Supporting Information

Solution-Phase Parallel Synthesis and SAR of Homopiperazinyl Analogs as Positive Allosteric Modulators of $mGlu_4$

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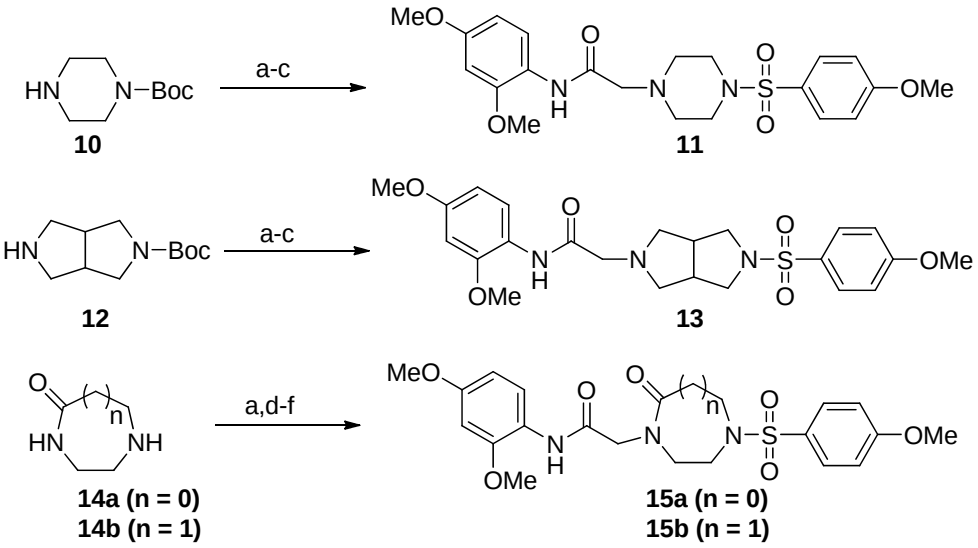
General Procedures. All NMR spectra were recorded on a Varian Inova 400 (400 MHz) spectrophotometer. ^{1}H chemical shifts are reported in δ values in ppm in either $CDCl_3$ or DMSO-d_6 solvent . Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br =broad, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an HP1100 MSD with electrospray ionization. High resolution mass spectra were recorded on a Bruker Daltonics 3T Fourier transform ion cyclotron resonance mass spectrometer (FT/ICR) with electrospray ionization. Analytical thin layer chromatography was performed on EM Reagent 0.25 mm silica gel 60-F plates. Analytical HPLC was performed on either an HP1100 with UV detection at 214 and 254 nm along with ELSD detection, LC/MS (J-Sphere80-C18, 3.0 x 50 mm, 4.1 min gradient, 5%[0.05%TFA/CH_3CN]: 95%[0.05%TFA/H_2O] to 100%[0.05%TFA/CH_3CN] or HP1100 with UV detection at 220 and 254 nm along with ELSD detection, LC/MS (Phenomenex Luna C18, 2.1 x 50 mm, 2.6□, 1.6 min gradient, 5% to 95% CH_3CN/(0.1% TFA/H_2O). Preparative purification was performed on either a custom HP1100 purification system with collection triggered by mass detection or on a Phenomenex Luna C18(2), 30 x 50 mm, 5μ, 3.5 min gradient, 10% to 90% CH_3CN/(0.1% TFA/H_2O), UV detection at 220 nm. Purities of compounds were in all cases greater than 95%, as determined by reverse phase HPLC analysis. Compounds purified by reverse phase preparative HPLC or mass directed HPLC were isolated as TFA salts. Preparative purification was performed on a custom HP1100 purification system (reference 16) with collection triggered by mass detection. All chemical reactions and isolations were optimized for compound purity (>95%), not for chemical yield. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification.

Scheme 1



Reagent and Conditions (a) 4-Methoxybenzenesulfonyl chloride, CH_2Cl_2, $EtNiPr_2$ (43% -quant.); (b) 4M HCl dioxane (quant.); (c) 22 or Methyl bromoacetate, Cs_2CO_3, CH_3CN 80 °C (45-50%); (d) LiOH, THF, MeOH, H_2O (35%-quant.); (e) NaH, DMF, methyl bromoacetate; (f) R_1R_2NH, HATU, DMF, $EtNiPr_2$ (19-60%).

Synthesis of Compound 11

Step a: ***tert*-Butyl 4-(4-methoxyphenylsulfonyl)piperazine-1-carboxylate**: To a stirred solution of Boc-piperazine (1 g, 5.37 mmol) in methylene chloride (27 mL) and triethylamine (1.40 mL, 8.05 mmol) was added 4-methoxybenzene sulfonyl chloride (1.05 g, 5.10 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with methylene chloride (30 mL), washed with 2N HCl (30 mL), saturated sodium bicarbonate (2× 30 mL), dried ($MgSO_4$), filtered and concentrated under vacuum to give the product (1.41 g, 78%). LCMS: >98% @ 214 nm, R_T = 2.55 min., *m/z* 379.1 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (2 H, d, *J* = 8.9 Hz), 7.02 (2 H, d, *J* = 8.9 Hz), 3.90 (3 H, s), 3.52 (4 H, t, *J* = 5.0 Hz), 2.97 (4 H, t, *J* = 5.1 Hz), 1.43 (9 H, s).

Step b: **1-(4-Methoxyphenylsulfonyl)piperazine**: To a stirred solution of *tert*-butyl 4-(4-methoxyphenylsulfonyl)piperazine-1-carboxylate (1.41 g, 3.96 mmol) in methanol (25 mL) was added 4M HCl in dioxane (20 mL, 79.1 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in water (40 mL), adjusted to pH 9 and extracted with chloroform/isopropanol (3:1) (3× 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under

vacuum to give the product (1.23 g, quant.). LCMS: >98% @ 214 nm, R_T = 1.27 min., *m/z* 257.2 $[M + H]^+$.

Step c: ***N*-(2,4-Dimethoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)acetamide (11)**: To a stirred solution of 1-(4-methoxyphenylsulfonyl)piperazine (56 mg, 0.219 mmol) and cesium carbonate (214 mg, 0.656 mmol) in acetonitrile (3 mL) was added **22** (78 mg, 0.285 mmol) and the reaction mixture was heated at 80 °C overnight. The reaction mixture was cooled to room temperature, diluted with acetonitrile (15 mL), filtered and concentrated under vacuum. The residue was purified by reverse phase preparative HPLC to give the product (55 mg, 45%). LCMS: >98% @ 214 nm, R_T = 1.88 min., *m/z* 450.1 $[M + H]^+$.

Synthesis of Compound 13

Step a: ***tert*-Butyl 5-(4-methoxyphenylsulfonyl)hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate:** Following the procedure for the synthesis of compound **11** (Step a), *tert*-butyl hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate (100 mg, 0.471 mmol) was coupled with 4-methoxybenzene sulfonyl chloride (92 mg, 0.446 mmol) to give the product (156 mg, quant.). LCMS: >98% @ 214 nm, R_T = 2.42 min., *m/z* 327.1 $[M - (CH_3)_2C{=}CH_2) + H]^+$.

Step b: **2-(4-Methoxyphenylsulfonyl)octahydropyrrolo[3,4-*c*]pyrrole**: Following the procedure for the synthesis of compound **11** (Step b), *tert*-butyl 5-(4-methoxyphenylsulfonyl)hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate (156 mg, 0.408 mmol) was hydrolyzed with 4M HCl in dioxane (2 mL, 8.16 mmol) to give the product (103 mg, 90%). LCMS: >98% @ 214 nm, R_T = 1.32 min., *m/z* 283.1 $[M + H]^+$.

Step c: ***N*-(2,4-dimethoxyphenyl)-2-(5-(4-methoxyphenylsulfonyl)hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)acetamide (13):** Following the procedure for the synthesis of compound **11** (Step c), 2-(4-methoxyphenylsulfonyl)octahydropyrrolo[3,4-c]pyrrole (40 mg, 0.142 mmol) was coupled with **22** to give the product (41 mg, 49%). LCMS: >98% @ 214 nm, R_T = 1.82 min., *m/z* 476.1 $[M + H]^+$.

Synthesis of Compound 15a

Step a: **4-(4-Methoxyphenylsulfonyl)piperazin-2-one:** Following the procedure for the synthesis of compound **11** (Step a), piperazin-2-one (500 mg, 4.99 mmol) was coupled with 4-methoxybenzene sulfonyl chloride (1.13 g, 5.49 mmol) to give the product (943 mg, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (2 H, d, J = 8.9 Hz), 7.02 (2 H, d, J = 8.9 Hz), 5.93 (1 H, br. s), 3.90 (2 H, m), 3.37–3.29 (4 H, m), 2.74 (2 H, dd, J = 10.3 Hz, 5.3 Hz).

Step f: **Methyl 2-(4-(4-methoxyphenylsulfonyl)-2-oxopiperazin-1-yl)acetate:** To a stirred solution of 4-(4-methoxyphenylsulfonyl)piperazin-2-one (100 mg, 0.370 mmol) in DMF (2 mL) was added sodium hydride (60% in mineral oil, 0.555 mmol) and the mixture was stirred for 5 min and methyl bromoacetate (40.8 μL, 0.44 mmol). The reaction mixture was stirred at room temperature for 1 h and heated at 60 °C for 0.5 h. The reaction mixture was cooled to room temperature, quenched with water (2 mL) and extracted with ethyl acetate (2× 2 mL). The combined organic extracts were washed with brine (2× 2 mL), dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel eluting with 0 to 70% ethyl acetate/hexanes to give the product (58 mg, 48%). LCMS: >98% @ 214 nm, R_T = 1.75 min., *m/z* 343.1 $[M + H]^+$.

Step d: **2-(4-(4-Methoxyphenylsulfonyl)-2-oxopiperazin-1-yl)acetic acid:** To a stirred solution of methyl 2-(4-(4-methoxyphenylsulfonyl)-2-oxopiperazin-1-yl)acetate (58 mg, 0.169 mmol) in tetrahydrofuran (0.56 mL), methanol (0.14 mL), water (0.14 mL) was added lithium hydroxide (20 mg, 0.847 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in water (10 mL), adjusted to pH1 with 2N HCl and extracted with chloroform/isopropanol (3:1) (5× 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under vacuum to give the product which was used without further purification (19 mg, 34%). LCMS: 49% @ 214 nm, R_T = 1.52 min., *m/z* 329.0 $[M + H]^+$.

Step e: ***N*-(2,4-Dimethoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)-2-oxopiperazin-1-yl)acetamide (15a):** To a stirred solution of 2-(4-(4-methoxyphenylsulfonyl)-2-oxopiperazin-1-yl)acetic acid (17 mg, 0.052 mmol), HATU (20 mg, 0.052 mmol), diisopropylethylamine (13.5 μL, 0.078 mmol) in DMF (0.5 mL) was added 2,4-dimethoxyaniline (7.4 μL, 0.052 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (10 mL), brine (2× 10 mL), dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by reverse phase preparative

HPLC to give the product (4.6 mg, 19%). LCMS: >98% @ 214 nm, R_T = 2.10 min., *m/z* 464.1 $[M + H]^+$.

Synthesis of Compound 15b

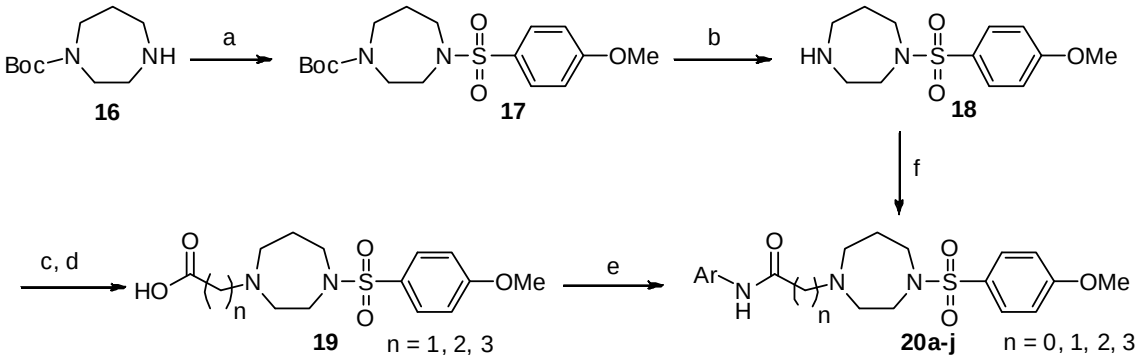
Step a: **1-(4-Methoxyphenylsulfonyl)-1,4-diazepan-5-one:** Following the procedure for compound **15a** (Step a), [1,4]-diazepan-5-one (500 mg, 4.38 mmol) was coupled with 4-methoxybenzene sulfonyl chloride (996 mg, 4.82 mmol) to give the product (531 mg, 43%). LCMS: >98% @ 214 nm, R_T = 1.55 min., *m/z* 285.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (2 H, d, *J* = 8.9 Hz), 7.02 (2 H, d, *J* = 8.9 Hz), 5.93 (1 H, br. s), 3.90 (2 H, m), 3.37–3.29 (4 H, m), 2.74 (2 H, dd, *J* = 10.3 Hz, 5.3 Hz).

Step f: **Methyl 2-(4-(4-methoxyphenylsulfonyl)-7-oxo-1,4-diazepan-1-yl)acetate:** Following the procedure for compound **15a** (Step f), 1-(4-methoxyphenylsulfonyl)-1,4-diazepan-5-one (100 mg, 0.352 mmol) was coupled with methyl bromoacetate (42 μL, 0.458 mmol) to give the product (41 mg, 33%). LCMS: >98% @ 214 nm, R_T = 1.76 min., *m/z* 357.1 $[M + H]^+$.

Step d: **2-(4-(4-Methoxyphenylsulfonyl)-7-oxo-1,4-diazepan-1-yl)acetic acid:** Following the procedure for compound **15a** (Step d), methyl 2-(4-(4-methoxyphenylsulfonyl)-7-oxo-1,4-diazepan-1-yl)acetate (41 mg, 0.115 mmol), was hydrolyzed with lithium hydroxide (13.8 mg, 0.58 mmol) to give the product (39 mg, quant.). LCMS: >98% @ 214 nm, R_T = 1.55 min., *m/z* 343.1 $[M + H]^+$.

Step e: ***N*-(2,4-Dimethoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)-7-oxo-1,4-diazepan-1-yl)acetamide (15b):** Following the procedure for compound **15a** (Step e), 2-(4-(4-methoxyphenylsulfonyl)-7-oxo-1,4-diazepan-1-yl)acetic acid (39 mg, 0.115 mmol) was coupled with 2,4-dimethoxyaniline (20 μL, 0.138 mmol) to give the product (41 mg, 60%). LCMS: >98% @ 214 nm, R_T = 2.10 min., *m/z* 478.1 $[M + H]^+$.

Scheme 2



Reagent and Conditions (a) 4-Methoxybenzenesulfonyl chloride, CH_2Cl_2, $EtNiPr_2$ (98%); (b) 4M HCl dioxane (quant.); (c) $Br(CH_2)_nCO_2Me$, Cs_2CO_3, CH_3CN 80 °C (40-76%); (d) LiOH, THF, MeOH, H_2O (quant.); (e) R_1R_2NH, HATU, DMF, $EtNiPr_2$ (2-80%); (f) 2,4-dimethoxyphenyl isocyanate, CH_2Cl_2, $EtNiPr_2$ (80%)

Synthesis of Compound 9

***tert*-Butyl 4-(4-methoxyphenylsulfonyl)-1,4-diazepane-1-carboxylate (17):** To a stirred solution of Boc-homopiperazine (2.14 g, 10.6 mmol) in methylene chloride (53 mL) and triethyl amine (2.76 mL; 15.9 mmol) was added 4-methoxybenzene sulfonyl chloride (2.0 g, 9.68 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with methylene chloride (30 mL), washed with 2N HCl (30 mL), saturated sodium bicarbonate (2× 30 mL), dried ($MgSO_4$), filtered and concentrated under vacuum to give the product, 17, (3.47g, 97%). LCMS: >98% @ 214 nm, R_T = 3.17 min., *m/z* 393.2 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (2 H, d, *J* = 8.7 Hz), 6.99 (2 H, d, *J* = 8.5 Hz), 3.88 (3 H, s), 3.61–3.42 (4 H, m), 3.36–3.17 (4 H, m), 1.93 (2 H, t, *J* = 6.1 Hz), 1.44 (9 H, s).

1-(4-Methoxyphenylsulfonyl)-1,4-diazepane (18): To a stirred solution of *tert*-butyl 4-(4-methoxyphenylsulfonyl)-1,4-diazepane-1-carboxylate (3.47 g, 9.37 mmol) in methanol (59 mL) was added 4 M HCl in dioxane (24 mL, 96 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in water (40 mL), adjusted to pH 9 and extracted with chloroform/isopropanol (3:1) (3× 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under vacuum to give the product, **18**, as a pale yellow oil (2.53 g, quant.). LCMS: >98% @ 214 nm, R_T = 1.85 min., *m/z* 271.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (2 H, d, *J* = 8.9 Hz),

6.99 (2 H, d, *J* = 8.9 Hz), 3.89 (3 H, s), 3.72 (1 H, s), 3.37 (2 H, t, *J* = 6.1 Hz), 3.35–3.30 (2 H, m), 3.00–2.96 (2 H, m), 2.94 (1 H, t, *J* = 5.8 Hz), 1.87–1.78 (2 H, m).

Step c: **Methyl 2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetate:** To a stirred solution of 1-(4-methoxyphenylsulfonyl)-1,4-diazepane (2.53 g, 9.36 mmol) and cesium carbonate (9.15 g; 28.0 mmol) in acetonitrile (128 mL) was added methyl bromoacetate (1.72 mL, 18.7 mmol) and the reaction mixture was heated at 80 °C overnight. The reaction mixture was cooled to room temperature, diluted with acetonitrile (15 mL), filtered and concentrated under vacuum to give methyl 2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetate as a yellow oil (2.44 g, 76%). LCMS: >98% @ 214 nm, R_T = 1.94 min., *m/z* 343.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (2 H, d, *J* = 8.8 Hz), 6.99 (2 H, d, *J* = 8.9 Hz), 3.89 (3 H, s), 3.72 (3 H, s), 3.43–3.33 (6 H, m), 2.91–2.85 (2 H, m), 2.82 (2 H, t, *J* = 5.6 Hz), 1.93–1.82 (2 H, m).

Step d: **2-(4-(4-Methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetic acid (19):** To a stirred solution of methyl 2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetate (2.44 g, 7.13 mmol) in THF (24 mL), MeOH (5.9 mL), water (5.9 mL) was added lithium hydroxide (853 mg, 35.6 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in water (10 mL), adjusted to pH1 with 2N HCl and extracted with chloroform/isopropanol (3:1) (5× 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under vacuum to give the product, **19**, as an off-white solid (2.43 g, quant.). LCMS: >98% @ 214 nm, R_T = 1.85 min., *m/z* 329.2 $[M + H]^+$. ^1H NMR 400 MHz, DMSO-d_6 δ 7.75 (2 H, d, *J* = 8.9 Hz), 7.16 (2 H, d, *J* = 8.9 Hz), 4.12 (2 H, s), 3.86 (3 H, s), 3.71–3.11 (11 H, m).

Step e: ***N*-(2,4-Dimethoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (9):** To a stirred solution of 2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetic acid, **19**, (70 mg, 0.213 mmol), HATU (81.9 mg, 0.213 mmol), diisopropylethylamine (55.3 μL, 0.320 mmol) in DMF (2.1 mL) was added 2,4-dimethoxyaniline (33.4 μL, 0.243 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (10 mL), brine (2× 10 mL), dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by reverse phase preparative HPLC to give the product (61 mg, 62%). LCMS: >98% @ 214 nm, R_T = 2.33 min., *m/z* 464.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 9.45 (1 H, br. s), 8.25 (1 H, d, *J* = 9.5 Hz), 7.76 (2 H, d, *J* = 8.9 Hz), 7.01 (2 H, d, *J* = 8.9 Hz), 6.49 (1 H, m), 6.49 (1 H, s), 3.90 (3 H, s),

3.81 (6 H, 2s), 3.45 (4 H, t, *J* = 5.6 Hz), 3.27 (2 H, s), 2.84 (4 H, t, *J* = 5.2 Hz), 1.95 (2 H, m). HRMS: *m/z* calcd for $C_{22}H_{30}N_3O_6S$ 464.1855, found 464.1854.

Following the above procedure the following library of compounds were prepared in parallel:

***N*-(2-Methoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20a):** (64% yield). LCMS: >98% @ 214 nm, R_T = 2.36 min., *m/z* 434.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ8.65 (1 H, br. s), 8.20 (1 H, d, *J* = 8.0 Hz), 7.73 (2 H, d, *J* = 8.9 Hz), 7.14 (1 H, t, *J* = 8.0 Hz), 7.02 (2 H, d, *J* = 8.9 Hz), 6.97 (1 H, t, *J* = 7.8 Hz), 6.91 (1 H, d, *J* = 8.2 Hz), 4.11 (2 H, s), 3.90 (3 H, s), 3.87 (3 H, s), 3.68 (2 H, t, *J* = 5.1 Hz), 3.64 (4 H, br. s), 3.43 (2 H, br. s), 2.34–2.34 (2 H, m). HRMS: *m/z* calcd for $C_{21}H_{28}N_3O_5S$ 434.175, found 434.175.

***N*-(4-Methoxyphenyl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20b):** (20% yield). LCMS: >98% @ 214 nm, R_T = 2.32 min., *m/z* 434.2 $[M + H]^+$.

***N*-(2,4-Difluorophenyl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20c):** (3% yield). LCMS: >98% @ 214 nm, R_T = 2.35 min., *m/z* 440.2 $[M + H]^+$.

***N*-(2-Fluorophenyl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20d):** (2% yield). LCMS: >98% @ 214 nm, R_T = 2.28 min., *m/z* 422.2 $[M + H]^+$.

2-(4-(4-Methoxyphenylsulfonyl)-1,4-diazepan-1-yl)-*N*-(pyridin-4-yl)acetamide (20e): (2% yield). LCMS: >98% @ 214 nm, R_T = 1.84 min., *m/z* 329.2 $[M + H]^+$.

***N*-Cyclohexyl-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20f):** (12% yield). LCMS: >98% @ 214 nm, R_T = 2.35 min., *m/z* 410.3 $[M + H]^+$.

***N*-Isopropyl-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20g):** (19% yield). LCMS: >98% @ 214 nm, R_T = 2.07 min., *m/z* 370.2 $[M + H]^+$.

***N*-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-2-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)acetamide (20h):** (12% yield). LCMS: >98% @ 214 nm, R_T = 2.31 min., *m/z* 462.2 $[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-4-(4-methoxyphenylsulfonyl)-1,4-diazepane-1-carboxamide (20i):** To a stirred solution of 1-(4-methoxyphenylsulfonyl)-1,4-diazepane, **18** (30 mg, 0.111 mmol) in methylene chloride (0.6 mL) was added triethylamine (29 μL, 0.166 mmol) followed by 2,4-dimethoxyphenylisocyanate (21.8 mg, 0.122 mmol) and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with methylene chloride (3 mL), with saturated sodium bicarbonate (1 mL) and filtered through phase separator cartridge. The filtrate was concentrated and the residue was purified by reverse phase preparative HPLC to give the product (40 mg, 80%). LCMS: >98% @ 214 nm, R_T = 2.24 min., *m/z* 450.1 $[M + H]^+$.

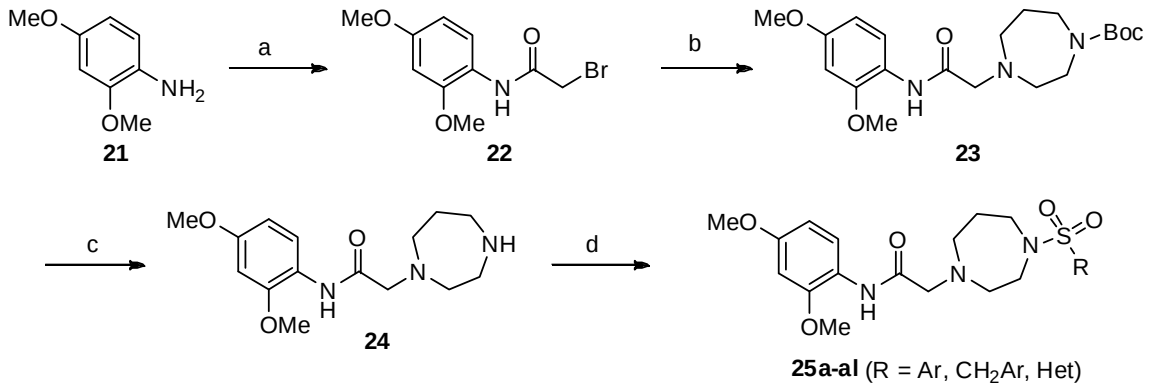
Synthesis of Compound 20j

Step c: **Methyl 4-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)butanoate:** Following the procedure for compound **11** (Step c), 1-(4-methoxyphenylsulfonyl)-1,4-diazepane (102 mg, 0.381 mmol) was coupled with methyl bromobutyrate (57.7 μL, 10.3 mmol) to give the product as a yellow oil (82 mg, 58%). LCMS: >98% @ 214 nm, R_T = 1.55 min., *m/z* 371.1 $[M + H]^+$.

Step d: **4-(4-(4-Methoxyphenylsulfonyl)-1,4-diazepan-1-yl)butanoic acid (19b):** Following the procedure for compound **15a** (Step d), methyl 4-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)butanoate, **19b** (82 mg, 0.222 mmol) was hydrolyzed with lithium hydroxide (26.5 mg, 1.11 mmol) to give the product (0.88 mg, quant.). LCMS: >98% @ 214 nm, R_T = 1.43 min., *m/z* 357.1 $[M + H]^+$.

Step e: ***N*-(2,4-Dimethoxyphenyl)-4-(4-((4-methoxyphenyl)sulfonyl)-1,4-diazepan-1-yl)butanamide (20j):** Following the procedure for compound **15a** (Step d), 4-(4-(4-methoxyphenylsulfonyl)-1,4-diazepan-1-yl)butanoic acid, **19b** (88 mg, 0.222 mmol) was coupled with 2,4-dimethoxyaniline (35 uM, 0.244 mmol) to give the product (94 mg, 70%). LCMS: >98% @ 214 nm, R_T = 1.87 min., *m/z* 492.1 $[M + H]^+$.

Scheme 3:



Reagent and Conditions (a) Bromoacetyl bromide, CH_2Cl_2, $EtNiPr_2$(81%); (b) Boc-homopiperazine, CH_3CN, Cs_2CO_3, 80 °C (79%); (c) 4M HCl dioxane (quant.); (d) RSO_2Cl, EtNi-Pr_2, CH_2Cl_2 (45-85%).

2-Bromo-*N*-(2,4-dimethoxyphenyl)acetamide (22): To a stirred solution of 2,4-dimethoxyaniline, **21**, (2 g, 13.1 mmol) in methylene chloride (60 mL) at 0 °C was added triethylamine (2.91 mL, 20.9 mmol) followed by 2-bromoacetyl bromide (1.42 mL, 16.3 mmol) and the reaction mixture was warmed to room temperature over 2 h. The reaction mixture was diluted with methylene chloride (60 mL), washed with saturated ammonium chloride (50 mL), dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel eluting with 0 to 20% ethyl acetate/hexanes to give the product (2.90 g, 81%). LCMS: >98% @ 214 nm, R_T = 2.56 min., *m/z* 276.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.60 (1 H, br. s), 8.20 (1 H, d, *J* = 7.9 Hz), 6.51 (1 H, s), 6.50 (1 H, d, *J* = 7.8 Hz), 4.04 (2 H, s), 3.91 (3 H, s), 3.83 (3 H, s).

***tert*-Butyl 4-(2-((2,4-dimethoxyphenyl)amino)-2-oxoethyl)-1,4-diazepane-1-carboxylate (23):** To a stirred solution of Boc-homopiperazine (2.95 mmol) and cesium carbonate (2.88 g, 8.84 mmol) in acetonitrile (40 mL) was added 2-bromo-*N*-(2,4-dimethoxyphenyl)acetamide, **22** (766 mg, 3.83 mmol) and the reaction mixture was heated at 80 °C overnight. The reaction mixture was cooled to room temperature, diluted with acetonitrile (15 mL), filtered and concentrated under vacuum giving the product, **23**, (0.90 g, 90%). LCMS: >98% @ 214 nm, R_T = 2.34 min., *m/z* 394.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 9.57 (1 H, br. s), 8.35–8.21 (1 H, m), 6.55–6.46 (2 H, m), 3.89 (3 H, d, *J* = 2.9 Hz), 3.82 (3 H, s), 3.66–3.37 (7 H, m), 2.97–2.70 (6 H, m), 1.99–1.86 (2 H, m), 1.86–1.72 (1 H, m), 1.49 (9 H, s).

2-(1,4-Diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)acetamide (24): To a stirred solution of *tert*-butyl 4-(2-((2,4-dimethoxyphenyl)amino)-2-oxoethyl)-1,4-diazepane-1-carboxylate, **23**, (0.90 g, 2.29 mmol) in methanol (14.5 mL) was added 4 M HCl in dioxane (11.5 mL, 45.8 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in water (40 mL), adjusted to pH 9 and extracted with chloroform/isopropanol (3:1) (3× 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under vacuum to give the product, **24**, (0.67 g, quant.). LCMS: >98% @ 214 nm, R_T = 1.59 min., *m/z* 294.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 9.67 (1 H, br. s), 8.30 (1 H, d, *J* = 9.2 Hz), 6.55–6.47 (2 H, m), 3.90 (3 H, s), 3.80 (3 H, s), 3.32 (2 H, s), 3.08 (2 H, t, *J* = 6.1 Hz), 3.01 (2 H, dd, *J* = 9.7 Hz, 5.4 Hz), 2.87 (2 H, t, *J* = 6.1 Hz), 2.83 (2 H, dd, *J* = 5.4 Hz, 3.2 Hz), 1.93–1.83 (2 H, m).

***N*-(2,4-Dimethoxyphenyl)-2-(4-(phenylsulfonyl)-1,4-diazepan-1-yl)acetamide (25a):** To a stirred solution of 2-(1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)acetamide, **24** (50 mg, 0.170 mmol), diisopropylethylamine (44 μL, 0.256 mmol) in methylene chloride (1 mL) was added benzene sulfonyl chloride (24 μL, 0.187 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with methylene chloride (2 mL), washed with saturated sodium bicarbonate (2 mL), dried by phase separator cartridge and concentrated. The residue was purified by reverse phase preparative HPLC to give the product (75 mg, 80%). LCMS: >98% @ 214 nm, R_T = 2.30 min., *m/z* 434.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ8.60 (1 H, br. s), 8.06 (1 H, dd, *J* = 7.3 Hz, 2.1 Hz), 7.90 (1 H, d, *J* = 7.3 Hz), 7.79 (1 H, d, *J* = 1.4 Hz), 7.66 (1 H, dd, *J* = 7.4 Hz, 5.2 Hz), 7.62–7.52 (2 H, m), 6.53–6.43 (2 H, m), 4.03 (2 H, s), 3.84 (3 H, s), 3.82 (3 H, s), 3.70–3.63 (4 H, m), 3.63–3.57 (2 H, m), 3.46 (2 H, br. t, *J* = 6.3 Hz), 2.36–2.25 (2 H, m). HRMS: *m/z* calcd for $C_{21}H_{28}N_3O_5S$ 434.1750, found 434.1750.

Compounds **25b-25al** were prepared following the procedure for compound **25a** (Step d).

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2-fluorophenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25b):** (3% yield). LCMS: >98% @ 214 nm, R_T = 2.36 min., *m/z* 452.2 $[M + H]^+$.

2-(4-((2-Chlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)acetamide (25c): (58% yield). LCMS: >98% @ 220 nm, R_T = 1.86 min., *m/z* 468.1 $[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2-methoxyphenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25d):** (87% yield). LCMS: >98% @ 220 nm, R_T = 1.18 min., *m/z* 464.2$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2-(trifluoromethyl)phenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25e):** (24% yield). LCMS: >98% @ 214 nm, R_T = 2.51 min., *m/z* 502.2$[M + H]^+$.

***N*-(2,4-dimethoxyphenyl)-2-(4-((3-(trifluoromethyl)phenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25f):** (6% yield). LCMS: >98% @ 214 nm, R_T = 2.60 min., *m/z* 502.2$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((4-(trifluoromethyl)phenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25g):** (36% yield). LCMS: 91.2% @ 214 nm, R_T = 2.07 min., *m/z* 502.2$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((4-(trifluoromethoxy)phenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25h):** (5% yield). LCMS: >98% @ 214 nm, R_T = 2.69 min., *m/z* 518.2$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-tosyl-1,4-diazepan-1-yl)acetamide (25i):** (77% yield). LCMS: >98% @ 214 nm, R_T = 2.42 min., *m/z* 448.2$[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (1 H, br. s), 8.06 (1 H, d, *J* = 7.6 Hz), 7.67 (2 H, d, *J* = 8.2 Hz), 7.35 (2 H, d, *J* = 8.1 Hz), 6.49 (1 H, s), 6.48 (1 H, m), 4.03 (3 H, s), 3.84 (3 H, s), 3.82 (3 H, s), 3.68–3.56 (6 H, m), 3.48–3.38 (2 H, m), 2.47 (3 H, s), 2.33–2.25 (2 H, m). HRMS: *m/z* calcd for $C_{22}H_{30}N_3O_5S$ 448.1906, found 448.1905.

***N*-(2,4-dimethoxyphenyl)-2-(4-((4-fluorophenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25j):** (77% yield). LCMS: >98% @ 214 nm, R_T = 2.36 min., *m/z* 452.2$[M + H]^+$.

2-(4-((4-Chlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)acetamide (25k): (41% yield). LCMS: >98% @ 214 nm, R_T = 1.97 min., *m/z* 482.1$[M + H]^+$.

2-(4-((4-(*tert*-Butyl)phenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25l): (32% yield). LCMS: >98% @ 214 nm, R_T = 2.22 min., *m/z* 490.2$[M + H]^+$.

2-(4-((4-Acetylphenyl)sulfonyl)-1,4-diazepan-1-yl)-N-(2,4-dimethoxyphenyl)acetamide (25m): (65% yield). LCMS: >98% @ 214 nm, R_T = 1.77 min., *m/z* 476.1$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2,4-dimethylphenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25n):** (58% yield). LCMS: >98% @ 214 nm, R_T = 2.01 min., *m/z* 462.1$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2,5-dimethylphenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25o):** (67% yield). LCMS: 93.6% @ 214 nm, R_T = 2.01 min., *m/z* 462.1$[M + H]^+$.

2-(4-((2,5-Dichlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-N-(2,4-dimethoxyphenyl)-acetamide (25p): (67% yield). LCMS: >98% @ 214 nm, R_T = 2.07 min., *m/z* 502.0$[M + H]^+$.

2-(4-((2-Chloro-6-methylphenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25q): (75% yield). LCMS: >98% @ 214 nm, R_T = 1.98 min., *m/z* 482.1$[M + H]^+$.

2-(4-((2,6-Dichlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25r): (23% yield). LCMS: >98% @ 214 nm, R_T = 1.97 min., *m/z* 502.0$[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((3,4-dimethylphenyl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25s):** (67% yield). LCMS: 94.5% @ 214 nm, R_T = 2.00 min., *m/z* 462.1$[M + H]^+$.

2-(4-((3-Chloro-4-methylphenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25t): (43% yield). LCMS: >98% @ 214 nm, R_T = 2.09 min., *m/z* 482.0$[M + H]^+$.

2-(4-((3,4-Dichlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25u): (31% yield). LCMS: >98% @ 214 nm, R_T = 2.11 min., *m/z* 502.0$[M + H]^+$.

2-(4-((3,4-difluorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25v): (64% yield). LCMS: >98% @ 214 nm, R_T = 1.75 min., *m/z* 476.1$[M + H]^+$.

2-(4-((4-Chloro-3-fluorophenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25w): (53% yield). LCMS: >98% @ 214 nm, R_T = 2.02 min., *m/z* 486.0$[M + H]^+$.

2-(4-((2,4-Dichloro-5-methylphenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl) acetamide (25x): (44% yield). LCMS: >98% @ 214 nm, R_T = 2.20 min., *m/z* 516.0$[M + H]^+$.

2-(4-((4-Chloro-2-fluoro-5-methylphenyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxy phenyl)acetamide (25y): (36% yield). LCMS: >98% @ 214 nm, R_T = 2.13 min., *m/z* 500.1[M + H]$^+$.

2-(4-(Benzylsulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)acetamide (25z): (12% yield). LCMS: >98% @ 214 nm, R_T = 2.33 min., *m/z* 448.2 [M + H]$^+$.

2-(4-((2,4-Dichlorobenzyl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25aa): (44% yield). LCMS: 90.6% @ 214 nm, R_T = 2.09 min., *m/z* 516.1 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(isopropylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ab):** (22% yield). LCMS: >98% @ 214 nm, R_T = 2.08 min., *m/z* 400.2 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(isobutylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ac):** (27% yield). LCMS: >98% @ 214 nm, R_T = 2.25 min., *m/z* 414.2 [M + H]$^+$.

***N*-(2,4-dimethoxyphenyl)-2-(4-(pyridin-2-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ad):** (36% yield). LCMS: >98% @ 214 nm, R_T = 1.59 min., *m/z* 435.1 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(thiophen-2-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ae):** (83% yield). LCMS: >98% @ 214 nm, R_T = 2.27 min., *m/z* 440.2 [M + H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (1 H, br. s), 8.06 (1 H, d, *J* = 9.3 Hz), 7.66 (1 H, d, *J* = 4.5 Hz), 7.60 (1 H, d, *J* = 3.7 Hz), 7.17 (1 H, t, *J* = 4.1 Hz), 6.50 (1 H, s), 6.48 (1 H, m), 4.05 (3 H, s), 3.84 (3 H, s), 3.82 (3 H, s), 3.72–3.59 (6 H, m), 3.50 (2 H, br. t, *J* = 6.8 Hz), 2.38–2.27 (2 H, m). HRMS: *m/z* calcd for $C_{19}H_{26}N_3O_5S_2$ 440.1314, found 440.1317.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(furan-2-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25af):** (98% yield). LCMS: >98% @ 214 nm, R_T = 1.65 min., *m/z* 424.1 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(pyridin-3-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ag):** (20% yield). LCMS: >98% @ 214 nm, R_T = 1.59 min., *m/z* 435.1 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(furan-3-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ah):** (70% yield). LCMS: >98% @ 220 nm, R_T = 0.95 min., *m/z* 424.2 [M + H]$^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-(thiophen-3-ylsulfonyl)-1,4-diazepan-1-yl)acetamide (25ai):** (79% yield). LCMS: >98.0% @ 220 nm, R_T = 0.96 min., *m/z* 440.1 $[M + H]^+$.

2-(4-((2-Acetamidothiazol-5-yl)sulfonyl)-1,4-diazepan-1-yl)-*N*-(2,4-dimethoxyphenyl)-acetamide (25aj): (60% yield). LCMS: >98.0% @ 220 nm, R_T = 0.95 min., *m/z* 498.2 $[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2,4-dimethylthiazol-5-yl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25ak):** (73% yield). LCMS: >98.0% @ 220 nm, R_T = 0.96 min., *m/z* 462.2 $[M + H]^+$.

***N*-(2,4-Dimethoxyphenyl)-2-(4-((2-methyl-4-(trifluoromethyl)thiazol-5-yl)sulfonyl)-1,4-diazepan-1-yl)acetamide (25al):** (49% yield). LCMS: >98.0% @ 220 nm, R_T = 1.95 min., *m/z* 523.2 $[M + H]^+$.

In Vitro Pharmacology:

Cell culture. Human $mGlu_4$ ($hmGlu_4$)/CHO cells stably transfected expressing the chimeric G protein Gqi5 2 in pIRESneo3 (Invitrogen, Carlsbad, CA) were cultured in 90% Dulbecco's Modified Eagle Media (DMEM), 10% dialyzed fetal bovine serum (FBS), 100 units/ml penicillin/streptomycin, 20 mM HEPES (pH 7.3), 1 mM sodium pyruvate, 2 mM glutamine, 400 μg/ml G418 sufate (Mediatech, Inc., Herndon, VA) and 5 nM methotrexate (Calbiochem, EMD Chemicals, Gibbstown, NJ). Human Embryonic Kidney (HEK-293) cell lines co-expressing rat mGluR 2, 3, 4, 7 or 8 and GIRK potassium channels3 were grown in Growth Media containing 45% DMEM, 45% F-12, 10% FBS, 20 mM HEPES, 2 mM Lglutamine, antibiotic/antimycotic non-essential amino acids, 700 μg/ml G418, and 0.6 μg/ml puromycin at 37ºC in the presence of 5% CO2. Rat mGluR1 and 5 cells were culture as described in Hempstapat et al., 2007. All cell culture reagents were purchased from Invitrogen Corp. (Carlsbad, CA) unless otherwise noted.

Calcium mobilization assays. Assays were performed within Vanderbilt University's High-Throughput Screening Center and the primary mGluR4 HTS has been described in detail.4 Human mGluR4/Gqi5/CHO cells (30,000 cells/20 •l/well) were plated in blackwalled, clear-bottomed, TC treated, 384 well plates (Greiner Bio-One, Monroe, North Carolina) 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% CO2. During the day of assay, the medium was replaced with 20 μL of 1 μM Fluo-4, AM (Invitrogen, Carlsbad, CA) prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in Assay Buffer (Hank's balanced salt solution, 20 mM HEPES and 2.5 mM Probenecid (Sigma-Aldrich, St. Louis, MO)) for 45 minutes at 37 °C. Dye was removed and replaced with 20 μL of Assay Buffer. Test compounds were transferred to daughter plates using an Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA) and then diluted into Assay Buffer. Ca2+ flux was measured using the Functional Drug Screening System 6000 (FDSS6000, Hamamatsu, Japan). Baseline readings were taken (10 images at 1 Hz, excitation, 470±20 nm, emission, 540±30 nm) and then 20 □l/well test compounds were 14 added using the FDSS's integrated pipettor. Cells were incubated with compounds for approximately 2.5 minutes and then an EC20 concentration of glutamate was applied; 2 minutes later an EC80 concentration of glutamate was added. For concentration-response curve experiments, compounds were serially diluted 1:3 into 10 point concentration response curves and were transferred to daughter plates using the Echo. Test compounds were again applied and

followed by EC20 concentrations of glutamate. For fold shift experiments, compounds were added at 2X their final concentration and then increasing concentrations of glutamate were added in the presence of vehicle or the appropriate concentration of test compound. Curves were fitted using a four point logistical equation using Microsoft XLfit (IDBS, Bridgewater, NJ). Subsequent confirmations of concentration response parameters were performed using independent serial dilutions of source compounds and data from multiple days experiments were integrated and fit using a four point logistical equation in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

In vitro PK Analysis:

Microsomal stability: The metabolic stability of each compound was investigated in rat hepatic microsomes (BD Biosciences, Billerica, MA) using substrate depletion methodology (% parent compound remaining). In separate 96-well plates for each time point, a mixture of 0.1M potassium phosphate-buffered (pH 7.4), 1µM test compound, 0.5 mg/mL microsomes, and 1mM NADPH (t=3, 7, 15, 25, or 45min) or buffer (t=0) were continually incubated at 37°C under ambient oxygenation. At the respective time, each plate's reaction was precipitated by the addition of 2 volumes of ice-cold acetonitrile containing glyburide as an internal standard (50 ng/mL). The plates were centrifuged at 3000 rpm (4°C) for 10 min. The resulting supernatants were transferred and diluted 1:1 (supernatant: water) into new 96-well plates in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. The in vitro half-life ($t_{1/2}$, min, Eq. 1), intrinsic clearance (*CLint*, mL/min/kg, Eq. 2) and subsequent predicted hepatic clearance (*CLhep*, mL/min/kg, Eq. 3) was determined employing the following equations:

1) $t_{1/2} = \mathrm{Ln}(2) / k$; where k represents the slope from linear regression analysis (% test compound remaining)

2) $CLint = (0.693 / t_{1/2})$ (rxn volume / mg of microsomes) (45 mg microsomes / gram of liver) (20^a gm of liver / kg body weight); ^{a}scale-up factors of 20 (human) and 45 (rat)

3) $$CLhep = \frac{Q \cdot CL\,\mathrm{int}}{Q + CL\,\mathrm{int}}$$

Plasma Protein Binding. The protein binding of each compound was determined in rat plasma via equilibrium dialysis employing Single-Use RED Plates with inserts (ThermoFisher Scientific, Rochester, NY). Plasma (220 µL) was added to the 96 well plate containing test compound (5 µL) and mixed thoroughly. Subsequently, 200 µL of the plasma-compound mixture was transferred to the *cis* chamber (red) of the RED plate, with an accompanying 350 µL of phosphate buffer (25 mM, pH 7.4) in the *trans* chamber. The RED plate was sealed and incubated for 4 hours at 37°C with shaking. At completion, 50 µL aliquots from each chamber were diluted 1:1

(50 µL) with either plasma (*cis*) or buffer (*trans*) and transferred to a new 96 well plate, at which time ice-cold acetonitrile (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rpm, 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.

Microsomal stability and plasma protein binding samples were analyzed on a Thermo Electron TSQ Quantum Ultra triple quad detector via electrospray ionization (ESI) with two Themo Electron Accella pumps (San Jose, CA), and a Leap Technologies CTC PAL autosampler. Analytes were separated by gradient elution on a dual column system with two Waters Acquity BEH C18, 2.1x50mm, 1.7µm columns (Milford, MA) heated at 50°C. HPLC mobile phase A was 95:5:0.1 Water:Acetonitrile:Formic Acid, while mobile phase B was 95:5:0.1 Acetonitrile:Water:Formic Acid. Pump 1 runs the gradient: 95:5 (A:B) at 800µL/min hold 0 to 0.5min, linear ramp to 5:95 (A:B) 0.5 to 1.0min, 5:95 (A:B) hold 1.0 to 1.9min, return to 95:5 (A:B) at 1.9 min. While pump 1 runs the gradient method, pump 2 equilibrates the other column isocratically with 95:5 (A:B). The total run time is 2.0 minutes per injection. All compounds are optimized using Thermo Electron's QuickQuan software.

Cytochrome P450 inhibition: A four-in-one, 96-well plate assay for determining IC_{50} values against human P450s 1A2, 2C9, 2D6 and 3A4 was developed based on previous reports (1,2). Human liver microsomes (final concentration of 0.1 mg/mL) and a substrate mixture containing the P450 probe substrates phenacetin (10µM), diclofenac (5µM), dextromethorphan (5µM) and midazolam (2µM) were added to a potassium phosphate buffered solution (0.1M, pH 7.4) and warmed to 37^{o}C. The reaction mixture was divided evenly into the 96-well plate and various dilutions of each inhibitor/compound of interest (in duplicate) were then added to this reaction mixture such that the final concentration of each compound ranged from 100nM to 30µM. This mixture was allowed to pre-incubate for 15 minutes while shaking at 37^{o}C. Buffer or NADPH (1mM) was added and the reaction mixture was incubated for an additional 8 minutes at 37^{o}C prior to quenching with 2 volumes of ice-cold acetonitrile containing 50ng/mL of carbamazepine as internal standard. The plates were centrifuged at 4000 rpm (4^{o}C) for 10 minutes and the supernatant was removed and diluted with water (1:4, v/v) in preparation for LC/MS/MS analysis. The IC_{50} values for each compound were obtained for the individual P450 enzymes by quantitating the inhibition of metabolite formation for each probe; acetaminophen (1A2), 4-hydroxydiclofenac (2C9), dextrorphan tartrate (2D6) and 1-hydroxymidazolam (3A4).

Miconazole was included as a positive control broad spectrum P450 inhibiton (REF). For discrete 2C19 inhibition experiments, a similar assay design was employed with the following exceptions: the probe substrate was S-mephenytoin (40μM), the NADPH incubation with the reaction mixture went for 30 minutes, the supernatant was reconstituted 1:1 with water for analysis, and the metabolite used for quantitation was 4-hydroxymephenytoin.

Cytochrome P450 inhibition 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 2.1 x 50 mm, 3 μm column (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40°C. 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 1.3 min; 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°C 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). All data were analyzed using AB Sciex Analyst 1.4.2 software.

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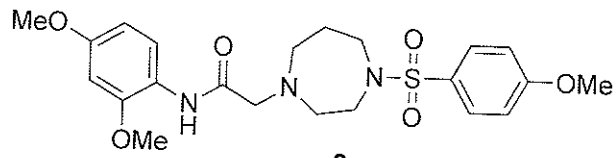
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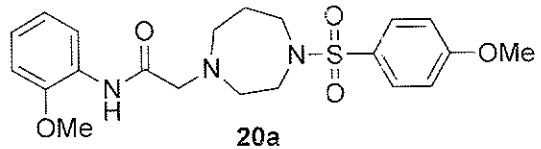
YYC-F-032-1



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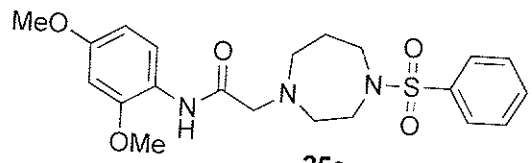

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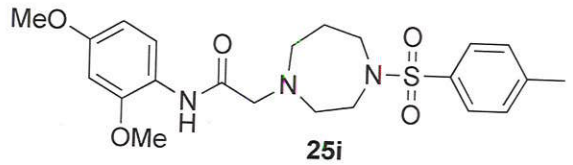

25i

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TD                 32768
SOLVENT             CDCl3
NS                     16
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FIDRES           0.158946 Hz
AQ               3.1458740 sec
RG                    256
DW                 96.000 usec
DE                  6.00 usec
TE                 298.0 K
D1               2.00000000 sec
TD0                     1

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P1                   8.60 usec
PL1                 -3.50 dB
SFO1          400.1318857 MHz
ND0                     2
TD                    512
SFO1             400.132 MHz
FIDRES          10.172506 Hz
SW                13.017 ppm
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SF            400.1300000 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
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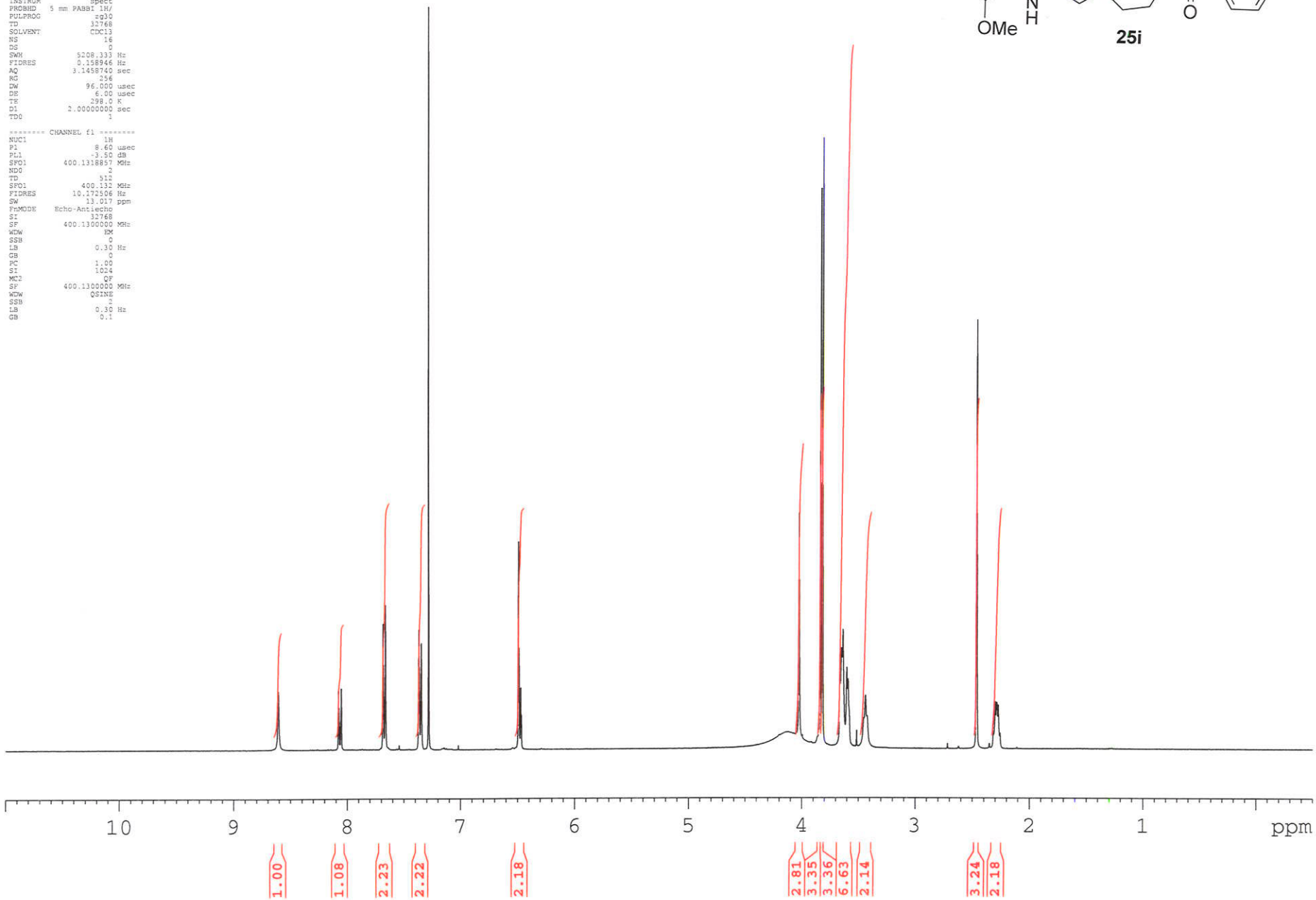

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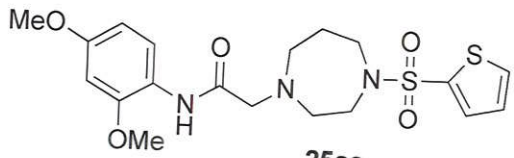

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