Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 demonstrates robust chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity

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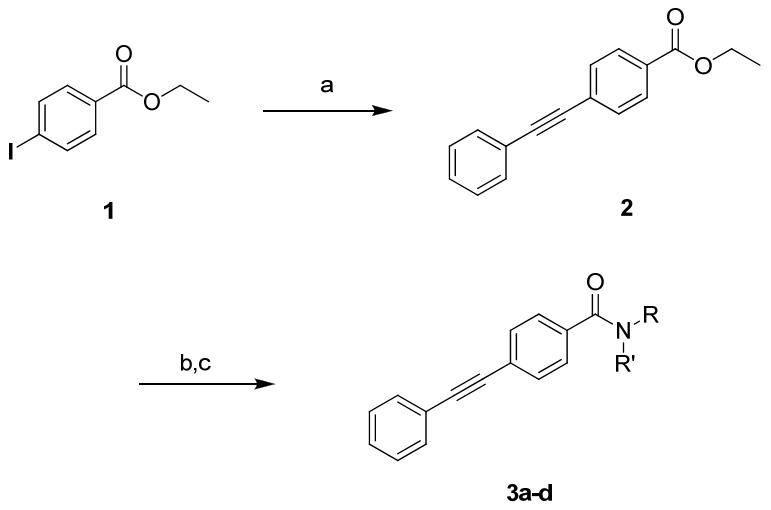
Molecular Pharmacology

Supporting Information

Chemistry

General. All NMR spectra were recorded on a Bruker 400 mHz instrument. ^{1}H chemical shifts are reported in δ values in ppm downfield from DMSO as the internal standard in DMSO. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constant (Hz). ^{13}C chemical shifts are reported in δ values in ppm with the DMSO carbon peak set to 39.5 ppm. Low resolution mass spectra were obtained on an agilent 1200 series 6130 mass spectrometer. High resolution mass spectra were recorded on a Waters Q-TOF API-US. Analytical thin layer chromatography was performed on Analtech silica gel GF 250 micron plates. Analytical HPLC was performed on an agilent 1200 series. Preparative purification was performed on combi-flash companion. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Argonaut Technologies.

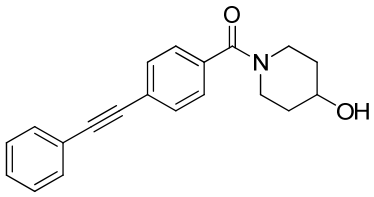
Experimental Procedures for mGluR5 PAMs:



Reaction conditions: a) phenylacetylene, $Pd(Ph_3P)_4$ (5 mol%), CuI (10 mol%), DEA, DMF, 80 ^oC, 1h (86%); b) LiOH, MeOH, THF (83%); c) RR'NH, EDC, HOBt, DIPEA, DMF (73-98%).

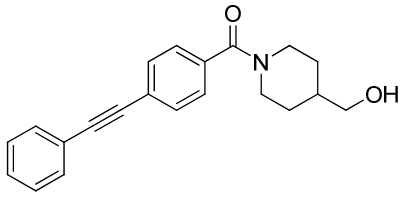
Ethyl 4-(phenylethynyl)benzoate (2). To a solution of ethyl 4-Iodobenzoate **1** (5.0 g, 18.2 mmol) in DMF (8 mL) was added phenylacetylene (2.25 g, 22.1 mmol), $Pd(Ph_3P)_4$ (502 mg, 0.45 mmol), CuI (172 mg, 0.91 mmol) and diethylamine (2 mL). The reaction vessel was sealed and heated at 60 °C for 1h in a microwave reactor. The reaction was cooled to rt, diluted with EtOAc:hexanes (2:1, 150 mL) and washed with water (2 x 100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified by column chromatography (silica gel) using 0 to 10 % EtOAc/hexanes to afford ester **2** (7.89 g, 86%) as a pale yellow solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.56 (dd, *J* = 8.0, 2.0 Hz, 2H), 7.41-7.37 (m, 3H), 4.41 (q, *J* = 7.0 Hz, 2H), 1.44 (t, *J* = 7.0 Hz, 3H); LC (214 nm) 5.79 min (>98%); MS (ESI) *m/z* = 250.9.

4-(phenylethynyl)benzoic acid. To a solution of ester **2** (7.81 g, 31.2 mmol) in THF (80 mL) was added MeOH (15 mL) and a solution of LiOH (5.24 g, 124 mmol) in water (15 mL). The reaction was stirred at room temperature and for 4h. The reaction was acidified with 1 N HCl (50 mL) and isolated benzoic acid (5.78 g, 83%) as a white solid: mp 190.1 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.0 Hz, 2H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.62-7.56 (m, 2H), 7.52-7.47 (m, 1H), 7.43-7.36 (m, 3H); ^{13}C-NMR (100 MHz, d_6-DMSO) δ 167.3, 138.0, 134.5, 131.9, 131.4, 130.9, 130.6, 130.2, 130.0, 129.6, 127.0, 122.15, 101.6, 92.3, 89.0; LC (214 nm) 5.12 min (>98%); MS (ESI) *m/z* = 222.9.



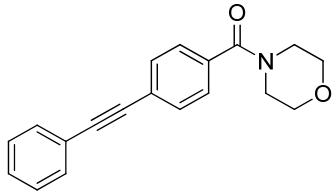
3a
VU0092273

(4-hydroxypiperidin-1-yl)(4-phenylethynyl)phenyl)methanone, VU0092273 (3a). To a solution of acid **9** (1.40 g, 6.30 mmol) and DIPEA (2.70 g, 20.8 mmol) in DMF (25 mL) was added EDC (1.41 g, 7.56 mmol), HOBt (850 mg, 6.30 mmol) and 4-hydroxypiperidine hydrochloride (1.29 g, 9.46 mmol). The reaction was stirred at room temperature for 18 h. The reaction was diluted with water (100 mL) and isolated amide **3a** (1.84 g, 98%) as a white solid by vacuum filtration: mp 157.7 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.0 Hz, 2H), 7.56-7.52 (m, 2H), 7.44-7.34 (m, 5H), 4.21-4.08 (m, 1H), 4.03-3.96 (m, 1H), 3.81-3.48 (m, 1H), 3.47-3.16 (m, 2H), 2.08-1.79 (m, 3H), 1.71-1.42 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.7, 135.5, 131.6, 131.5, 128.5, 128.3, 126.9, 124.7, 122.8, 90.8, 88.5, 66.9, 44.8, 39.3, 34.4, 33.8; LC (214 nm) 2.86 min (>98%); MS (ESI) *m/z* = 306.1; HRMS = 306.1496 (calc. 306.1494), $C_{20}H_{20}N_1O_2$.



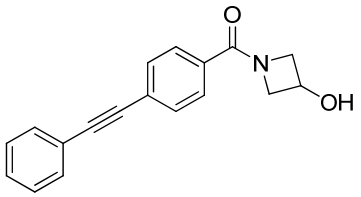
3b
VU0366024

(4-methanolpiperidin-1-yl)(4-phenylethynyl)phenyl)methanone, VU0366024 (3b). Amide **3b** (1.26 g, 73%) isolated as a white solid was prepared in a manner similar to **3a**: mp 144.8 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57-7.50 (m, 4H), 7.40-7.31 (m, 5H), 4.76 (br s, 1H), 3.77 (br d, 1H), 3.54-3.49 (m, 2H), 3.12-2.70 (m, 2H), 1.95 (br s, 4H), 1.22 (br s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.7, 135.8, 131.6, 131.5, 128.5, 128.3, 126.9, 124.6, 122.8, 120.2, 90.7, 88.6, 67.1, 47.7, 42.2, 38.8, 38.4, 36.9, 29.4, 28.8, 28.3; LC (214 nm) 3.02 min (>98%); MS (ESI) *m/z* = 320.0; HRMS = 320.1645 (calc. 320.1651), $C_{21}H_{22}N_1O_2$.



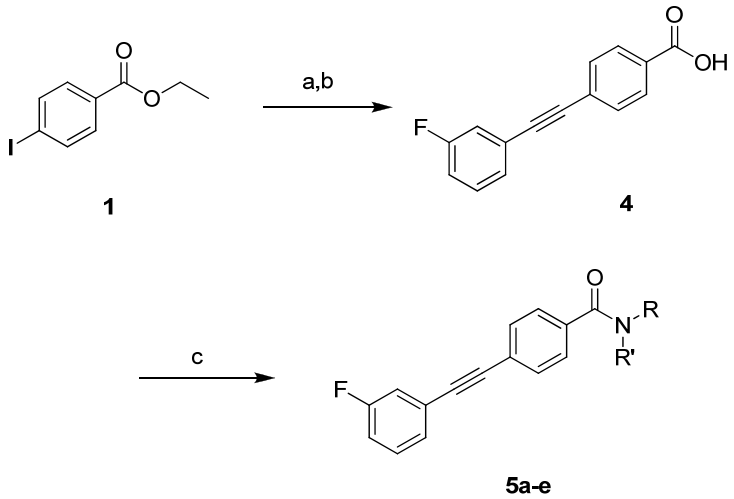
3c
VU0240381

(4-morphonyl)(4-phenylethynyl)phenyl)methanone, VU0240381 (3c). Amide **3c** (2.01 g, 86%) isolated as a white solid was prepared in a manner similar to **3a**: mp 118.4 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60-7.53 (m, 4H), 7.43-7.34 (m, 5H), 3.65 (br s, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.7, 134.7, 131.7, 131.6, 128.5, 128.3, 127.2, 125.0, 122.7, 90.9, 88.4, 66.8; LC (214 nm) 3.11 min (>98%); MS (ESI) *m/z* = 292.2; HRMS = 292.1332 (calc. 292.1338), $C_{19}H_{18}N_1O_2$.



3d
VU0366025

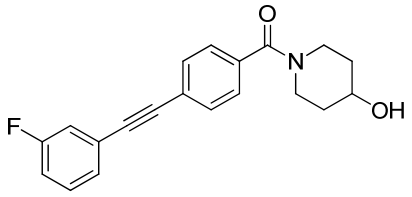
(3-hydroxyazetidin-1-yl)(4-phenylehtynyl)phenyl)methanone, VU0366025 (3d). Amide **3d** (1.68 g, 88%) isolated as a white solid was prepared in a manner similar to **3a**: mp 161.2 C, ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.68-7.61 (m, 4H), 7.49-7.41 (m, 3H), 7.34-7.26 (m, 1H), 5.78 (br s, 1H), 4.52-4.43 (m, 2H), 4.27-4.23 (m, 1H), 4.09-4.00 (m, 1H), 3.82-3.76 (m, 1H); ^{13}C-NMR (100 MHz, d_6-DMSO) δ168.5, 133.5, 131.9, 131.7, 129.5, 129.2, 128.5, 125.1, 122.3, 91.5, 89.0, 63.1, 60.8, 58.9; LC (214 nm) 2.81 min (>98%); MS (ESI) *m/z* = 278.1; HRMS = 278.1189 (calc. 278.1181), $C_{18}H_{16}N_1O_2$.



Reaction conditions: a) 3-fluorophenylacetylene, $Pd(Ph_3P)_4$ (5 mol%), CuI (10 mol%), DEA, DMF, 80 °C, 1h; b) LiOH, MeOH, THF (79%, two steps); c) RR'NH, EDC, HOBt, DIPEA, DMF (71-98%).

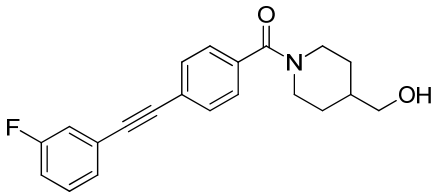
4-((3-fluorophenyl)ethynyl) benzoic acid (4). To a solution of ethyl 4-iodobenzoate **1** (10.0 g, 36.2 mmol) in DMF (30 mL) was added 3-fluorophenylacetylene (5.22 g, 43.4 mmol), $Pd(Ph_3P)_4$ (1.04 g, 0.91 mmol), CuI (346 mg, 1.82 mmol) and diethylamine (6 mL). The reaction vessel was sealed and heated at 60 °C for 1h in a microwave reactor. The reaction was cooled to room temperature, diluted with EtOAc:hexanes (2:1, 250 mL) and washed with water (2 x 200 mL) and brine (200 mL). The organic phase was dried

over $MgSO_4$, filtered and concentrated under vacuum. The crude product was dissolved in THF (130 mL), MeOH (28 mL) and a solution of LiOH (3.04 g, 72.4 mmol) in water (28 mL) added. The reaction was stirred at room temperature and for 4h. The reaction was acidified with 1 N HCl (100 mL) and isolated benzoic acid **4** (6.89 g, 79%) as a light tan solid: mp 157.1 °C (with decomp.); ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.97 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 1H), 7.71-7.63 (m, 2H), 7.52-7.40 (m, 2H), 7.36-7.29 (m, 1H); ^{13}C-NMR (100 MHz, d_6-DMSO) δ 167.0, 163.5, 138.0, 132.0, 131.4, 130.6, 129.9, 128.3, 126.5, 124.1, 118.6, 117.0, 101.6, 90.9, 89.8; LC (214 nm) 3.29 min (>85%); MS (ESI) *m/z* = 241.1.



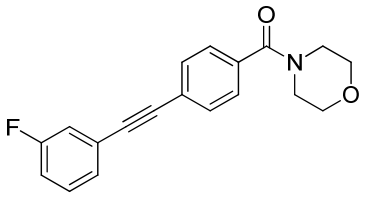
5a
VU0366026

(4-hydroxypiperidin-1-yl)(4-(3-fluorophenyl)ethynyl)phenyl)methanone, VU0366026 (5a). Amide **5a** (2.35 g, 91%) isolated as a white solid and prepared in a manner similar to **3a**: mp 143.3 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.38-7.31 (m, 2H), 7.28-7.21 (m, 1H), 7.11-7.03 (m, 1H), 4.21-4.08 (m, 1H), 4.03-3.96 (m, 1H), 3.81-3.48 (m, 1H), 3.47-3.15 (m, 2H), 2.14 (s, 1H), 2.05-1.78 (m, 2H), 1.71-1.42 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6, 163.5, 161.1, 135.8, 131.7, 130.0, 127.5, 127.2, 126.9, 124.7, 124.6, 124.2, 118.3, 115.6, 89.4, 66.8, 44.8, 39.4, 34.4, 33.8; LC (214 nm) 2.98 min (>98%); MS (ESI) *m/z* = 324.2; HRMS = 324.1399 (calc. 324.1400), $C_{20}H_{19}N_1O_2F_1$.



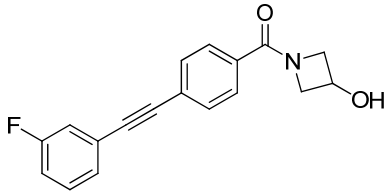
5b
VU0366027

(4-((3-fluorophenyl)ethynyl)phenyl)(4-hydroxymethyl)piperidin-1-yl)methanone, VU0366027 (5b). Amide **5b** (2.32 g, 78%) light cream solid as prepared in identical fashion to **3a**; mp 144.9 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.38-7.30 (m, 2H), 7.28-7.21 (m, 1H), 7.09-7.02 (m, 1H), 3.89-3.71 (m, 1H), 3.61-3.52 (m, 2H), 3.12-2.71 (m, 2H), 1.96-1.69 (m, 3H), 1.41-1.12 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6, 163.5, 136.1, 131.6, 129.9, 127.5, 127.0, 124.0, 118.5, 115.8, 89.4, 89.3, 67.2, 48.2, 42.1, 37.8, 29.4, 28.3; LC (215 nm) 4.98 min (>98%); MS (ESI) *m/z* = 338.1; HRMS = 338.1556 (calc. 338.1556), $C_{21}H_{21}N_1O_2F_1$.



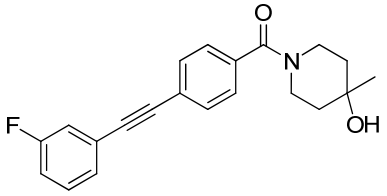
5c
VU0366031

(4-morphonyl)(4-(3-fluorophenyl)ethynyl)phenyl)methanone, VU0366031 (5c). Amide **5c** (3.11 g, 97%) white solid as prepared in identical fashion to **3a;** mp 97.0 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.38-7.31 (m, 2H), 7.28-7.21 (m, 1H), 7.13-7.05 (m, 1H), 4.00-3.31 (bd s, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6, 163.5, 161.1, 135.0, 131.8, 130.0, 129.9, 127.5, 127.4, 124.5, 118.3, 115.9, 89.6, 89.5, 66.8; LC (214 nm) 3.18 min (>98%); MS (ESI) *m/z* = 310.3; HRMS = 310.1236 (calc. 310.1243), $C_{19}H_{17}N_1O_2F_1$.



5d
VU0366029

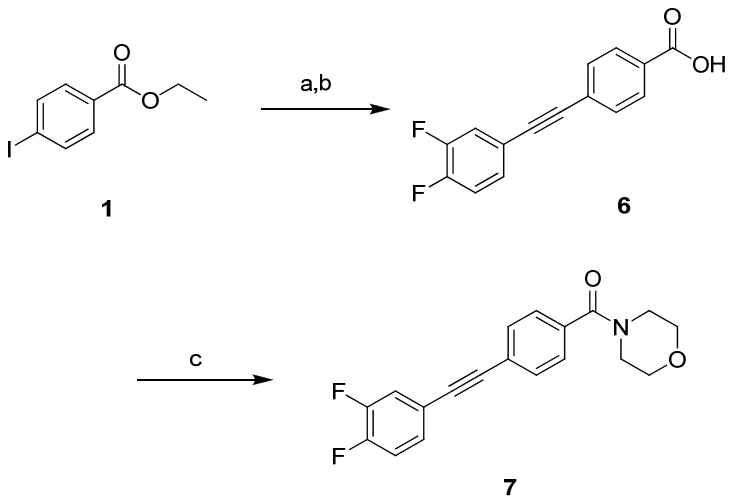
Synthesis of (3-hydroxyazetidin-1-yl)(4-(3-fluorophenyl)ethynyl)phenyl)methanone, VU0366029 (5d). Amide **5d** (2.12g, 83%) white solid as prepared in identical fashion to **3a;** mp 151.6 °C (with decomp.); ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.68-7.61 (m, 4H), 7.49-7.41 (m, 3H), 7.34-7.26 (m, 1H), 5.78 (br s, 1H), 4.52-4.43 (m, 2H), 4.27-4.23 (m, 1H), 4.09-4.00 (m, 1H), 3.82-3.76 (m, 1H); ^{13}C-NMR (100 MHz, d_6-DMSO) δ 168.4, 163.5, 161.0, 137.6, 133.8, 131.8, 131.4, 131.3, 130.0, 128.5, 128.3, 124.6, 124.3, 118.4, 118.3, 117.0, 90.1, 89.9, 63.1, 60.8, 58.9; LC (215 nm) min (>98%); MS (ESI) *m/z* = 296.1; HRMS = 296.1088 (calc. 296.1087), $C_{18}H_{15}N_1O_2F_1$.



5e
VU0366030

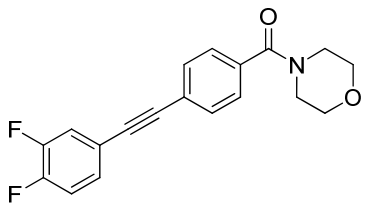
(4-((3-fluorophenyl)ethynyl)phenyl)(4-hydroxy-4-methylpiperidin-1-yl)methanone, VU0366030 (5e). Amide **5e** (120 mg, 73%) white solid as prepared in identical fashion to **3a**; mp 143.3 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.39-7.32 (m, 2H), 7.25 (dd, *J* = 9.5, 2.0 Hz, 1H), 7.11-7.05 (m, 1H), 4.37 (s, 1H), 3.57-3.48 (m, 1H), 2.81-2.42 (m, 3H), 1.74-1.52 (m, 3H), 1.32 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.9, 163.4, 135.5, 132.5, 131.7, 130.0, 129.9, 127.5, 126.9, 124.3,

118.5, 118.3, 115.9, 115.8, 112.9, 89.3, 68.0, 54.2, 39.8, 39.6, 30.3; LC (214 nm) 3.14 min (>98%); MS (ESI) *m/z* = 338.2; HRMS = 338.1556 (calc. 338.1556), $C_{21}H_{21}N_1O_2F_1$.



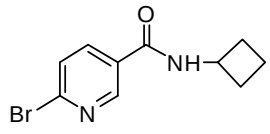
Reaction conditions: a) 3,4-difluorophenylacetylene, $Pd(Ph_3P)_4$ (5 mol%), CuI (10 mol%), DEA, DMF, 80 ^oC, 1h; b) LiOH, MeOH, THF (74%, two steps); c) Morpholine, EDC, HOBt, DIPEA, DMF (96%).

4-((3,4-fluorophenyl)ethynyl) benzoic acid (6). To a solution of ethyl 4-Iodobenzoate **1** (5.0 g, 18.1 mmol) in DMF (20 mL) was added 3,4-difluorophenylacetylene (2.97 g, 21.5 mmol), $Pd(Ph_3P)_4$ (520 mg, 0.45 mmol), CuI (173 mg, 0.91 mmol) and diethylamine (3 mL). The reaction vessel was sealed and heated at 60 °C for 1h in a microwave reactor. The reaction was cooled to room temperature, diluted with EtOAc:hexanes (2:1, 130 mL) and washed with water (2 x 100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was dissolved in THF (70 mL) added MeOH (14 mL) and a solution of LiOH (870 mg, 36.2 mmol) in water (14 mL). The reaction was stirred at room temperature and for 4h. The reaction was acidified with 1 N HCl (60 mL) and isolated benzoic acid **6** (3.45 g, 74%) as a light tan solid; ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.97 (d, *J* = 8.0 Hz, 2H), 7.76-7.70 (m, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.55-7.42 (m, 2H); ^{13}C-NMR (100 MHz, d_6-DMSO) δ 167.0, 151.7, 150.9, 149.2, 148.4, 132.0, 131.1, 129.9, 129.6, 126.4, 121.1, 119.5, 118.7, 90.1, 89.4; LC (214 nm) 3.35 min (>98%); MS (ESI) *m/z* = 259.1.



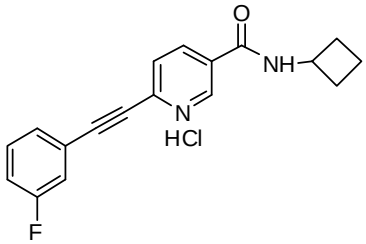
7
VU0366028

Synthesis of (4-((3,4-difluorophenyl)ethynyl)phenyl)(morpholino)methanone VU0366028, (7a). Amide **7a** (2.91 g, 96%) white solid was prepared in identical fashion to **3a;** mp 132.8 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.31-7.25 (m, 1H), 7.15 (dd, *J* = 18.0, 8.0 Hz, 1H), 4.00-3.31 (bd s, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6, 151.9, 149.4, 135.1, 131.7, 128.2, 127.2, 124.3, 120.6, 119.6, 117.5, 88.8, 88.7, 66.8; LC (215 nm) 5.10 min (>98%); MS (ESI) *m/z* = 327.9; HRMS = 328.1126 (calc. 328.1149), $C_{19}H_{16}N_1O_2F_2$.



8

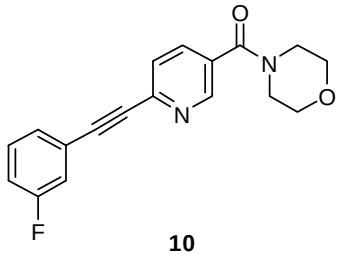
6-Bromo-*N*-cyclobutylnicotinamide (8). Commercially available 6-bromonicotinic acid (4.71 g, 23.4 mmol), cyclobutanamine (2.0 mL, 23.4 mmol) and HATU (10.6 g, 28.1 mmol) were mixed in DMF (50.0 mL) at room temperature. *N*,*N*-Diisopropylethylamine (12.2 mL, 70.3 mmol) was then added into the mixture. The reaction was allowed to stir at room temperature for 4 h. Analysis of the reaction mixture after 4 h by LC-MS indicated full conversion. The reaction was quenched with H_2O (100 mL). White precipitate was formed upon the addition of H_2O and was filtered. NMR and LC-MS indicated the white precipitate was the desired pure product **8** in 76% yield. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.71 (d, *J* = 2.4 Hz, 1H), 7.97 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 1H), 6.23 (br s, NH), 4.60 (m, 1H), 2.48 (m, 2H), 1.99 (m, 2H), 1.84 (m, 2H); LC-MS (ESI), single peak, 1.10 min, *m/z* = 255.0 ($[M+1]^+$).



9
VU0360172

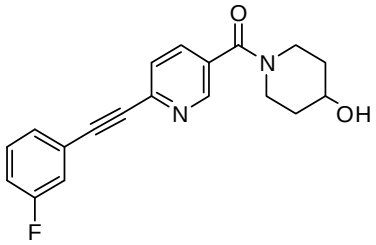
***N*-Cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide hydrochloride, VU0360172 (9).** *N*-Cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide hydrochloride (**9**) was prepared by Sonogashira coupling. 6-Bromo-*N*-cyclobutylnicotinamide (**8**) (1.33g, 5.26 mmol), 1-ethynyl-3-fluorobenzene (0.61 mL, 5.26 mmol), $Pd(PPh_3)_4$ (0.30 g, 0.26 mmol), CuI (0.10 g, 0.53 mmol), and diethylamine (1.64 mL, 15.8 mmol) were mixed in 13.0 mL of DMF in a sealed 10 – 20 mL microwave tube. The reaction was subjected to microwave irradiation at 80 ^{o}C for 1 h. Analysis of the reaction by LC-MS indicated 93% conversion. The crude reaction was diluted by EtOAc (60.0 mL), and the resulting mixture was passed through a celite pad. The filtrate was extracted with H_2O (40.0 mL x

3), and the organic layer was separated and washed with brine (50 mL). The volatile component was removed under reduced pressure to give the crude residue. The crude mixture was purified by column chromatography and afforded product *N*-cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide as an orange color powder in 77% yield.
Pure product (*N*-cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide (1.19 g, 4.05 mmol) was dissolved in 1,4-dioxane (15.0 mL), and HCl (4.0 mL, 4.0 M in 1,4-dioxane) was added to the mixture dropwisely. Off-white precipitate formed upon the addition of HCl. The mixture was allowed to stir at room temperature for 1 h, and filtered. The filtrand was the desired product VU0360172, **9** as an off-white powder. ^{1}H NMR (400MHz, DMSO): δ 9.04 (d, *J* = 1.2 Hz, 1H), 8.93 (d, *J* = 7.8 Hz, 1H), 8.27 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.57 – 7.49 (overlapped, 3H), 7.37 (m, 1H), 4.43 (m, 1H), 2.25 (m, 2H), 2.11 (m, 2H), 1.70 (m, 2H); ^{13}C NMR (100MHz, DMSO): δ163.08, 162.22 (d, *J* = 244.0 Hz, 1C), 148.82, 143.31, 136.89, 131.52 (d, *J* = 9.0 Hz, 1C), 129.72, 128.67 (d, *J* = 3.0 Hz, 1C), 127.68, 123.33 (d, *J* = 9.0 Hz, 1C), 120.35, 118.82 (d, *J* = 23.0 Hz, 1C), 117.64 (d, *J* = 21.0 Hz, 1C), 90.08 (d, *J* = 3.0 Hz, 1C), 89.01, 45.07, 30.38 (2C), 15.18; LC-MS (ESI), single peak, 1.35 min, *m/z* = 295.10 ([M+1]$^{+}$); HRMS = 295.1248 ([M+1]$^{+}$, 100%) calcd for $C_{18}H_{16}N_2OF$, 295.1247.



10
VU0360175

(6-((3-Fluorophenyl)ethynyl)pyridin-3-yl)(morpholino)methanone, VU0360175, (10). Amide **10** was prepared in similar fashion to **9;** ^{1}H NMR (400MHz, $CDCl_3$): δ 8.70 (d, *J* = 1.2 Hz, 1H), 7.82 (dd, *J* = 8.0, 2.6 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 6.6, 6.6 Hz, 1H), 7.37 (dd, *J*= 8.0, 2.0 Hz, 1H), 7.34 (m, 1H), 7.14 (m, 1H), 3.79 (br m, 6H), 3.52 (br m, 2H); LC-MS (ESI), single peak, 1.25 min, *m/z* = 311.1 ([M+1]$^{+}$); HRMS = 311.1184 ([M+1]$^{+}$, 100%), calcd for $C_{18}H_{16}FN_2O_2$, 311.1196.

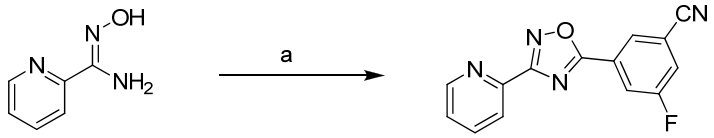


11
VU0361747

(6-((3-Fluorophenyl)ethynyl)pyridin-3-yl)(4-hydroxypiperidin-1-yl)methanone, VU0361747 (11). Amide **11** was prepared in similar fashion to **9;** ^{1}H NMR (400MHz, $CDCl_3$): δ 8.70 (d, *J* = 2.0 Hz, 1H), 7.81 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.41 (dd, *J* = 8.2, 8.2 Hz, 1H), 7.37 (dd, *J*= 8.0, 2.4 Hz, 1H), 7.34 (m, 1H), 7.13 (m, 1H),

4.15 (br m, 1H), 4.06 (m, 1H), 3.68 (br m, 1H), 3.53 (br m, 1H), 3.32 (br m, 1H), 2.03 – 1.90 (overlapped, 4H); LC-MS (ESI), single peak, 1.18 min, *m/z* = 325.1 ($[M+1]^+$); HRMS = 325.1351 ($[M+1]^+$, 100%), calcd for $C_{19}H_{18}FN_2O_2$, 325.1352.

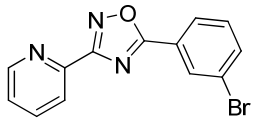
Standard Experimental Procedures for mGluR5 NAMs:



12a-f
VU0285683

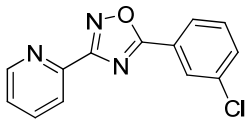
Reaction conditions: a) i) 3-cyano-5-fluorobenzoic acid, EDC, HOBt, 1,4-dioxane, rt, 6h, ii) reflux, 18 h (65%).

Synthesis of 3-fluoro-5-(3-(pyridine-2-yl)-1,2,4-oxadiazol-5-yl)benzonitrile, VU0285683 (12a). To a mixture of 2-pyridylamidoxime (2.00 g, 14.6 mmol) in 1,4-dioxane (100 mL) was added 3-cyano-5-fluorobenzoic acid (2.40 g, 14.6 mmol), EDC (4.50 g, 23.3 mmol) and HOBt (2.00 g, 14.6 mmol). The reaction mixture was stirred at room temperature for 6h, then at reflux for 18 h. The reaction mixture was cool, diluted with water (250 mL) and extracted with EtOAc (3 x 150 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was recrystallized from EtOAc/hexanes to afford **12a** as a yellow solid (2.53 g, 65%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 5 Hz, 1H), 8.43 (s, 1H), 8.29-8.24 (m, 2H), 7.94 (td, *J* = 8.0, 1.5 Hz, 1H), 7.65 (ddd, *J* = 8.0, 2.5, 1.5 Hz, 1H), 7.52 (ddd, *J* = 8.0, 5.0, 1.5 Hz, 1H); LC-MS (214 nm) 2.91 min (>98%); MS (ESI) *m/z* = 267.1; HRMS = 267.0679 (calc. 267.0682), $C_{14}H_8N_4O_1F_1$.



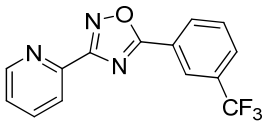
12b
VU0255037

Synthesis of 5-(3-bromophenyl)-3-(pyridine-2-yl)-1,2,4-oxadiazole, VU0255037 (12b). Oxadiazole **12b** (120 mg, 96%) white solid as prepared in identical fashion to **12a;** ^{1}H-NMR (400 MHz, *d*$_4$-MeOH) δ 8.76 (d, *J* = 5.0 Hz, 1H), 8.43 (t, *J* = 1.5 Hz, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.25 (dt, *J* = 8.0, 1.5 Hz, 1H), 8.08 (td, *J* = 8.0, 1.5 Hz, 1H), 7.89 (ddd, *J* = 8.0, 1.5, 1.0 Hz, 1H), 7.64 (ddd, *J* = 8.0, 5.0, 1.0 Hz, 1H), 7.58 (t, *J* = 8.0 Hz, 1H); LC-MS (214 nm) 1.54 min (>98%); MS (ESI) *m/z* = 302.0 and 304.0; HRMS = 301.9925 (calc. 301.9929), $C_{13}H_9N_3O_1Br$.



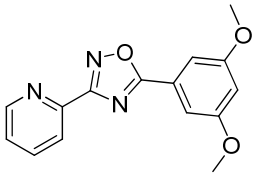
12c
VU0067144

Synthesis of 5-(3-chlorophenyl)-3-(pyridine-2-yl)-1,2,4-oxadiazole, VU0067144 (10c). Oxadiazole **12c** (96 mg, 91%) white solid as prepared in identical fashion to **12a;** ^{1}H-NMR (400 MHz, d_4-MeOH) δ 8.77 (d, *J* = 5.0 Hz, 1H), 8.32-8.26 (m, 2H), 8.21 (dt, *J* = 8.0, 1.5 Hz, 1H), 8.08 (td, *J* = 8.0, 1.5 Hz, 1H), 7.74 (ddd, *J* = 8.0, 1.5, 1.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.64-7.62 (m, 1H); LC-MS (214 nm) 1.51 min (>98%); MS (ESI) *m/z* = 258.0; HRMS = 258.0436 (calc. 258.0434), $C_{13}H_9N_3O_1Cl$.



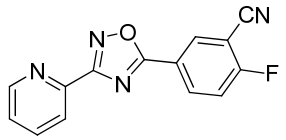
12d
VU0255038

Synthesis of 3-(pyridine-2-yl)-5-(3-(trifluoromethyl)phenyl)-1,2,4-oxadiazole, VU0255038 (12d). Oxadiazole **12d** (102 mg, 93%) white solid as prepared in identical fashion to **12a;** ^{1}H-NMR (400 MHz, d_4-MeOH) δ 8.77 (d, *J* = 5.0 Hz, 1H), 8.56 (s, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.08 (td, *J* = 8.0, 1.5 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 7.64 (ddd, *J* = 8.0, 5.0, 1.0 Hz, 1H); LC-MS (214 nm) 1.49 min (>98%); MS (ESI) *m/z* = 292.0; HRMS = 292.0697 (calc. 292.0698), $C_{14}H_9N_3O_1F_3$.



12e
VU00040228

Synthesis of 5-(3,5-dimethoxyphenyl)-3-(pyridine-2-yl)-1,2,4-oxadiazole, VU00040228 (12e). Oxadiazole **12e** (98 mg, 89%) white solid as prepared in identical fashion to **12a;** ^{1}H-NMR (400 MHz, d_4-MeOH) δ 8.77 (dd, *J* = 5.0, 1.0 Hz, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.07 (td, *J* = 8.0, 1.5 Hz, 1H), 7.64 (ddd, *J* = 8.0, 5.0, 1.0 Hz, 1H), 7.41 (d, *J* = 2.5 Hz, 1H), 6.82 (t, *J* = 2.5 Hz, 1H), 3.92 (s, 6H); LC-MS (214 nm) 1.45 min (>98%); MS (ESI) *m/z* = 284.1; HRMS = 284.1035 (calc. 284.1035), $C_{15}H_{14}N_3O_3$.



12f
VU0255036

Synthesis of 2-fluoro-5-(3-(pyridin-2-yl)-1,2,4-oxadiazol-5-yl)benzonitrile, VU0255036 (12f). Oxadiazole **12f** (46 mg, 78%) white solid as prepared in identical fashion to **12a;** ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.88 (ddd, *J* = 4.8, 0.8, 0.4 Hz, 1H), 8.64 (dd, *J* = 6.0, 2.0 Hz, 1H), 8.57 (m,1H), 8.24 (d, *J* = 7.8 Hz, 1H), 7.93 (td, *J* = 7.8, 2.0 Hz, 1H), 7.52 (ddd, *J* = 7.8, 4.8, 1.2 Hz, 1H), 7.48 (t, *J* = 8.5 Hz, 1H); LC-MS (214 nm) 1.36 min (>98%); MS (ESI) *m/z* = 267.0; HRMS 289.0503 (calc. 289.0502), $C_{14}H_7N_4OFNa$.

Figure S1. VU0040228 Selectivity

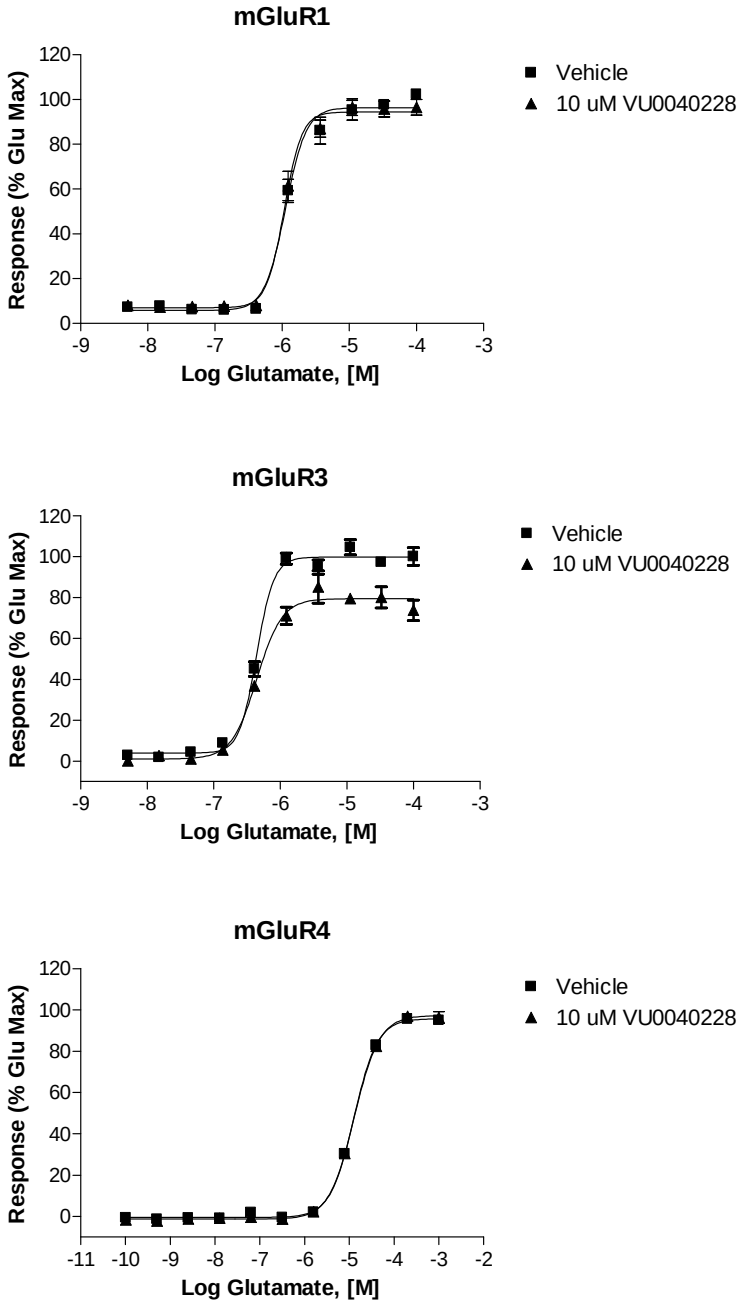


Figure S1. VU0040228 is selective for mGluR5 versus other mGluR subtypes. Agonist concentration response relationship of mGluRs1, 3, and 4 in the presence and absence of 10 μM VU0040228 was measured.

Figure S2. VU0092273 Selectivity

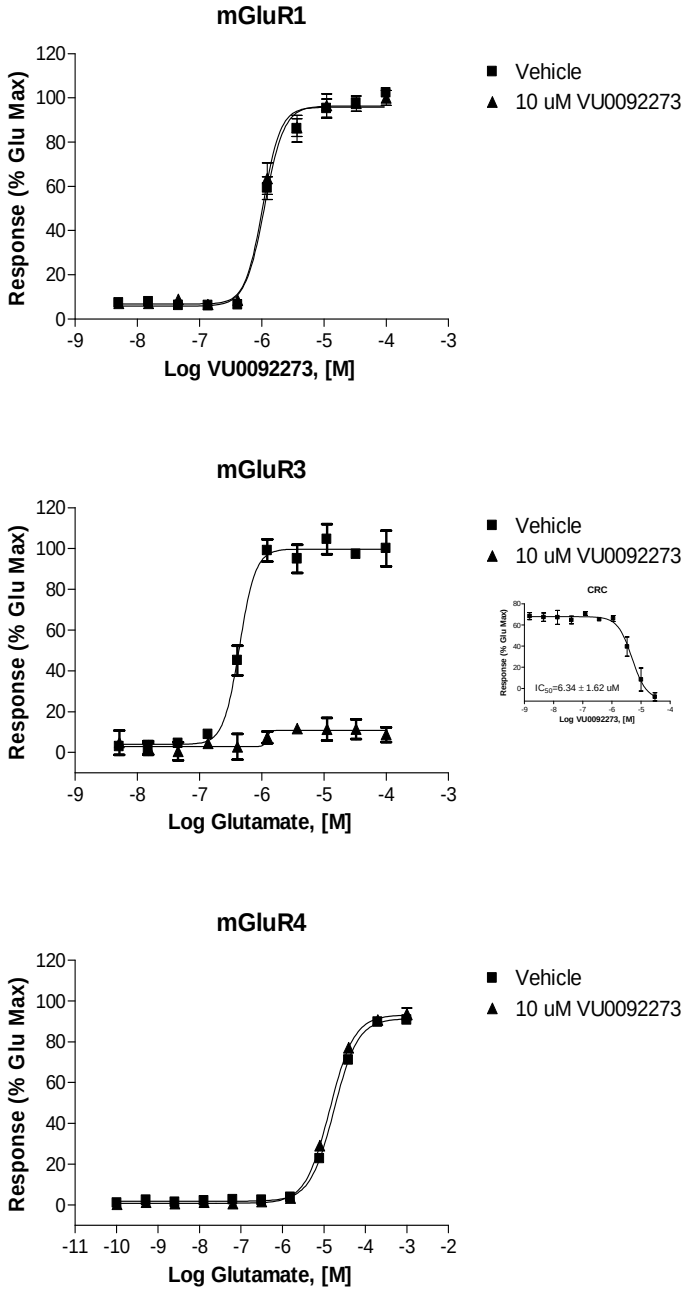


Figure S2. VU0092273 selectivity profile. Agonist concentration response relationship of mGluRs1, 3, and 4 in the presence and absence of 10 μM VU0092273 was measured.

Figure S3. VU0285683 Selectivity

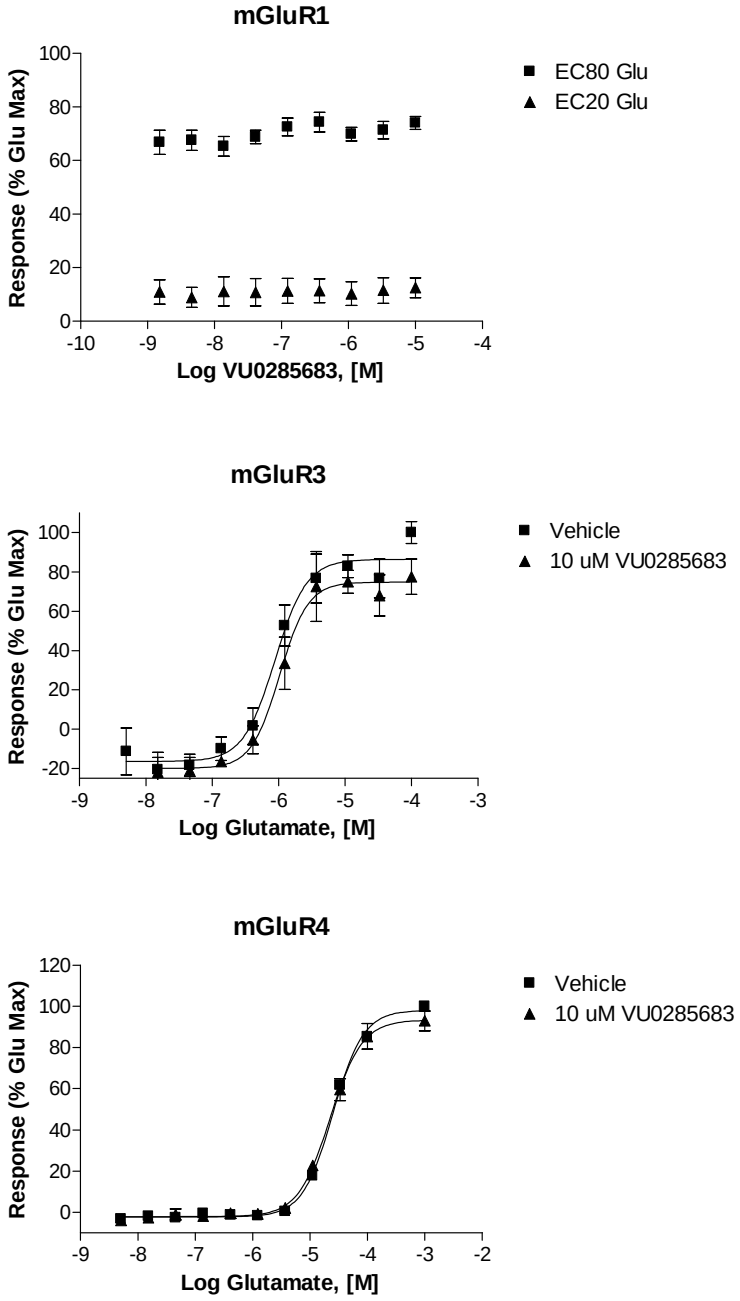


Figure S3. VU0285683 is selective for mGluR5 versus other mGluR subtypes. Effect of 10 μM VU0285683 on EC20 and EC80 agonist (top panel), or agonist concentration response relationship (bottom panels) of mGluRs1, 3, and 4 was measured.

Figure S4. VU0360172 Selectivity

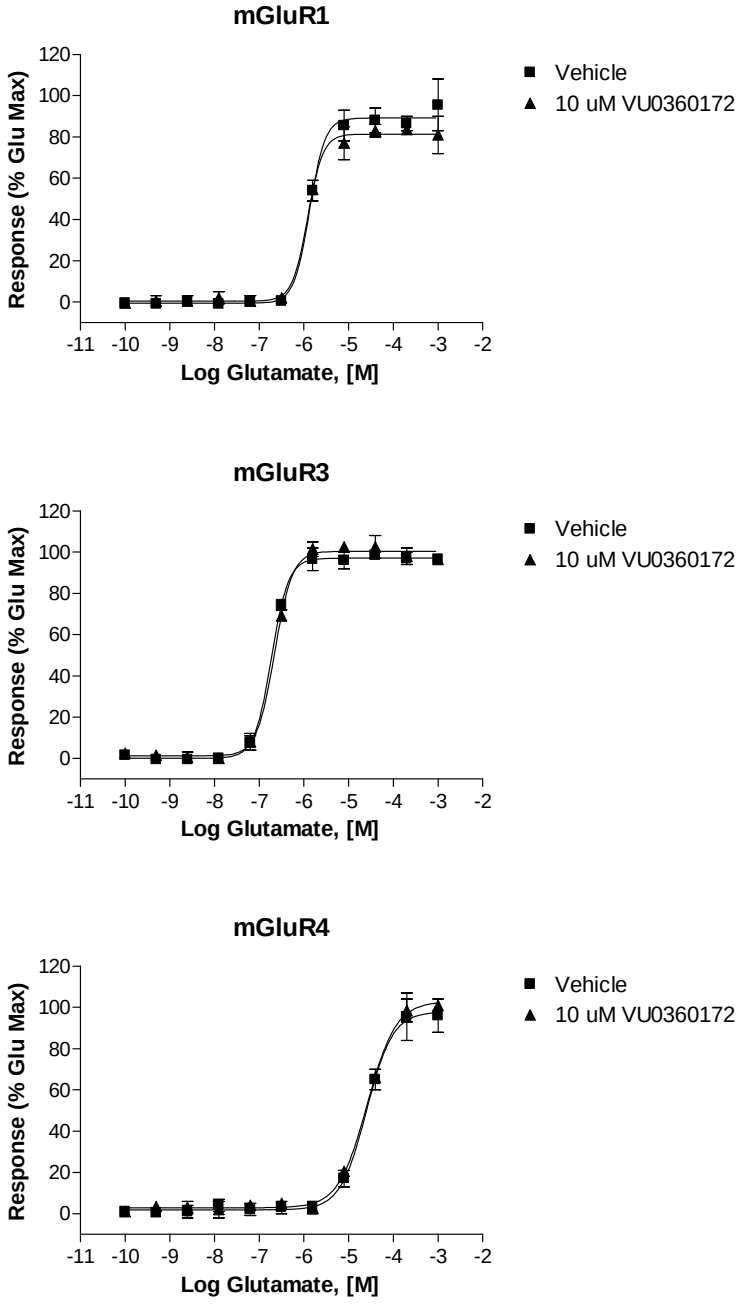


Figure S4. VU0360172 is selective for mGluR5 versus other mGluR subtypes. Agonist concentration response relationship of mGluRs1, 3, and 4 in the presence and absence of 10 μM VU0360172 was measured.

Figure S5. VU0285683 competes with [^{3}H]methoxyPEPy

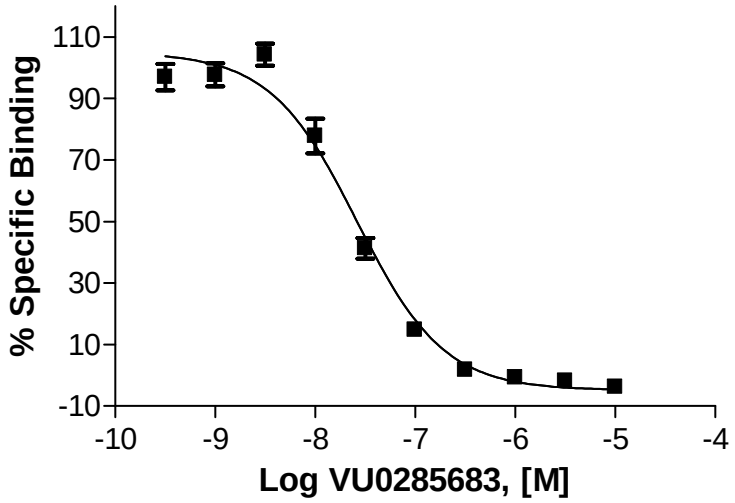


Figure S5. Antagonist VU0285683 fully competes with the equilibrium of [^{3}H]methoxyPEPy (K_i = 16.9 ± 1.1). Competition binding curves for allosteric modulators were obtained in the presence of 2 nM [^{3}H]methoxyPEPy using membranes harvested from mGluR5-expressing HEK293 cells.

Figure S6. In vivo pharmacokinetic profile of VU0360172

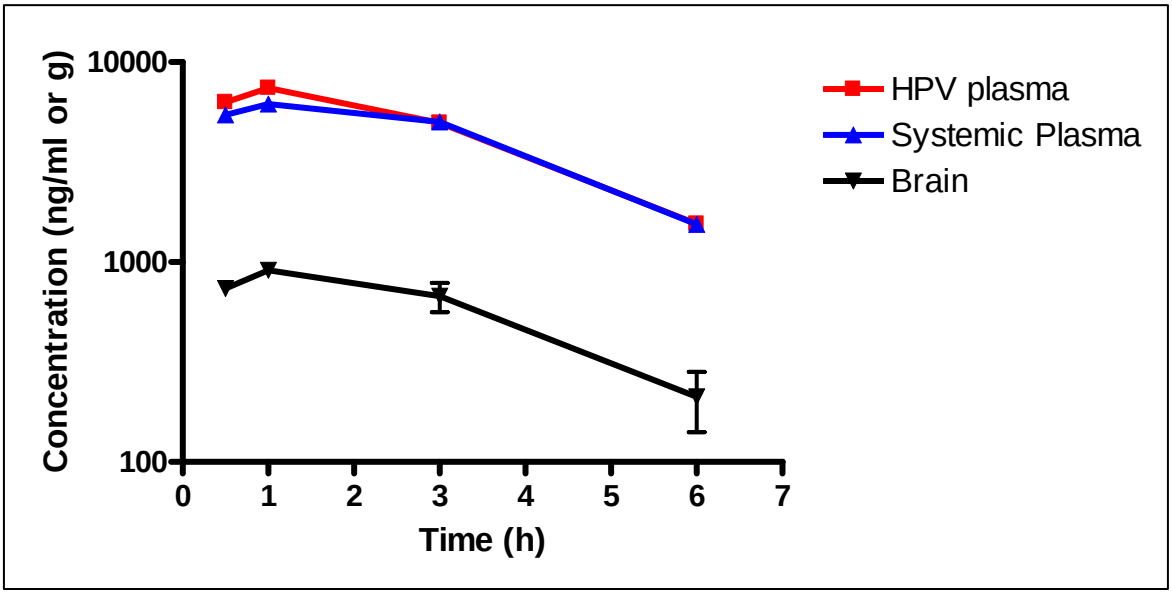


Figure S6. In vivo pharmacokinetics of VU0360172 was studied in male SD rats after oral administration of 10 mg/kg dose in 20% hydroxypropyl β-cyclodextrin (BCD) solution. At times 0.5h, 1h, 3h and 6h after dosing, the concentrations of VU0360172 were measured in hepatic portal vein (HPV) plasma, systemic plasma (cardiac puncture), and whole brain tissues.