.4 ppm. HRMS (TOF, ES+) calc'd for $C_{23}H_{23}F_3N_4O_4$, 476.1671; found, 476.1680.



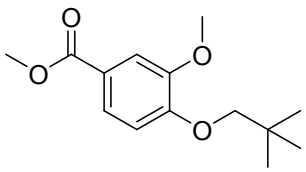
Synthesis of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(benzyloxy)-3-methoxybenzamide (7g) (VU6013340)

This compound was synthesized according to general procedure 4. White solid (19.8 mg, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (bs, 1H), 8.75 (d, J = 2.2 Hz, 1H), 8.50 (s, 1H), 8.28 (s, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.45-7.31 (m, 7H), 7.08 (dd, J = 8.8, 1.6 Hz, 1H), 6.94 (d, J = 8.4, 1H), 5.25 (s, 2H), 3.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.0, 153.1, 151.9, 149.9, 149.7 (d, J_{CF} = 2.0 Hz), 143.9, 136.4, 133.6, 128.8, 128.3, 127.3, 126.8, 123.8, 123.6, 120.5 (q, J_{CF} = 257.2 Hz), 119.9, 116.0, 115.6, 112.9, 111.2, 71.0, 56.2 ppm. HRMS (TOF, ES+) calc'd for $C_{24}H_{19}F_3N_4O_4$, 484.1358; found, 484.1364.



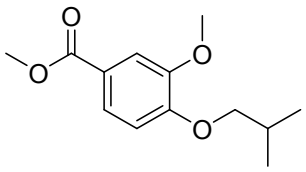
Synthesis of methyl 3-methoxy-4-(2,2,2-trifluoroethoxy)benzoate (16a)

This compound was synthesized according to general procedure 5. White solid (273 mg, 94% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.60 (d, J = 1.9 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 4.45 (q, 2H), 3.93 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.6, 150.7, 149.9, 125.7, 123.4 (q, J_{CF} = 277.0 Hz), 123.3, 115.6, 113.5, 67.4 (q, J_{CF} = 35.5 Hz), 56.2, 52.3 ppm. HRMS (TOF, ES+) calc'd for $C_{11}H_{11}F_3O_4$, 264.0609; found, 264.0613.



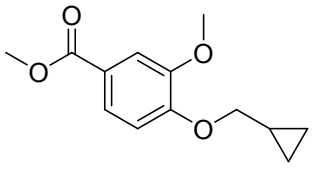
Synthesis of methyl 3-methoxy-4-(neopentyloxy)benzoate (16b)

This compound was synthesized according to general procedure 5. Colorless oil (219 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.67 (s, 2H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 153.7, 149.3, 123.7, 122.4, 113.2, 112.0, 78.9, 56.4, 52.0, 32.2, 26.7 ppm. HRMS (TOF, ES+) calc'd for $C_{14}H_{20}O_4$, 252.1362; found, 252.1366.



Synthesis of methyl 4-isobutoxy-3-methoxybenzoate (16c)

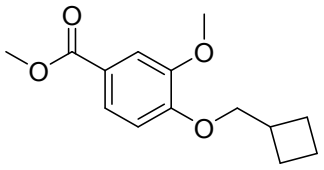
This compound was synthesized according to general procedure 5. White solid (249 mg, 95% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.82 (d, 2H), 2.18 (m, 1H), 1.04 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 153.0, 149.1, 123.7, 122.5, 112.7, 111.7, 75.4, 56.3, 52.1, 28.2, 19.4 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{18}O_4$, 238.1205; found, 238.1206.



Synthesis of methyl 4-(cyclopropylmethoxy)-3-methoxybenzoate (16d)

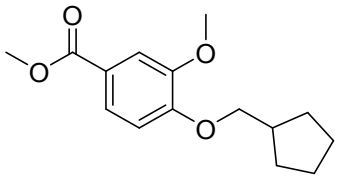
This compound was synthesized according to general procedure 5. White solid (249 mg, 96% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.54 (d, *J* = 1.9 Hz, 1H), 6.86

(d, J = 8.4 Hz, 1H), 3.92 (s, 3H), 3.91 (d, 2H), 3.88 (s, 3H), 1.37-1.31 (m, 1H), 0.69-0.64 (m, 2H), 0.39-0.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 152.7, 149.0, 123.6, 122.7, 112.4, 111.8, 74.0, 56.2, 52.1, 10.2, 3.6 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{16}O_4$, 236.1049; found, 236.1052.



Synthesis of methyl 4-(cyclobutylmethoxy)-3-methoxybenzoate (16e)

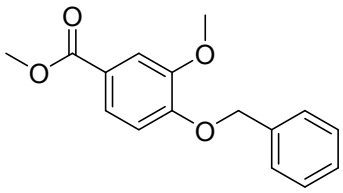
This compound was synthesized according to general procedure 5. White solid (258 mg, 94% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 4.04 (d, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 2.91-2.81 (m, 1H), 2.21-2.13 (m, 2H), 2.00-1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 153.0, 149.1, 123.6, 122.6, 112.7, 111.9, 73.3, 56.2, 52.1, 34.5, 25.2, 18.8 ppm. HRMS (TOF, ES+) calc'd for $C_{14}H_{18}O_4$, 250.1205; found, 250.1207.



Synthesis of methyl 4-(cyclopentylmethoxy)-3-methoxybenzoate (16f)

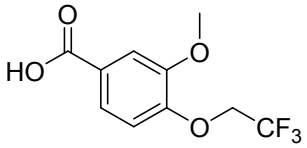
This compound was synthesized according to general procedure 5. Colorless oil (244 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.54 (d, J = 2.0 Hz, 1H), 6.88

(d, J = 8.4 Hz, 1H), 3.93 (d, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 2.45 (m, 1H), 1.90-1.83 (m, 2H), 1.68-1.57 (m, 4H), 1.41-1.34 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.1, 153.1, 149.1, 123.7, 122.5, 112.7, 111.8, 73.4, 56.3, 52.1, 39.0, 29.7, 25.4 ppm. HRMS (TOF, ES+) calc'd for $C_{15}H_{20}O_4$, 264.1362; found, 264.1367.



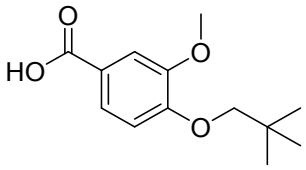
Synthesis of methyl 4-(benzyloxy)-3-methoxybenzoate (16g)

This compound was synthesized according to general procedure 5. White solid (278 mg, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61(dd, J = 8.4, 2.0 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.44-7.30 (m, 5H), 6.90 (d, J = 8.4 Hz, 1H), 5.21 (s, 2H), 3.94 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.0, 152.2, 149.3, 136.5, 128.8, 128.2, 127.3, 123.5, 123.1, 112.58, 112.56, 70.9, 56.2, 52.1 ppm. HRMS (TOF, ES+) calc'd for $C_{16}H_{16}O_4$, 272.1049; found, 272.1049.



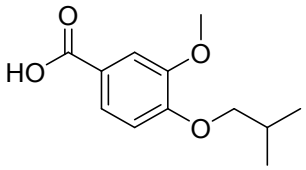
Synthesis of 3-methoxy-4-(2,2,2-trifluoroethoxy)benzoic acid (17a)

This compound was synthesized according to general procedure 6. White solid (225 mg, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (dd, J = 8.4, 1.9 Hz, 1H), 7.51 (d, J = 1.9 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 4.81 (q, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 166.9, 149.9, 148.6, 125.1, 123.9 (q, J_{CF} = 277.0 Hz), 122.7, 113.6, 112.7, 65.3 (q, J_{CF} = 34.1 Hz), 55.7 ppm. HRMS (TOF, ES+) calc'd for $C_{10}H_9F_3O_4$, 250.0453; found, 250.0454.



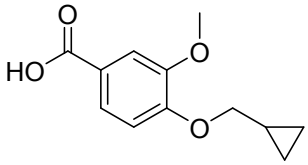
Synthesis of 3-methoxy-4-(neopentyloxy)benzoic acid (17b)

This compound was synthesized according to general procedure 6. White solid (166 mg, 88% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.44 (d, *J* = 1.9 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 3.81 (s, 3H), 3.68 (s, 2H), 1.00 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.6, 148.5, 123.3, 122.8, 112.5, 112.1, 77.9, 55.8, 31.7, 26.3 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{18}O_4$, 238.1205; found, 238.1207.



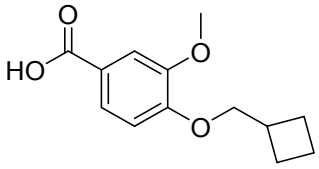
Synthesis of 4-isobutoxy-3-methoxybenzoic acid (17c)

This compound was synthesized according to general procedure 6. White solid (188 mg, 91% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.44 (d, *J* = 1.8 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 3.80 (s, 3H), 3.79 (d, 2H), 2.04 (m, 1H), 0.97 (d, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.2, 148.5, 123.2, 122.8, 112.2, 111.9, 74.4, 55.6, 27.7, 19.0 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{16}O_4$, 224.1049; found, 224.1051.



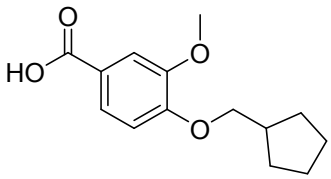
Synthesis of 4-(cyclopropylmethoxy)-3-methoxybenzoic acid (17d)

This compound was synthesized according to general procedure 6. White solid (199 mg, 96% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.44 (d, *J* = 1.9 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 3.86 (d, 2H), 3.81 (s, 3H), 1.28-1.19 (m, 1H), 0.60-0.55 (m, 2H), 0.34-0.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.0, 148.3, 123.1, 122.8, 112.0, 111.8, 72.8, 55.4, 10.1, 3.2 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{14}O_4$, 222.0892; found, 222.0894.



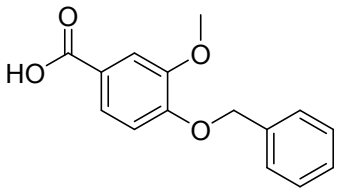
Synthesis of 4-(cyclobutylmethoxy)-3-methoxybenzoic acid (17e)

This compound was synthesized according to general procedure 6. White solid (202 mg, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.43 (d, *J* = 1.9 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 4.00 (d, 2H), 3.79 (s, 3H), 2.72 (m, 1H), 2.11-2.03 (m, 2H), 1.94-1.78 (m, 4H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.2, 148.4, 123.2, 122.9, 112.2, 112.0, 72.3, 55.5, 33.9, 24.4, 18.1 ppm. HRMS (TOF, ES+) calc'd for $C_{13}H_{16}O_4$, 236.1049; found, 236.1051.



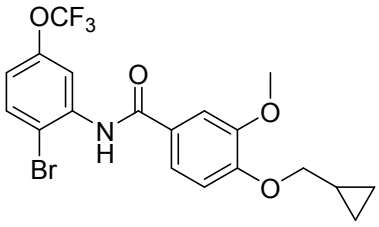
Synthesis of 4-(cyclopentylmethoxy)-3-methoxybenzoic acid (17f)

This compound was synthesized according to general procedure 6. White solid (190 mg, 91% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.44 (d, *J* = 1.6 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.89 (d, 2H), 3.80 (s, 3H), 2.32 (m, 1H), 1.80-1.72 (m, 2H), 1.62-1.51 (m, 4H), 1.36-1.28 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 152.2, 148.4, 123.2, 122.8, 112.2, 112.0, 72.3, 55.6, 38.4, 29.0, 24.9 ppm. HRMS (TOF, ES+) calc'd for $C_{14}H_{18}O_4$, 250.1205; found, 250.1211.



Synthesis of 4-(benzyloxy)-3-methoxybenzoic acid (17g)

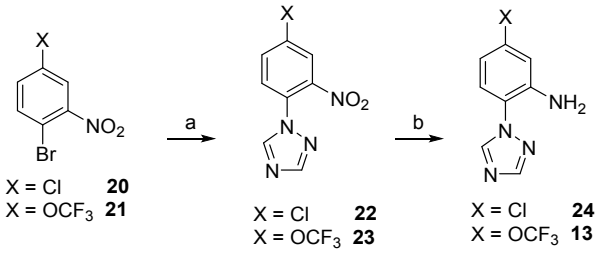
This compound was synthesized according to general procedure 6. White solid (242 mg, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.45-7.32 (m, 5H), 7.13 (d, *J* = 8.5 Hz, 1H), 5.16 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 167.1, 151.6, 148.6, 136.6, 128.5, 128.0, 127.9, 123.2, 123.0, 112.4, 112.2, 69.9, 55.5 ppm. HRMS (TOF, ES+) calc'd for $C_{15}H_{14}O_4$, 258.0892; found, 258.0896.

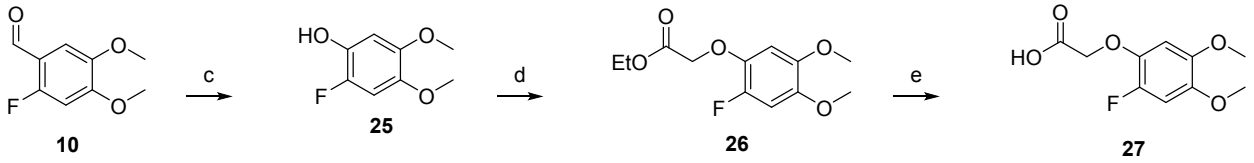


Synthesis of *N*-(2-bromo-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (19)

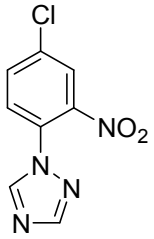
This compound was synthesized according to general procedure 4. White solid (1.86 g, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 2.3 Hz, 1H), 8.47 (bs, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.43 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.91-6.87 (m, 1H), 3.97 (s, 3H), 3.94 (d, 2H), 1.40-1.33 (m, 1H), 0.71-0.66 (m, 2H), 0.42-0.38 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.0, 152.4, 149.9, 149.1, 137.3, 132.9, 126.6, 120.5 (q, J_{CF} = 256.6 Hz), 119.6, 117.1, 114.2, 112.2, 111.2, 110.7, 74.1, 56.3, 10.2, 3.6 ppm. HRMS (TOF, ES+) calc'd for $C_{19}H_{17}BrF_3NO_4$, 459.0293; found 459.0296.

<u>Supplementary Schemes: Experimental Procedures and Spectroscopic Data of Intermediates 22-27</u>



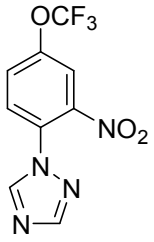


Reagents and conditions: (a) 1H-1,2,4-triazole, trans-N,N'-dimethylcyclohexane-1,2-diamine, K3PO4, CuI, DMF, 100 °C, 16h, 49-56%; (b) Fe powder, NH4Cl, EtOH, H2O, 75 °C, 4h, 53-58%; (c) i. mCPBA, CH2Cl2, rt, 5h, ii. LiOH, THF:H2O (1:1), rt, 1h, 73%; (d) ethyl bromoacetate, K2CO3, MeCN, 170 °C, mw, 1h, 81%; (e) LiOH, THF:H2O (1:1), rt, 3h, 97%



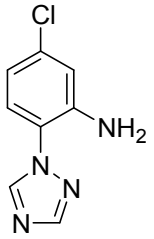
Synthesis of 1-(4-chloro-2-nitrophenyl)-1*H*-1,2,4-triazole (22)

This compound was synthesized according to general procedure 2. Beige solid (2.13 g, 56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.11 (s, 1H), 8.02 (d, *J* = 2.3 Hz, 1H), 7.74 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 153.4, 144.8, 144.0, 136.4, 133.9, 128.8, 128.6, 125.9 ppm. HRMS (TOF, ES+) calc'd for $C_8H_5ClN_4O_2$, 224.0101; found, 224.0103.



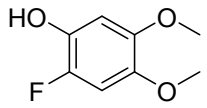
Synthesis of 1-(2-nitro-4-(trifluoromethoxy)phenyl)-1*H*-1,2,4-triazole (23)

This compound was synthesized according to general procedure 2. White solid (2.35 g, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.12 (s, 1H), 7.90 (d, *J* = 2.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.63-7.61 (m, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 153.5, 149.4 (d, J_{CF} = 2.0 Hz), 145.0, 144.1, 129.2, 128.7, 125.7, 120.3 (q, J_{CF} = 259.0 Hz), 118.4 ppm. HRMS (TOF, ES+) calc'd for $C_9H_5F_3N_4O_3$, 274.0314; found, 274.0315.



Synthesis of 5-chloro-2-(1*H*-1,2,4-triazol-1-yl)aniline (24)

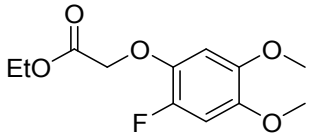
This compound was synthesized according to general procedure 3. White solid (1.01 g, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.14 (s, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.2 Hz, 1H), 4.64 (bs, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 152.7, 143.5, 142.2, 135.7, 125.3, 121.3, 118.2, 117.1 ppm. HRMS (TOF, ES+) calc'd for $C_8H_7ClN_4$, 194.0359; found, 194.0360.



Synthesis of 2-fluoro-4,5-dimethoxyphenol (25)

To a solution of 6-fluoroveratraldehyde (300 mg, 1.63 mmol) in DCM (7.50 mL) was added 3-chloroperoxybenzoic acid (562 mg, 2.44 mmol) at room temperature portionwise. Upon completion of addition, the reaction was stirred at room temperature for 5 hr whereupon LCMS indicated complete consumption of starting materials and formation of the desired formate ester. The reaction was diluted with DCM and quenched with the addition of saturated sodium thiosulfate. The layers were separated, and the aqueous layer was washed with DCM x 3. The

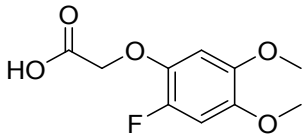
combined organic material was passed through a phase separator and concentrated. The crude formate ester was redissolved in THF : H_2O (1:1), and lithium hydroxide (4.89 mmol) was added. The reaction was stirred at room temperature for 1 hr before dilution with EtOAc and acidification to pH ~1 using 2 M HCl. The layers were separated, and the aqueous layer was washed with EtOAc x 3. The combined organic material was passed through a phase separator, concentrated, and purified via flash chromatography (Teledyne ISCO system; silica gel column; hexanes:EtOAc; 0-30% EtOAc gradient) to afford the desired product as a beige solid (205 mg, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (d, J_{HF} = 11.6 Hz, 1H), 6.59 (d, J_{HF} = 8.4 Hz, 1H), 4.79 (bd, 1H), 3.82 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 145.7 (d, J_{CF} = 2.5 Hz), 144.5 (d, J_{CF} = 228.3 Hz), 142.5 (d, J_{CF} = 7.8 Hz), 136.6 (d, J_{CF} = 15.0 Hz), 102.0 (d, J_{CF} = 2.1 Hz), 101.0 (d, J_{CF} = 23.2 Hz), 56.8, 56.5 ppm. HRMS (TOF, ES+) calc'd for $C_8H_9FO_3$, 172.0536; found, 172.0536.



Synthesis of ethyl 2-(2-fluoro-4,5-dimethoxyphenoxy)acetate (26)

To a solution of 2-fluoro-4,5-dimethoxyphenol (**25**) (190 mg, 1.10 mmol) in MeCN (3.17 mL) in a Biotage microwave vial was added potassium carbonate (310 mg, 2.21 mmol) and ethyl bromoacetate (244 uL, 2.21 mmol). The vial was sealed and heated to 170 °C for 1 hr using a Biotage microwave reactor whereupon LCMS indicated complete consumption of starting material and formation of the desired product. The reaction was diluted with EtOAc, filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system; silica gel column; hexanes:EtOAc; 0-30% EtOAc gradient) to afford the desired product as a white solid (231 mg, 81% yield). ^{1}H

NMR (400 MHz, $CDCl_3$) δ 6.70 (d, J_{HF} = 5.6 Hz, 1H), 6.67 (d, J_{HF} = 10.2 Hz, 1H), 4.64 (s, 2H), 4.26 (q, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 1.30 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.2, 147.4 (d, J_{CF} = 237 Hz), 145.1 (d, J_{CF} = 3.0 Hz), 144.9 (d, J_{CF} = 8.0 Hz), 138.5 (d, J_{CF} = 12.0 Hz), 104.5 (d, J_{CF} = 2.0 Hz), 101.5 (d, J_{CF} = 24.0 Hz), 69.0 (d, J_{CF} = 3.0 Hz), 61.4, 56.6, 56.5, 14.3 ppm. HRMS (TOF, ES+) calc'd for $C_{12}H_{15}FO_5$, 258.0904; found, 258.0906.



Synthesis of 2-(2-fluoro-4,5-dimethoxyphenoxy)acetic acid (27)

This compound was synthesized according to general procedure 6. White solid (173 mg, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.95 (d, J_{HF} = 12.8 Hz, 1H), 6.80 (d, J_{HF} = 8.4 Hz, 1H), 4.70 (s, 2H), 3.72 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 170.1, 145.6 (d, J_{CF} = 235.0 Hz), 144.9 (d, J_{CF} = 2.0 Hz), 143.2 (d, J_{CF} = 8.0 Hz), 138.4 (d, J_{CF} = 12.0 Hz), 102.3 (d, J_{CF} = 2.0 Hz), 102.1 (d, J_{CF} = 23.0 Hz), 66.4, 56.4, 56.3 ppm. HRMS (TOF, ES+) calc'd for $C_{10}H_{11}FO_5$, 230.0591; found, 230.0592.

Molecular Pharmacology Methods

Cell lines and cell culture

As described in Jalan-Sakrikar et al.,[1] rat $mGlu_7/G_{\alpha 15}$/HEK cells were obtained by stable co-expression of $G_{\alpha 15}$/pCMV with the rat $mGlu_7$ receptor cDNA that had been cloned into a pIRESpuro3 vector (Invitrogen). $mGlu_7$ polyclonal cells were grown in 90% DMEM, 10% FBS, 100 units/ml penicillin/streptomycin, 20 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine, 1X non-essential amino acids, 700 μg/mL G418 and 0.6 μg/mL puromycin. All cell culture reagents were purchased from Invitrogen (Carlsbad, CA) unless otherwise noted.

Calcium assays. Rat $mGlu_7/G_{a15}$/HEK cells (20,000 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). 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 50 minutes at room temperature. 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), 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 Max) for compounds were determined using a four parameter logistical equation in the Dotmatics software platform (Dotmatics, Bishop's Stortford, UK). mGlu receptor selectivity profiling assays were performed as previously reported[1-3] using a high (10 uM)

concentration of compound followed by the application of a full concentration-response curve of orthosteric agonist. This allows determine of compound activity alone (ranked as agonist) as well as the ability to potentiate (shifts the orthosteric agonist curve to the left,) or antagonize (shifts the orthosteric curve to the right and/or down) receptor activity.

DMPK Methods

In-Vitro DMPK Methods

Intrinsic clearance in liver microsomes

Hepatic 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] species specific scale up factors

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

where Q_h = hepatic blood flow in each species..

Plasma Protein Binding

The protein binding of each compound was determined in 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 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 brain tissue with three volumes 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.

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.

Time Course PK and Single Time Point Tissue Distribution Studies in Rat

IV cassette PK experiments in rats were carried out according to methods described previously.[4] 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).

Discrete Rat PK and Tissue Distribution Studies

For discrete rat pharmacokinetic experiments, compounds were formulated in 10% EtOH/60% PEG400/30% saline (IV, 0.2 mg/kg) and in 10% Tween 80 in 0.5% MC (PO, 10 mg/kg) and dosed via the jugular vein to two dual-cannulated (carotid artery and jugular vein) adult male Sprague–Dawley rats (IV) or by oral gavage (PO). 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. Tissue distribution studies were performed by formulating the compound in 10% Tween 80 in 0.5% MC and dosing via intraperitoneal injection (30 mg/kg) to male SD rats (n =

3-4 per time point). At 1 hr post dose, animals were euthanized and decapitated, blood was collected via cardiac puncture, CSF was obtained, and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice.

Discrete Mouse Tissue Distribution Studies

Single time point, discrete tissue distribution studies were performed by formulating the compound in 10% Tween 80 in water and dosing via intraperitoneal injection (3 mg/kg) to male mice (n = 6 per time point). At 1 hr 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), brain homogenate (20 µL), or CSF (where appropriate, 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

Animals: All studies were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Vanderbilt University Institutional Animal Care and Use Committee. Separate cohorts of C57Bl/6J male mice (8 weeks old) were used for behavioral testing. All mice were grouped housed on a 12:12 light–dark cycle with lights on at 0600. All experiments were conducted during the light phase. Food and water were available ad libitum.

Elevated Zero Maze (EZM): Mice received i.p. injections of either vehicle (10% Tween 80) or various doses of VU6012962 (VU'692; 1-10 mg/kg) 60 minutes prior to testing. To start the EZM test, mice were lowered by their tail and placed at a randomly chosen boundary between an open

and a closed zone, facing the inside of the closed zone on the elevated circular platform (40 cm off the ground, 50 cm in diameter). Activity of the mouse was monitored for five minutes via an overhead camera connected to a computer in a separate room using video acquisition and ANY-maze analysis software (Stoelting, Wood Dale, IL, USA). Data analyzed included the time spent in the open versus closed arm, the total distance traveled in the maze, and the number of arm entries. Time spent in the open versus closed arm is shown as percentage of time in open to total time. Two mice were excluded from analysis at the highest dose (10 mg/kg) as they failed to initiate the trial, which was defined as a lack of movement from the starting location within 120 seconds. These animals were immediately removed from the testing apparatus after 120 seconds had elapsed, and therefore did not complete the full five minute test trial.

Light-Dark Box: Sixty minutes prior to behavioral testing, mice received one of the following treatment conditions: vehicle (10% Tween 80; i.p.), 15 mg/kg fluoxetine (FLX; i.p.) or 3 mg/kg VU6012962 (VU'692; i.p.). Mice were individually placed into sound-attenuating chambers (27.9 × 27.9 cm; MED-OFA-510; MED Associates, St. Albans, VT, USA) containing dark box inserts that split the chamber into light (~25 lux) and dark (<5 lux) halves (Med Associates ENV-511). Beam breaks from 16 infrared beams were recorded by Activity Monitor v5.10 (MED Associates) to monitor position and behavior during a 10-min testing period. Data analyzed only included the first five minutes of the session and included total distance traveled and time spent in light to total time. Data are presented as Mean ± S.E.M and is shown as a ratio of time in light to total time.

Marble Burying: Mice were pretreated with either vehicle (10% Tween 80; i.p.), 15 mg/kg fluoxetine (FLX; i.p.) or 3 mg/kg VU6012962 (VU'692; i.p.). Forty-five minutes after injection,

mice were habituated for 15 minutes to 2.5 cm Diamond Soft Bedding (Harlan Teklad, Madison, WI, USA). Mice were then placed back in Plexiglass cages in which 15 blue marbles (14 mm diameter) were distributed in a 3 x 5 layout with 1.5 cm between each marble on top of the Diamond Soft Bedding. The amount of marbles buried was quantified following a 30 minute interval. The mice were then removed from the cages and the number of buried marbles was counted by two blinded experimenters using a criterion of greater than 2/3rd covered by bedding. Data are presented as Mean ± S.E.M and are presented as total marbles buried or the ratio of marbles buried to total marbles. Two mice were excluded from analysis for being outliers as assessed by the Grubb's Outlier Test.

Data Analysis: Behavioral data were analyzed by a one-way analysis of variance (ANOVA) followed by post hoc Dunnett's or Bonferroni's multiple comparison tests unless otherwise specified. All statistical analyses were conducted with Prism GraphPad 6 (San Diego, CA, USA). Data sets in Prism were run through the Grubb's test. Results are shown as Mean ± S.E.M. Statistical significance was set at $P<0.05$.

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 **7d** (VU6012962) from two independent determinations.

Supporting Table 1: Eurofins Profiling of **7d** (VU6012962).

Target/Protein	Species	% Inhibition
Adenosine A_1	Human	9
Adenosine A_{2A}	Human	-1
Adenosine A_3	Human	45
Adrenergic α_{1A}	Rat	-3
Adrenergic α_{1B}	Rat	-5
Adrenergic α_{1D}	Human	8
Adrenergic α_{2A}	Human	-6
Adrenergic β_1	Human	6
Adrenergic β_2	Human	6
Androgen (Testosterone)	Human	-9
Bradykinin B_1	Human	-5
Bradykinin B_2	Human	-5
Calcium Channel L-Type, Benzothiazepine	Rat	-3
Calcium Channel L-Type, Dihydropyridine	Rat	14
Calcium Channel N-Type	Rat	-5
Cannabinoid CB_1	Human	49
Dopamine D_1	Human	5
Dopamine D_{2S}	Human	3
Dopamine D_3	Human	-4
Dopamine $D_{4.2}$	Human	0
Endothelin ET_A	Human	0
Endothelin ET_B	Human	-5
Epidermal Growth Factor (EGF)	Human	9
Estrogen ERα	Human	-3
$GABA_A$, Flunitrazepam, Central	Rat	3
$GABA_A$, Muscimol, Central	Rat	23
$GABA_{B1A}$	Human	5
Glucocorticoid	Human	-4
Glutamate, Kainate	Rat	-1
Glutamate, NMDA, Agonism	Rat	10
Glutamate, NMDA, Glycine	Rat	-5
Glutamate, NMDA, Phencyclidine	Rat	8
Histamine H_1	Human	-3
Histamine H_2	Human	2
Histamine H_3	Human	-14
Imidazoline I_2, Central	Rat	5
Interleukin IL-1	Mouse	2
Leukotriene, Cysteinyl $CysLT_1$	Human	-5
Melatonin MT_1	Human	6
Muscarinic M_1	Human	-2
Muscarinic M_2	Human	-18
Muscarinic M_3	Human	-2

Neuropeptide Y Y_1	Human	3
Neuropeptide Y Y_2	Human	-6
Nicotinic Acetylcholine	Human	-6
Nicotinic Acetylcholine α1, Bungarotoxin	Human	11
Opiate δ_1 (OP1, DOP)	Human	0
Opiate κ (OP2, KOP)	Human	14
Opiate μ (OP3, MOP)	Human	-6
Phorbol Ester	Mouse	-3
Platelet Activating Factor (PAF)	Human	22
Potassium Channel [K_{ATP}]	Hamster	-5
Potassium Channel hERG	Human	17
Prostanoid EP_4	Human	10
Purinergic P2X	Rabbit	10
Purinergic P2Y	Rat	-3
Rolipram	Rat	25
Serotonin (5-HT_{1A})	Human	-1
Serotonin (5-HT_{2B})	Human	73
Serotonin (5-HT_3)	Human	-3
Sigma σ_1	Human	-9
Sodium Channel, Site 2	Rat	30
Tachykinin NK_1	Human	7
Thyroid Hormone	Rat	6
Transporter, Dopamine (DAT)	Human	0
Transporter, GABA	Rat	5
Transporter, Norepinephrine (NET)	Human	4
Transporter, Serotonin (SERT)	Human	-9

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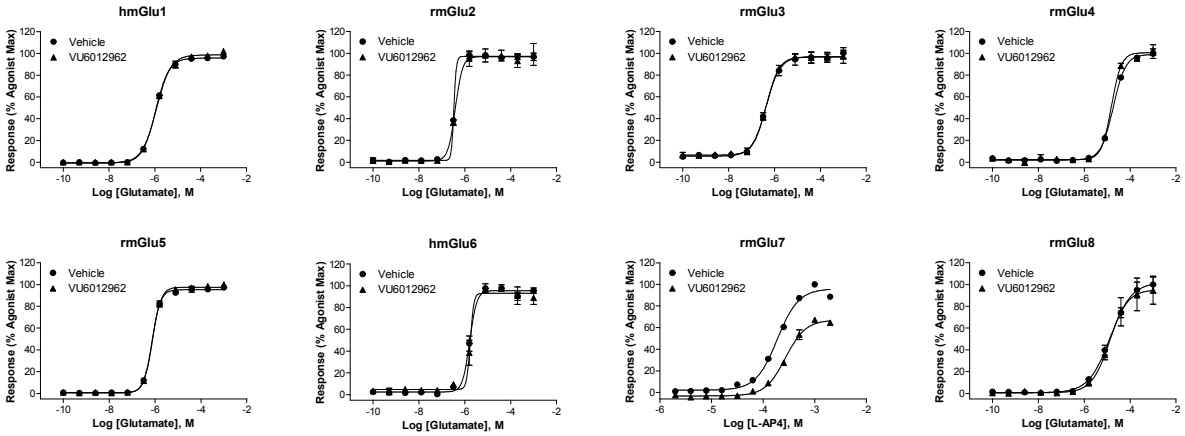
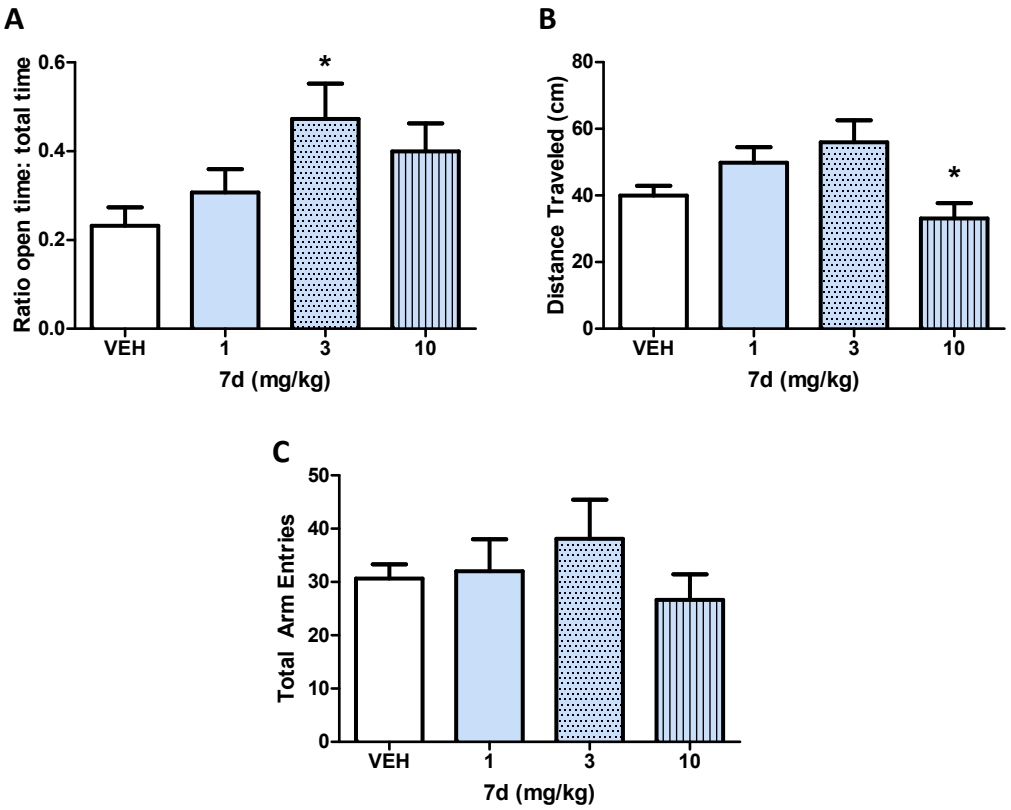
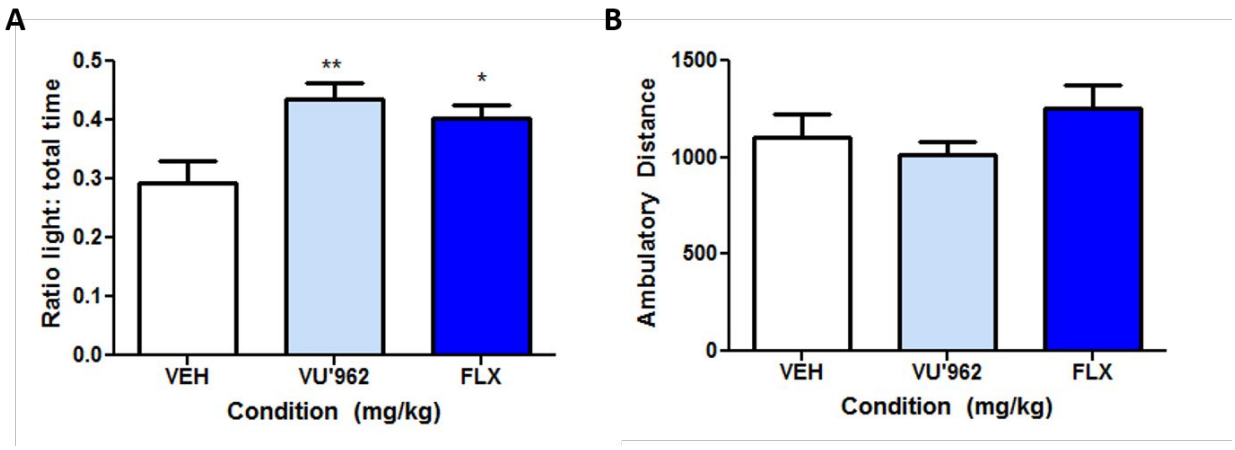


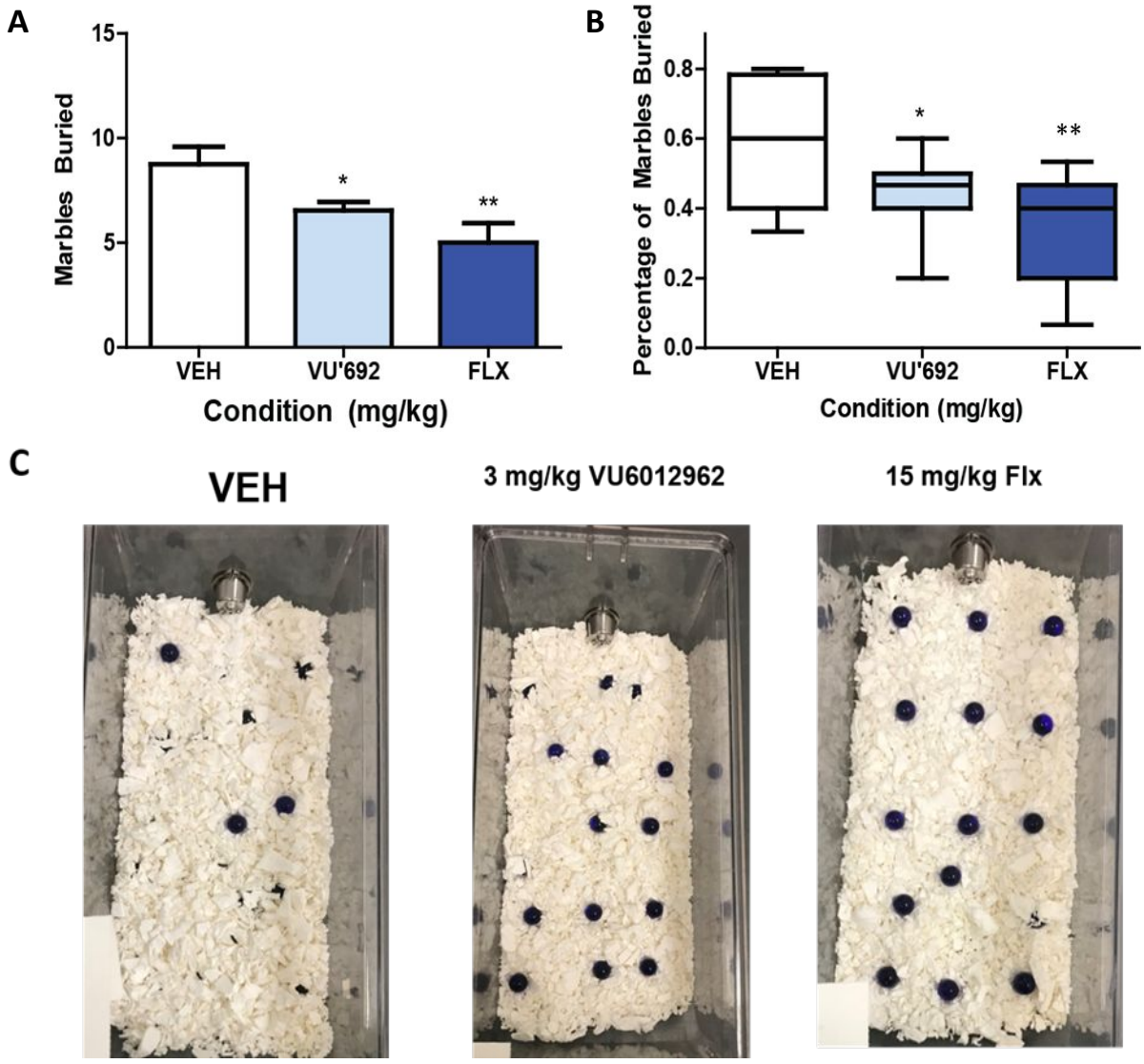
Supporting Figure 1. mGlu receptor selectivity for **7d** (VU6012962) in 10 μM, fold-shift assay.



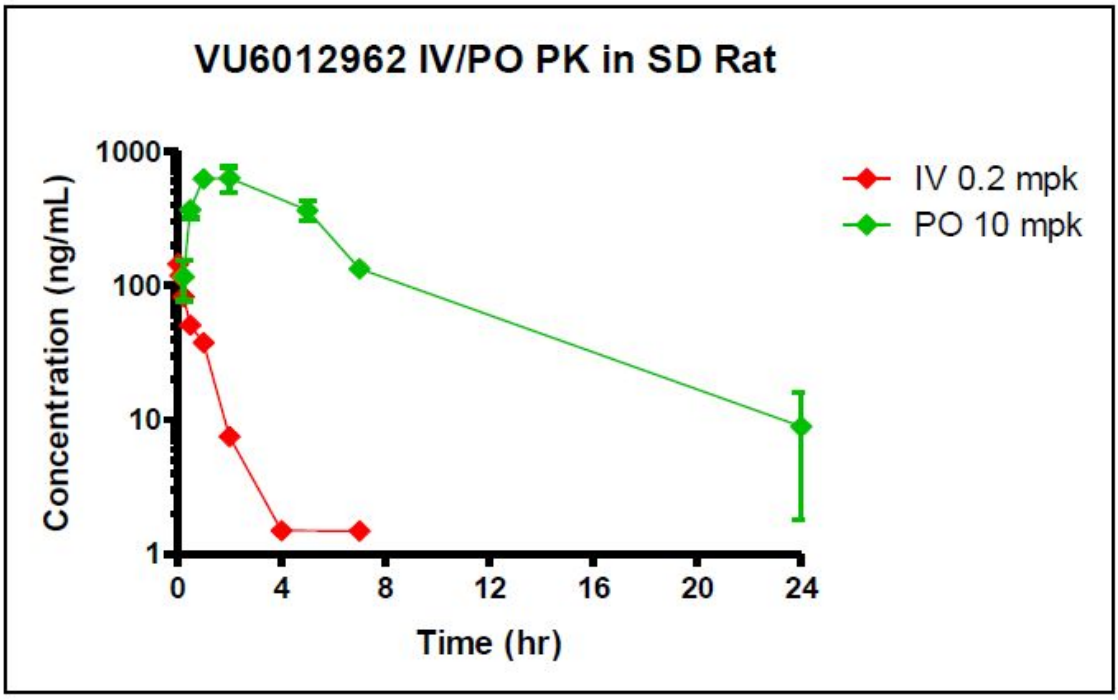
Supplemental Figure 2. NAM **7d** decreases anxiety in the elevated zero maze (EZM) assay. A) Administration of **7d** increases time spent in the open arms (MED = 3 mg/kg); One-way ANOVA; ($F[3,39]=3.112$, $p = 0.0382$), Bonferroi's post hoc test; VEH vs. 3 mg/kg, $p<0.05$. B) The 10 mg/kg dose decreased distance traveled; One-way ANOVA; ($F[3,39]=4.613$, $p = 0.0079$), Bonferroni's post hoc test; 3 mg/kg vs. 10 mg/kg, $p<0.05$. C). There was no significant difference in total arm entries for any treatment group; One-way ANOVA; ($F[3,39]=0.8141$, $p = 0.4945$), Bonferroni's post hoc test).



Supplemental Figure 3. 7d (VU6012962) exhibits efficacy in a Light/Dark box anxiolytic assay. A) Administration of both 3 mg/kg **7d** and 15 mg/kg fluoxetine (FLX) increased total time spent in the light side of the chamber compare to vehicle (VEH) controls (One-way ANOVA; ($F[2,20]=6.160$, $p = 0.0092$) Dunnett's Multiple Posthoc Test; B) VEH vs. VU962, $p<0.01$; VEH s. FLX, $p<0.05$), no significant differences in locomotor activity were noted during the test period (B) (One-way ANOVA; $F[2,20]=1.361$, $p=0.2816$).



Supplemental Figure 4. **7d** (VU6012962) decreases anxiety in the marble-burying assay and is comparable to fluoxetine (FLX). A) Administration of 3 mg/kg of the $mGlu_7$ NAM (**7d**) reduced the number of marbles buried (One-way ANOVA; ($F[2,31]=6.376$, $p = 0.0051$) Dunnett's Multiple Posthoc Test; VEH vs. VU962, $p<0.05$; VEH s. FLX, $p<0.01$. All data points were run with Grubb's outlier test). B) Administration of the $mGlu_7$ NAM (**7d**) reduced the percentage of marbles buried (One-way ANOVA; ($F[2,31]=6.376$, $p = 0.0051$) Dunnett's Multiple Posthoc Test; VEH vs. VU962, $p<0.05$; VEH vs. FLX, $p<0.01$. All data points were run with Grubb's outlier test). C) Representative photo after the 30 minute behavioral session from an animal in each treatment condition.



Supplemental Figure 5. 7d (VU6012962) rat IV/PO PK