Supporting Information

Discovery of sulfonamidebenzamides as selective apoptotic CHOP pathway activators of the unfolded protein response

Daniel P. Flaherty,§ Justin R. Miller,† Danielle M. Garshott,‡ Michael Hedrick,§ Palak Gosalia,‡ Yujie Li,‡ Monika Milewski,‡ Eliot Sugarman,§ Stefan Vasile,‖ Sumeet Salaniwal,‡ Ying Su,‡ Layton H Smith,‖ Thomas D. Y. Chung,‡ Anthony B. Pinkerton,‡ Jeffrey Aubé,§ Michael U. Callaghan,† Jennifer E. Golden,§* Andrew M. Fribley§* and Randal J. Kaufman*§

§University of Kansas Specialized Chemistry Center, Delbert M. Shankel Structural Biology Center, 2034 Becker Dr. Lawrence, KS, USA 66047
†Wayne State University, Carmen and Ann Adams Department of Pediatrics, Division of Hematology and Oncology, and the Karmanos Cancer Institute Molecular Therapeutics Group, 2228 Elliman Building, 421 E. Canfield, Detroit, MI, USA, 48201
‡Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, 92037
‖Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute at Lake Nona, Orlando, FL, USA, 32827
§Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Rd., La Jolla, CA, USA, 92037

*corresponding authors

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General Experimental Details:

Purity of all final compounds was confirmed by HPLC/MS analysis. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AM 400 spectrometer (operating at 400 and 101 MHz respectively) or a Bruker AVIII spectrometer (operating at 500 and 126 MHz respectively) in CDCl$_3$ with 0.03% TMS as an internal standard or DMSO-$d_6$. The chemical shifts ($\delta$) reported are given in parts per million (ppm) and the coupling constants (J) are in Hertz (Hz). The spin multiplicities are reported as s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet and m = multiplet. The LCMS analysis was performed on an Agilent 1200 RRL chromatograph with photodiode array UV detection and an Agilent 6224 TOF mass spectrometer. The chromatographic method utilized the following parameters: a Waters Acquity BEH C$^18$ 2.1 x 50 mm, 1.7 $\mu$m column; UV detection wavelength = 214 nm; flow rate = 0.4ml/min; gradient = 5 - 100% acetonitrile over 3 minutes with a hold of 0.8 minutes at 100% acetonitrile; the aqueous mobile phase contained 0.15% ammonium hydroxide (v/v). The mass spectrometer utilized the following parameters: an Agilent multimode source which simultaneously acquires ESI+/APCI+; a reference mass solution consisting of purine and hexakis(1H, 1H, 3H-tetrafluoropropoxy) phosphazine; and a make-up solvent of 90:10:0.1 MeOH:Water:Formic Acid which was introduced to the LC flow prior to the source to assist ionization.

$\text{N-(4-(((4-Chloropiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (3).}$ To a microwave vial was added commercially available 4-(morpholinosulfonyl)aniline (0.065 g, 0.27 mmol), 5-nitro-2-furoyl chloride (0.052 g, 0.29 mmol) and acetonitrile (3 mL). The vial was sealed and heated to 150 °C in a microwave reactor for 20 minutes. The reaction then cooled to room temperature and was diluted with CH$_2$Cl$_2$ (10 mL) and washed with saturated NaHCO$_3$ (10 mL). The CH$_2$Cl$_2$ layer was collected, dried with MgSO$_4$, filtered and adsorbed to silica gel. The crude product was purified by silica gel flash column chromatography (0 – 5% v/v CH$_3$OH/CH$_2$Cl$_2$) to produce 3 (0.094 g, 0.24 mmol, 84 % yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.03 (s, 1H), 8.05 (d, $J$ = 8.8 Hz, 2H), 7.85 (d, $J$ = 3.9 Hz, 1H), 7.79 (d, $J$ = 8.8 Hz, 2H), 7.72 (d, $J$ = 3.9 Hz, 1H), 3.67 – 3.55 (m, 4H), 2.92 – 2.82 (m, 4H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 155.03, 151.97, 147.27, 142.36, 129.36, 128.92, 120.40, 117.33, 113.43, 65.27, 45.92. LCMS retention time: 2.777 min. LCMS Purity at 214 nm: 97.5 %. HRMS: m/z calcd for C$_{15}$H$_{15}$N$_3$O$_7$S (M + H$^+$) 382.0631, found 382.0699.

$\text{N-(4-((2,6-Dimethylmorpholino)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (7).}$ Step 1: 4-((2,6-Dimethylmorpholino)sulfonyl)aniline. To a vial was added 4-nitrobenzenesulfonyl chloride (0.19 g, 0.87 mmol), triethylamine (0.24 mL, 1.74 mmol), 2,6-dimethylmorpholine (0.12 mL, 0.96 mmol) and THF (1.5 mL). The reaction was subsequently heated to 60 °C for 20 minutes then allowed to cool to room temperature, was diluted with EtOAc (10 mL) and washed with saturated NaHCO$_3$ (10 mL). The EtOAc extract was separated, dried with MgSO$_4$, filtered and adsorbed to silica and purified by silica gel flash column chromatography (15 min, 0 - 30% v/v EtOAc/hexanes) to produce 2,6-dimethyl-4-((4-nitrophenoxy)sulfonyl) morpholine (0.21 g, 0.69 mmol, 80 % yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.46 (d, $J$ = 8.8 Hz, 2H), 8.02 (d, $J$ = 8.9 Hz, 2H), 7.71 – 3.45 (m, 4H), 2.92 – 1.88 (m, 2H), 1.05 (d, $J$ = 6.1 Hz, 6H).

![N-(4-((4-Chloropiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide](image1.png)

![N-(4-((2,6-Dimethylmorpholino)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide](image2.png)
Step 2: To a vial containing 2,6-dimethyl-4-((4-nitrophenyl)sulfonyl)morpholine (0.21 g, 0.69 mmol) was added CH₃OH:CH₂Cl₂ (4 mL:2 mL) and the reaction vessel was cooled to 0 °C. Raney nickel (0.004 g, 0.069 mmol) was added followed by portionwise addition of sodium borohydride (0.052 g, 1.39 mmol). The reaction stirred at 0 °C for 30 minutes and was then diluted with CH₂Cl₂ (10 mL) and filtered slowly. The CH₂Cl₂ layer was washed with water (10 mL), dried with MgSO₄, filtered, adsorbed to silica and purified by silica gel flash column chromatography (15 min, 0 - 5 % v/v CH₃OH/CH₂Cl₂) to produce 4-((2,6-dimethylmorpholino)sulfonyl)aniline (0.18 g, 0.68 mmol, 98 % yield). ¹H NMR (400 MHz, CDCl₃) δ 7.50 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 4.19 (s, 2H), 3.73 – 3.63 (m, 2H), 3.56 – 3.46 (m, 2H), 1.92 (dd, J = 11.4, 10.2 Hz, 2H), 1.12 (d, J = 6.3 Hz, 6H).

Step 3: To a microwave vial was added 4-((2,6-dimethylmorpholino)sulfonyl)aniline (0.15 g, 0.62 mmol), 5-nitrofuroyl chloride (0.56 g, 2.50 mmol), pyridine (0.30 mL, 3.70 mmol), 4-((4-nitrophenyl)sulfonyl)morpholine (0.21 g, 0.69 mmol) to produce tert-butyl 4-((4-aminophenyl)sulfonyl)piperazine-1-carboxylate (0.12 g, 0.35 mmol, 38 % yield) as a yellow solid. The BOC group was advantageously removed under these same conditions and did not require further manipulation. ¹H NMR (400 MHz, DMSO-d₆) δ 11.02 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 3.9 Hz, 1H), 3.70 – 3.56 (m, 2H), 3.55 – 3.47 (m, 2H), 1.91 – 1.73 (m, 2H), 1.06 (d, J = 6.2 Hz, 6H). ¹³C NMR (100 MHz, DMSO-d₆): δ 155.0, 152.0, 147.3, 142.3, 129.6, 128.9, 120.4, 117.3, 113.4, 70.6, 50.6, 18.5. LCMS retention time: 2.961 min. LCMS Purity at 214 nm: 95 %. HRMS: m/z calcd for C_{17}H_{19}N_{3}O_{2}S (M + H⁺) 410.0944, found 410.0997.

N-(4-(Piperazin-1-ylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (8). Prepared as described for compound 7, Step 1, except using 4-nitrobenzenesulfonyl chloride (0.56 g, 2.50 mmol), pyridine (0.30 mL, 3.70 mmol), 4-N-Boc-piperazine (0.51 g, 2.80 mmol) and THF (1.5 mL) to produce tert-butyl 4-((4-nitrophenyl)sulfonyl)piperazine-1-carboxylate (0.35 g, 0.94 mmol, 38 % yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.45 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H), 3.46 – 3.36 (m, 4H), 2.96 (t, J = 5.1 Hz, 4H), 1.35 (s, 9H). The tert-butyl 4-((4-nitrophenyl)sulfonyl)piperazine-1-carboxylate was then treated as described for compound 7, Step 2, except tert-butyl 4-((4-nitrophenyl)sulfonyl)piperazine-1-carboxylate (0.14 g, 0.36 mmol) in CH₃OH:CH₂Cl₂ (3 mL:1 mL) with Raney nickel (0.002 g, 0.036 mmol) and sodium borohydride (0.028 g, 0.73 mmol) was used to produce tert-butyl 4-((4-amino phenyl)sulfonyl)piperazine-1-carboxylate (0.12 g, 0.337 mmol, 96 % yield). ¹H NMR (400 MHz, DMSO-d₆) δ 7.35 (d, J = 8.7 Hz, 2H), 6.65 (d, J = 8.8 Hz, 2H), 6.12 (s, 2H), 3.37 (d, J = 5.3 Hz, 4H), 2.75 (t, J = 5.1 Hz, 4H), 1.35 (s, 9H). As described for compound 7, Step 3, except tert-butyl 4-((4-amino phenyl)sulfonyl) piperazine-1-carboxylate (0.23 g, 0.82 mmol), 5-nitro-2-furoyl chloride (0.16 g, 0.90 mmol) and acetonitrile (3 mL) was used to produce 8 (0.11 g, 0.26 mmol, 31 % yield) as a yellow solid. The BOC group was advantageously removed under these same conditions and did not require further manipulation. ¹H NMR (400 MHz, DMSO-d₆) δ 11.01 (s, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 4.0 Hz, 1H), 7.76 (d, J = 8.9 Hz, 2H), 7.71 (d, J = 3.9 Hz, 1H), 2.78 (d, J = 5.0 Hz, 4H), 2.73 (d, J = 5.0 Hz, 4H). ¹³C NMR (100 MHz, DMSO-d₆): δ 155.0, 151.9, 147.3, 142.1, 129.8, 128.8, 120.4, 117.3, 113.4, 46.6, 44.5. LCMS retention time: 2.453 min. LCMS Purity at 214 nm: 90.5 %. HRMS: m/z calcd for C_{13}H_{16}ClN_{4}O_{6}S (M + H⁺) 381.0791, found 381.0884.
N-(4-(4-Hydroxypiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (9). Prepared as described for compound 7, Step 1, except 4-nitrobenzenesulfonyl chloride (0.28 g, 1.27 mmol), triethylamine (0.26 mL, 1.91 mmol), 4-hydroxypiperidine (0.14 g, 1.40 mmol) in CH$_2$Cl$_2$ (4 mL) was stirred at rt to produce 1-((4-nitrophenyl)sulfonyl)piperidin-4-ol (0.32 g, 1.11 mmol, 88 % yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.45 (d, $J$ = 8.8 Hz, 2H), 8.02 (d, $J$ = 8.9 Hz, 2H), 3.60 – 3.50 (m, 1H), 3.27 – 3.15 (m, 2H), 2.89 – 2.76 (m, 2H), 1.81 – 1.70 (m, 2H), 1.53 – 1.36 (m, 2H). The 1-((4-nitrophenyl)sulfonyl)piperidin-4-ol was then treated as described for compound 7, Step 2, except 1-((4-nitrophenyl)sulfonyl)piperidin-4-ol (0.15 g, 0.54 mmol) in CH$_3$OH:CH$_2$Cl$_2$ (3 mL:1 mL) with Raney nickel (0.003 g, 0.053 mmol) and sodium borohydride (0.041 g, 1.07 mmol) was used to produce 1-((4-aminophenyl)sulfonyl)piperidin-4-ol (0.074 g, 0.29 mmol, 54 % yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.34 (d, $J$ = 8.7 Hz, 2H), 6.64 (d, $J$ = 8.7 Hz, 2H), 6.05 (s, 2H), 4.65 (d, $J$ = 3.9 Hz, 1H), 3.58 – 3.43 (m, 1H), 3.22 – 2.99 (m, 2H), 2.65 – 2.55 (m, 2H), 1.75 – 1.63 (m, 2H), 1.49 – 1.29 (m, 2H). As described for compound 7, Step 3, 1-((4-aminophenyl)sulfonyl)piperidin-4-ol (0.074 g, 0.29 mmol) was treated with 5-nitro-2-furoyl chloride (0.056 g, 0.32 mmol) and acetonitrile (2 mL) to produce 9 (0.058 g, 0.15 mmol, 51 % yield) as a white solid. 13C NMR (125 MHz, DMSO-$d_6$): $\delta$ 155.0, 151.9, 147.3, 142.0, 130.6, 128.6, 120.4, 117.3, 113.4, 63.8, 43.2, 32.9. LCMS retention time: 2.500 min. LCMS Purity at 214 nm: 99.0 %. HRMS: $m/z$ calcd for C$_{16}$H$_{17}$N$_3$O$_7$S (M + H$^+$) 396.0787, found 396.0866.

N-(4-(Piperidin-1-ylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (10). Prepared as described for compound 3, except commercially available 4-(piperidin-1-ylsulfonyl)aniline (0.13 g, 0.53 mmol), 5-nitro-2-furoyl chloride (0.10 g, 0.58 mmol) and acetonitrile (3 mL) was used to produce 10 (0.17 g, 0.44 mmol, 82 % yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.00 (s, 1H), 8.02 (d, $J$ = 8.8 Hz, 2H), 7.85 (d, $J$ = 3.9 Hz, 1H), 7.77 (d, $J$ = 8.8 Hz, 2H), 7.71 (d, $J$ = 4.0 Hz, 1H), 3.59 – 3.45 (m, 2H), 3.23 – 3.08 (m, 2H), 2.78 – 2.64 (m, 2H), 1.82 – 1.64 (m, 2H), 1.50 – 1.35 (m, 2H). 13C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.0, 151.9, 147.3, 142.0, 130.6, 128.6, 120.3, 117.2, 113.4, 46.6, 24.7, 22.9. LCMS retention time: 3.016 min. LCMS Purity at 214 nm: 100 %. HRMS: $m/z$ calcd for C$_{16}$H$_{17}$ClN$_3$O$_6$S (M + H$^+$) 380.0838, found 380.0907.

N-(4-((4-Fluoropiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (11). Step 1, 4-fluoro-1-((4-nitrophenyl)sulfonyl)piperidine: To a vial was added 4-nitrobenzenesulfonyl chloride (0.13 g, 0.58 mmol), triethylamine (0.20 mL, 1.5 mmol), 4-fluoropiperidine hydrochloride (0.097 g, 0.70 mmol) and CH$_2$Cl$_2$ (2 mL).
The reaction was stirred at rt for 18 h and was then diluted with saturated NaHCO₃ (5 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 8 mL) and the organic layers were combined then dried with MgSO₄. The crude product was adsorbed to silica and purified by silica gel flash chromatography (0 – 35% EtOAc:hexanes) to produce 4-fluoro-1-((4-nitrophenyl)sulfonyl)piperidine (0.15 g, 0.52 mmol, 89 % yield). ¹H NMR (400 MHz, DMSO-d₆): δ 8.45 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 9.0 Hz, 2H), 4.82 – 4.70 (m, 1H), 3.17 (dt, J = 11.2, 5.2 Hz, 2H), 2.99 (t, J = 9.3 Hz, 2H), 2.04 – 1.62 (m, 3H). The 4-fluoro-1-((4-nitrophenyl)sulfonyl)piperidine was treated as described for compound 7, Step 2, except 4-fluoro-1-((4-nitrophenyl)sulfonyl)piperidine (0.15 g, 0.52 mmol) in CH₃OH:CH₂Cl₂ (3 mL:1 mL) with Raney nickel (0.003 g, 0.052 mmol) and sodium borohydride (0.039 g, 1.04 mmol) was used to produce 4-((4-fluoropiperidin-1-yl)sulfonyl)aniline (0.13 g, 0.51 mmol, 98 % yield). ¹H NMR (400 MHz, CDCl₃): δ 8.45 (d, J = 9.0 Hz, 2H), 8.03 (d, J = 8.9 Hz, 2H), 4.83 – 4.68 (m, 1H), 3.15 (m, 2H), 2.97 (m, 2H), 1.98 – 1.78 (m, 4H). The 4-((4-fluoropiperidin-1-yl)sulfonyl)aniline was treated as compound 7, Step 3, except 4-((4-fluoropiperidin-1-yl)sulfonyl)aniline (0.039 g, 0.15 mmol), 5-nitro-2-furoyl chloride (0.029 g, 0.17 mmol) and acetonitrile (2 mL) was used to produce 11 (0.030 g, 0.076 mmol, 49 % yield) as a light orange solid. ¹H NMR (400 MHz, DMSO-d₆): δ 11.02 (s, 1H), 8.04 (d, J = 8.9 Hz, 2H), 7.84 (d, J = 3.9 Hz, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 4.0 Hz, 1H), 4.82 – 4.68 (m, 1H), 3.07 (m, 2H), 2.94 (m, 2H), 1.98 – 1.78 (m, 4H). ¹³C NMR (100 MHz, DMSO-d₆): δ 155.1, 152.0, 147.4, 142.4, 130.2, 128.7, 120.5, 117.3, 113.4, 86.8 (d, J = 163.5 Hz), 42.2 (d, J = 12.6), 30.1 (d, J = 32.4 Hz). LCMS retention time: 3.001 min. LCMS Purity at 214 nm: 96.7 %. HRMS: m/z calcd for C₁₆H₁₇N₃O₆S (M + H⁺) 398.0817, found 398.0845.

N-(4-((4-chloropiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (12). Step 1: To a vial was added 4-nitrobenzenesulfonyl chloride (0.32 g, 1.4 mmol), pyridine (0.12 mL, 1.4 mmol) and THF (1.5 mL). The reaction was stirred at room temperature while 4-chloropiperidine (0.13 g, 1.0 mmol) was added dropwise over 10 minutes. The reaction was subsequently heated to 60 °C for 20 minutes then allowed to cool to room temperature, was diluted with EtOAc (10 mL) and washed with saturated NaHCO₃ (10 mL). The EtOAc extract was separated, dried with MgSO₄, filtered and adsorbed to silica and purified by silica gel flash chromatography (15 min, 0 – 30% v/v EtOAc/hexanes) to produce 4-chloro-1-((4-nitrophenyl)sulfonyl)piperidine (0.29 g, 0.96 mmol, 96 % yield). ¹H NMR (400 MHz, CDCl₃): δ 8.40 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 9.0 Hz, 1H), 4.22 (m, 1H), 3.29 (m, 2H), 3.18 (m, 2H), 2.16 (m, 2H), 1.97 (m, 2H).

Step 2: To a vial containing 4-chloro-1-((4-nitrophenyl)sulfonyl)piperidine (0.29 g, 0.96 mmol) was added CH₃OH:CH₂Cl₂ (3 mL:3 mL) and the reaction was cooled to 0 °C. The Raney nickel (0.006 g, 0.096 mmol) was added followed by portionwise addition of sodium borohydride (0.073 g, 1.9 mmol). The reaction was stirred at 0 °C for 30 minutes and was then diluted with CH₂Cl₂ (10 mL) and filtered slowly. The CH₂Cl₂ layer was washed with water (10 mL), dried with MgSO₄, filtered, adsorbed to silica and purified by silica gel flash column chromatography (15 min, 0 - 5 % v/v CH₃OH/CH₂Cl₂) to produce 4-chloro-1-((4-nitrophenyl)sulfonyl)piperidine (0.29 g, 0.82 mmol, 86 % yield). ¹H NMR (400 MHz, CDCl₃): δ 7.66 (d, J = 8.7 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H), 7.03 (s, 1H), 5.32 (s, 1H), 4.12 (m, 1H), 3.16 (m, 2H), 3.10 (m, 2H), 2.13 (m, 2H), 1.95 (m, 2H). The 4-((4-chloropiperidin-1-yl)sulfonyl)aniline was treated as done for compound 7, Step 3, except 4-((4-chloropiperidin-1-yl)sulfonyl)aniline (0.23 g, 0.82 mmol), 5-nitro-2-furoyl chloride (0.16 g, 0.90 mmol) and acetonitrile (3 mL), were used to produce 12 (0.11 g, 0.26 mmol, 31 % yield). ¹H NMR (400 MHz, DMSO-d₆): δ 11.01 (s, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.83
(d, J = 3.9 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 3.9 Hz, 1H), 4.27 (m, 1H), 3.17 (m, 2H), 2.87 (m, 2H), 2.10 (m, 2H), 1.79 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆): δ 154.9, 151.9, 147.3, 142.2, 130.4, 128.6, 120.4, 117.2, 113.4, 56.1, 43.4, 33.9. LCMS retention time: 3.147 min. LCMS Purity at 214 nm: 97.5 %. HRMS: m/z calcd for C₁₆H₁₇ClN₃O₆S (M + H⁺) 414.0521, found 414.0522.

**N-(4-((4-Methylpiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (13).** Prepared as described for compound 3, commercially available 4-((4-methylpiperidin-1-yl)sulfonyl)aniline (0.11 g, 0.43 mmol), 5-nitro-2-furoyl chloride (0.082 g, 0.47 mmol) and acetonitrile (2 mL) was used to produce 13 (0.14 g, 0.37 mmol, 86 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ 11.00 (s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 3.9 Hz, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 3.9 Hz, 1H), 3.66 – 3.52 (m, 2H), 2.21 (td, J = 12.0, 2.5 Hz, 2H), 1.72 – 1.58 (m, 2H), 1.40 – 1.22 (m, 1H), 1.21 – 1.04 (m, 2H), 0.86 (d, J = 6.4 Hz, 3H). ¹³C NMR (100 MHz, DMSO-d₆): δ 155.0, 151.9, 147.3, 142.0, 130.6, 128.6, 120.4, 117.3, 113.4, 46.1, 32.8, 29.3, 21.3. LCMS retention time: 3.184 min. LCMS Purity at 214 nm: 96.6 %. HRMS: m/z calcd for C₁₇H₁₉N₃O₆S (M + H⁺) 394.0995, found 394.1082.

**N-(4-((4,4-Dimethylpiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (14).** Prepared as described for compound 7, Step 1, except 4-nitrobenzenesulfonyl chloride (0.23 g, 1.0 mmol), pyridine (0.13 mL, 1.6 mmol), 4,4-dimethylpiperidine (0.15 g, 1.2 mmol) and THF (3 mL) was used to produce 4,4-dimethyl-1-((4-nitrophenyl)sulfonyl)piperidine (0.25 g, 0.84 mmol, 81 % yield). ¹H NMR (400 MHz, CDCl₃) δ 8.39 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H), 3.11 – 2.95 (m, 4H), 1.50 – 1.42 (m, 4H), 0.86 (s, 6H). As described for compound 7, Step 2, 4,4-dimethyl-1-((4-nitrophenyl)sulfonyl)piperidine (0.12 g, 0.40 mmol) in CH₃OH:CH₂Cl₂ (3 mL:1 mL) was treated with Raney nickel (0.002 g, 0.040 mmol) and sodium borohydride (0.030 g, 0.80 mmol) to produce 4-((4,4-dimethylpiperidin-1-yl)sulfonyl)aniline (0.11 g, 0.39 mmol, 98 % yield). ¹H NMR (400 MHz, DMSO-d₆) δ 7.36 (d, J = 8.7 Hz, 2H), 6.65 (d, J = 8.7 Hz, 2H), 6.04 (s, 2H), 2.87 – 2.74 (m, 4H), 1.44 – 1.26 (m, 4H), 0.79 (s, 6H). As described for compound 7, Step 3, 4-((4,4-dimethylpiperidin-1-yl)sulfonyl)aniline (0.11 g, 0.39 mmol), 5-nitro-2-furyl chloride (0.076 g, 0.43 mmol) and acetonitrile (2 mL) was used to produce 14 (0.10 g, 0.25 mmol, 63 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 10.99 (s, 1H), 8.02 (d, J = 8.9 Hz, 2H), 7.84 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 3.9 Hz, 1H), 2.91 (t, J = 5.5 Hz, 4H), 1.36 (t, J = 5.6 Hz, 4H), 0.79 (s, 6H). ¹³C NMR (100 MHz, DMSO-d₆): δ 155.0, 151.9, 147.3, 141.9, 130.8, 128.5, 120.3, 117.2, 113.4, 46.1, 32.8, 29.3, 21.3. LCMS retention time: 3.307 min. LCMS Purity at 214 nm: 98.3 %. HRMS: m/z calcd for C₁₇H₁₉N₃O₆S (M + H⁺) 408.1224, found 408.124.

**N-(4-((4-Tert-butylpiperidin-1-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (15).** Prepared as described for
compound 7, Step 1, except 4-nitrobenzenesulfonyl chloride (0.20 g, 0.91 mmol), pyridine (0.22 mL, 2.72 mmol), 4-tert-butylpiperidine hydrochloride (0.16 g, 0.91 mmol) and THF (10 mL) was used to produce 4-(tert-butyl)-1-((4-nitrophenyl)sulfonyl)piperidine (0.088 g, 0.27 mmol, 30 % yield). ¹H NMR (400 MHz, CDCl₃): δ 8.37 (d, J = 8.9 Hz, 2H), 7.94 (d, J = 8.9 Hz, 2H), 3.93 – 3.89 (m, 2H), 2.22 (td, J₁ = 12.2 Hz, J₂ = 2.4 Hz, 2H), 1.76 – 1.72 (m, 2H), 1.38 (qd, J₁ = 12.4 Hz, J₂ = 4.1 Hz, 2H), 0.89 (m, 1H), 0.82 (s, 9H). As described for compound 7, Step 2, 4-((tert-butyl)piperidin-1-yl)sulfonyl)aniline (0.11 g, 0.39 mmol, 98 % yield). ¹H NMR (400 MHz, CDCl₃): δ 7.52 (d, J = 8.6 Hz, 2H), 6.68 (d, J = 8.7 Hz, 2H), 4.13 (s, 2H), 3.85 – 3.71 (m, 2H), 2.17 – 2.04 (m, 2H), 1.75 – 1.64 (m, 2H), 1.45 – 1.18 (m, 2H), 0.87 (tt, J₁ = 11.0, 2.8 Hz, 1H), 0.81 (s, 9H). As described for compound 7, Step 3, 5-nitro-2-furoyl chloride (0.032 g, 0.18 mmol) and acetonitrile (2 mL) to produce 16 (0.055 g, 0.20 mmol, 97 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 10.99 (s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 2.94 – 2.80 (m, 4H), 1.48 – 1.40 (m, 4H), 1.31 (s, 6H), 1.17 (s, 4H). LCMS retention time: 3.553 min. LCMS Purity at 214 nm: 99.4 %. HRMS: m/z calcd for C₂₁H₂₅N₃O₆S (M + H⁺) 448.1464, found 448.1535.

N-(4-(3-Azaspiro[5.5]undecan-3-ylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (16). Prepared as described for compound 7, Step 1, except 4-nitrobenzenesulfonlfyl chloride (0.058 g, 0.26 mmol), pyridine (0.064 mL, 0.79 mmol), 3-azaspiro[5.5]undecane hydrochloride (0.050 g, 0.26 mmol) and THF (10 mL) was used to produce 3-((4-nitrophenyl)sulfonyl)-3-azaspiro[5.5]undecane (0.070 g, 0.21 mmol, 78 % yield). ¹H NMR (400 MHz, CDCl₃): δ 8.38 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 3.12 – 2.95 (m, 4H), 1.57 – 1.47 (m, 4H), 1.41 – 1.31 (m, 6H), 1.25 – 1.11 (m, 4H). As described for compound 7, Step 2, 3-((4-nitrophenyl)sulfonyl)-3-azaspiro[5.5]undecane (0.070 g, 0.21 mmol) in CH₃OH:CH₂Cl₂ (3 mL:1 mL) with Raney nickel (0.002 g, 0.021 mmol) and sodium borohydride (0.016 g, 0.41 mmol) was used to produce 3-(3-azaspiro[5.5]undecan-3-ylsulfonyl)aniline (0.062 g, 0.20 mmol, 97 % yield). ¹H NMR (400 MHz, CDCl₃): δ 10.99 (s, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 2.96 – 2.88 (m, 4H), 1.54 – 1.47 (m, 4H), 1.36 (s, 6H), 1.21 (d, J = 6.6 Hz, 4H). As described for compound 7, Step 3, 4-(3-azaspiro[5.5]undecan-3-ylsulfonyl)aniline (0.062 g, 0.20 mmol), 5-nitro-2-furoyl chloride (0.032 g, 0.18 mmol) and acetonitrile (2 mL) to produce 16 (0.055 g, 0.20 mmol, 97 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 10.99 (s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 2.94 – 2.80 (m, 4H), 1.48 – 1.40 (m, 4H), 1.31 (s, 6H), 1.17 (s, 4H). ¹³C NMR (125 MHz, DMSO-d₆): δ 155.0, 152.0, 147.3, 142.0, 130.6, 128.7, 120.4, 117.3, 113.4, 54.9, 46.8, 44.8, 31.8, 27.0, 25.7. LCMS retention time: 3.553 min. LCMS Purity at 214 nm: 99.4 %. HRMS: m/z calcd for C₂₁H₂₅N₃O₆S (M + H⁺) 448.1464, found 448.1535.
N-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (17). Prepared as described for compound 3, commercially available 4-(pyrrolidin-1-ylsulfonyl)aniline (0.072 g, 0.32 mmol), 5-nitro-2-furoyl chloride (0.061 g, 0.35 mmol) and acetonitrile (2 mL) was used to produce 17 (0.060 g, 0.16 mmol, 52 % yield) as a white solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.98 (s, 1H), 8.01 (d, \(J = 8.8\) Hz, 2H), 7.88 – 7.80 (m, 3H), 7.71 (d, \(J = 4.0\) Hz, 1H), 3.23 – 3.00 (m, 4H), 1.74 – 1.51 (m, 4H). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 155.0, 152.0, 147.3, 142.0, 131.3, 128.5, 120.4, 117.3, 113.4, 47.8, 24.7. LCMS retention time: 2.866 min. LCMS Purity at 214 nm: 99.0 %. HRMS: \(m/z\) calcd for C\(_{15}\)H\(_{15}\)N\(_3\)O\(_6\)S (M + H\(^+\)) 366.0682, found 366.0759.

N-(4-(Phenylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (18). Prepared as described for compound 3, commercially available 4-(phenylsulfonyl)aniline (0.087 g, 0.37 mmol), 5-nitro-2-furoyl chloride (0.072 g, 0.41 mmol) and acetonitrile (2 mL) was used to produce 18 (0.085 g, 0.23 mmol, 61 % yield) as a white solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 11.00 (s, 1H), 8.00 (s, 4H), 7.98 – 7.93 (m, 2H), 7.83 (d, \(J = 3.9\) Hz, 1H), 7.73 – 7.67 (m, 2H), 7.67 – 7.58 (m, 2H). \(^{13}\)H NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 155.0, 151.9, 147.2, 142.6, 141.5, 135.9, 133.6, 129.7, 128.7, 127.1, 120.7, 117.4, 113.4. LCMS retention time: 2.936 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\(_{17}\)H\(_{12}\)N\(_2\)O\(_6\)S (M + H\(^+\)) 373.0416, found 373.0743.

N-(4-((Tetrahydro-2H-pyran-4-yl)sulfonyl)phenyl)-5-nitrofuran-2-carboxamide (19). 4-((4-Nitrophenyl)thio)tetrahydro-2H-pyran, Step 1: To a vial was added the 4-bromotetrahydropyran (0.10 mL, 0.91 mmol), 4-nitrothiophenol (0.17 g, 1.0 mmol), cesium carbonate (0.49 g, 1.5 mmol) and acetonitrile (5 mL). The reaction was then heated to 50 °C for 4 h then was allowed to cool to rt and was quenched with saturated NaHCO\(_3\) (7 mL). The reaction was extracted with EtOAc (3 x 10 mL). The organic layers were combined and dried with MgSO\(_4\), filtered and adsorbed to silica then purified by silica gel flash chromatography (0 – 35% EtOAc:hexanes) to produce 4-((4-nitrophenyl)thio)tetrahydro-2H-pyran (0.15 g, 0.63 mmol, 69 %). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.14 (d, \(J = 8.9\) Hz, 2H), 7.41 (d, \(J = 8.9\) Hz, 2H), 4.01 (dt, \(J = 12.1, 4.1\) Hz, 2H), 3.62 – 3.41 (m, 3H), 2.06 – 1.93 (m, 2H), 1.82 – 1.66 (m, 2H). Synthesis of 4-((4-nitrophenyl)sulfonyl)tetrahydro-2H-pyran, Step 2: To a vial was added 4-((4-nitrophenyl)thio)tetrahydro-2H-pyran (0.15 g, 0.63 mmol) and acetic acid (5 mL). A solution of 30% w/v hydrogen peroxide in water (0.22 mL, 1.94 mmol) was then slowly added to the reaction at rt and the reaction was heated to 50 °C for 5 h. The reaction was allowed to cool to rt and was extracted with CH\(_2\)Cl\(_2\) (3 x 10 mL). The combined organic layers were washed with water (30 mL), dried with MgSO\(_4\), filtered and concentrated to provide 4-((4-nitrophenyl)sulfonyl)tetrahydro-2H-pyran (0.16 g, 0.58 mmol, 92 %). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.43 (d, \(J =
8.8 Hz, 2H), 8.10 (d, J = 8.9 Hz, 2H), 4.12 – 4.03 (m, 2H), 3.34 (td, J = 11.8, 2.4 Hz, 2H), 3.20 (tt, J = 11.9, 4.1 Hz, 1H), 1.95 – 1.74 (m, 4H). Synthesis of 4-((tetrahydro-2H-pyran-4-yl)sulfonyl)aniline, Step 3 – as described for compound 7.

Step 2: 4-(4-nitrophenyl)sulfonyl)tetrahydro-2H-pyran (0.16 g, 0.58 mmol) in CH₂OH:CH₂Cl₂ (5 mL:5 mL) with Raney nickel (0.003 g, 0.060 mmol) and sodium borohydride (0.044 g, 1.15 mmol) was used to produce 4-((tetrahydro-2H-pyran-4-yl)sulfonyl)aniline (0.13 g, 0.52 mmol, 91% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 115.4, 65.4, 58.8, 25.3. LCMS retention time: 2.477 min. LCMS Purity at 214 nm: 94.4%. HRMS: m/z calcd for C₁₆H₁₄ClN₂O₅S (M + H⁺) 381.0678, found 381.0678.

N-(4-(cyclohexylsulfonyl)phenyl)-5-nitrofuran-2-carboxamide (20). Synthesis of cyclohexyl(4-nitrophenyl)sulfane, Step 1: Cyclohexyl(4-nitrophenyl)sulfane was prepared using a procedure adapted from Hayashi et al.¹ To a vial was added cyclohexyl mercaptan (0.14 mL, 1.13 mmol) and DMF (5 mL). The 60% w/w sodium hydride (0.045 g, 1.13 mmol) was added to the reaction portionwise over 3 minutes. After the addition the reaction was stirred at rt for 10 minutes then 4-iodo-nitrobenzene (0.31 g, 1.2 mmol) was added. The reaction was then heated to 70 °C and stirred for 4 hours then allowed to cool to rt and was quenched slowly with 1.0 M aqueous HCl to pH 3. The aqueous layer was then extracted with EtOAc (3 x 10 mL) and the organic layers were combined and washed with water (3 x 30 mL). The organic layers were then dried with MgSO₄, filtered and concentrated to provide 1-(cyclohexylsulfonyl)-4-nitrobenzene (0.26 g, 0.82 mmol) to produce 4-(cyclohexylsulfonyl)aniline (0.15 g, 0.62 mmol, 58% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.41 (d, J = 8.8 Hz, 2H), 8.08 (d, J = 8.9 Hz, 2H), 2.96 (tt, J = 12.2, 3.4 Hz, 1H), 2.08 – 2.01 (m, 2H), 1.89 (m, 2H), 1.74 – 1.61 (m, 1H), 1.43 (qd, J = 12.2, 3.2 Hz, 2H), 1.32 – 1.06 (m, 3H). Synthesis of 4-(cyclohexylsulfonyl)aniline, Step 3 – as described for compound 7.

Step 2: 4-(cyclohexylsulfonyl)aniline (0.15 g, 0.62 mmol), 5-nitro-2-furoyl chloride (0.12 g, 0.69 mmol) and acetonitrile (3 mL) was used to produce 20 (0.14 g, 0.38 mmol, 60% yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ 113.4, 65.4, 58.8, 25.3. LCMS retention time: 2.477 min. LCMS Purity at 214 nm: 94.4%. HRMS: m/z calcd for C₁₆H₁₄ClN₂O₅S (M + H⁺) 381.0678, found 381.0678.
DMSO-\textsubscript{d\textsubscript{6}} δ 11.02 (s, 1H), 8.04 (d, \(J = 8.9\) Hz, 2H), 7.91 – 7.81 (m, 3H), 7.71 (d, \(J = 3.9\) Hz, 1H), 3.26 – 3.05 (m, 1H), 1.98 – 1.84 (m, 2H), 1.83 – 1.67 (m, 2H), 1.64 – 1.49 (m, 1H), 1.37 – 1.16 (m, 4H), 1.15 – 1.00 (m, 1H). \textsuperscript{\textsuperscript{13}}C NMR (100 MHz, DMSO-\textsubscript{d\textsubscript{6}}): δ 155.0, 151.9, 147.3, 142.7, 131.9, 129.8, 120.3, 117.3, 113.4, 61.7, 39.5, 25.1, 24.7, 24.3. LCMS retention time: 3.047 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\textsubscript{17}H\textsubscript{18}ClN\textsubscript{2}O\textsubscript{6}S (M + H\textsuperscript{+}) 379.0886, found 379.0958.

\textbf{N-(4-(Morpholinosulfonyl)phenyl)-5-nitrothiophene-2-carboxamide (21).} Purchased from Ryan Scientific, Inc. \textsuperscript{1}H NMR (500 MHz, DMSO-\textsubscript{d\textsubscript{6}}) δ 11.03 (s, 1H), 8.25 (d, \(J = 4.4\) Hz, 1H), 8.12 (d, \(J = 4.4\) Hz, 1H), 8.04 (d, \(J = 8.8\) Hz, 2H), 7.79 (d, \(J = 8.8\) Hz, 2H), 3.68 – 3.55 (m, 4H), 2.94 – 2.76 (m, 4H). \textsuperscript{13}C NMR (125 MHz, DMSO-\textsubscript{d\textsubscript{6}}): δ 158.8, 153.8, 145.4, 142.4, 130.1, 129.4, 129.1, 128.9, 65.3, 54.9. LCMS retention time: 3.002 min. LCMS Purity at 214 nm: 98.3 %. HRMS: \(m/z\) calcd for C\textsubscript{15}H\textsubscript{15}N\textsubscript{3}O\textsubscript{4}S\textsubscript{2} (M + H\textsuperscript{+}) 398.0402 found 398.0348.

\textbf{N-(4-(Morpholinosulfonyl)phenyl)thiophene-2-carboxamide (22).} Prepared as described for compound 3, commercially available 4-(morpholino sulfonyl)aniline (0.13 g, 0.53 mmol), 2-thiophenecarbonyl chloride (0.062 mL, 0.58 mmol) and acetonitrile (2 mL) were used to produce 22 (0.094 g, 0.24 mmol, 84 % yield) as a white solid. \textsuperscript{1}H NMR (400 MHz, DMSO-\textsubscript{d\textsubscript{6}}) δ 10.63 (s, 1H), 8.10 (dd, \(J = 3.8, 1.2\) Hz, 1H), 8.04 (d, \(J = 8.8\) Hz, 2H), 7.93 (dd, \(J = 5.0, 1.1\) Hz, 1H), 7.75 (d, \(J = 8.8\) Hz, 2H), 7.27 (dd, \(J = 5.0, 3.8\) Hz, 1H), 3.70 – 3.50 (m, 4H), 2.92 – 2.78 (m, 4H). \textsuperscript{13}C NMR (126 MHz, DMSO-\textsubscript{d\textsubscript{6}}): δ 160.4, 143.3, 139.3, 132.8, 130.0, 128.9, 128.4, 128.2, 119.9, 65.3, 45.9. LCMS retention time: 2.745 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\textsubscript{15}H\textsubscript{16}N\textsubscript{2}O\textsubscript{4}S\textsubscript{2} (M + H\textsuperscript{+}) 353.0551, found 353.0623.

\textbf{N-(4-(Morpholinosulfonyl)phenyl)benzamide (23).} Prepared as described for compound 3, commercially available 4-(morpholino sulfonyl)aniline (0.12 g, 0.50 mmol), benzoyl chloride (0.064 mL, 0.55 mmol) and acetonitrile (2 mL) used to produce 23 (0.13 g, 0.36 mmol, 73 % yield) as a white solid. \textsuperscript{1}H NMR (400 MHz, DMSO-\textsubscript{d\textsubscript{6}}) δ 10.69 (s, 1H), 8.09 (d, \(J = 8.8\) Hz, 2H), 8.01 – 7.92 (m, 2H), 7.75 (d, \(J = 8.8\) Hz, 2H), 7.67 – 7.60 (m, 1H), 7.60 – 7.51 (m, 2H), 3.64 (dd, \(J = 5.8, 3.6\) Hz, 4H), 2.94 – 2.78 (m, 4H). \textsuperscript{13}C NMR (126 MHz, DMSO-\textsubscript{d\textsubscript{6}}): δ 166.2, 143.7, 134.4, 132.0, 128.8, 128.5, 128.4, 127.9, 119.9, 65.3, 45.9. LCMS retention time: 2.772 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\textsubscript{17}H\textsubscript{18}N\textsubscript{2}O\textsubscript{6}S (M + H\textsuperscript{+}) 347.0987, found 347.1063.
**N-(4-(Morpholinosulfonyl)phenyl)benzamide (24).** Prepared as described for compound 3, commercially available 4-(morpholino sulfonyl)aniline (0.062 g, 0.26 mmol), 4-nitrobenzoyl chloride (0.052 mL, 0.28 mmol) and acetonitrile (2 mL) used to produce 24 (0.038 g, 0.096 mmol, 38 % yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.99 (s, 1H), 8.40 (d, $J$ = 8.9 Hz, 2H), 8.22 (d, $J$ = 8.8 Hz, 2H), 8.07 (d, $J$ = 8.8 Hz, 2H), 7.76 (d, $J$ = 8.8 Hz, 2H), 3.70 – 3.55 (m, 4H), 2.94 – 2.79 (m, 4H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 164.7, 149.2, 144.3, 140.7, 129.4, 128.8, 128.4, 123.6, 120.4, 65.3, 45.9. LCMS retention time: 2.896 min. LCMS Purity at 214 nm: 100 %. HRMS: $m/z$ calcd for C$_{17}$H$_{17}$N$_3$O$_6$S (M + H$^+$) 392.0838, found 382.0906.

**N-(4-((4,4-Dimethylpiperidin-1-yl)sulfonyl)phenyl)-3-nitrobenzamide (25).** To a vial was added 3-nitrobenzoic acid (0.030 g, 0.180 mmol) and thionyl chloride (2.0 ml, 27.4 mmol). The reaction stirred at 80 °C for 4 h and was then allowed to cool to rt and concentrated in vacuo. The residue was added to a microwave vial with 4-((4,4-dimethylpiperidin-1-yl)sulfonyl)aniline – used in the preparation of 14 - (0.053 g, 0.20 mmol) and acetonitrile (3 mL). The reaction stirred at 150 °C for 20 min in the microwave reactor. The reaction was then allowed to cool to rt and was diluted with aqueous NaHCO$_3$ (10 mL) and extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried with MgSO$_4$, filtered and adsorbed to silica then purified by silica gel flash chromatography (0 – 40% v/v EtOAc:hexanes) to produce 25 (0.042 g, 0.10 mmol, 56 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.97 (s, 1H), 8.82 (t, $J$ = 2.0 Hz, 1H), 8.48 (ddd, $J$ = 8.2, 2.3, 1.0 Hz, 1H), 8.43 (ddd, $J$ = 7.8, 1.7, 1.0 Hz, 1H), 8.07 (d, $J$ = 8.8 Hz, 2H), 7.88 (t, $J$ = 8.0 Hz, 1H), 7.79 (d, $J$ = 8.8 Hz, 2H), 2.96 – 2.85 (m, 4H), 1.41 – 1.33 (m, 4H), 0.80 (s, 6H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 164.0, 147.8, 142.8, 135.8, 134.4, 130.32, 130.30, 128.5, 126.6, 122.6, 120.2, 42.3, 37.1, 27.8, 27.2. LCMS retention time: 3.383 min. LCMS Purity at 214 nm: 100 %. HRMS: $m/z$ calcd for C$_{20}$H$_{23}$N$_3$O$_5$S (M + H$^+$) 418.1358, found 418.1424.

**N-(4-((4,4-Dimethylpiperidin-1-yl)sulfonyl)phenyl)-1-methyl-5-nitro-1H-imidazole-2-carboxamide (26).** To a vial was added 3-nitrobenzoic acid (0.030 g, 0.180 mmol) and thionyl chloride (2.0 ml, 27.4 mmol). The reaction stirred at 80 °C for 4 h and was then allowed to cool to rt and concentrated in vacuo. The residue was added to a microwave vial with 4-((4,4-dimethylpiperidin-1-yl)sulfonyl)aniline – used in the preparation of 14 - (0.053 g, 0.20 mmol) and acetonitrile (3 mL). The reaction stirred at 150 °C for 20 min in the microwave reactor. The reaction was then allowed to cool to rt and was diluted with aqueous NaHCO$_3$ (10 mL) and extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried with MgSO$_4$, filtered and adsorbed to silica then purified by silica gel flash chromatography (0 – 40% v/v EtOAc:hexanes) to produce 25 (0.042 g, 0.10 mmol, 56 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.97 (s, 1H), 8.82 (t, $J$ = 2.0 Hz, 1H), 8.48 (ddd, $J$ = 8.2, 2.3, 1.0 Hz, 1H), 8.43 (ddd, $J$ = 7.8, 1.7, 1.0 Hz, 1H), 8.07 (d, $J$ = 8.8 Hz, 2H), 7.88 (t, $J$ = 8.0 Hz, 1H), 7.79 (d, $J$ = 8.8 Hz, 2H), 2.96 – 2.85 (m, 4H), 1.41 – 1.33 (m, 4H), 0.80 (s, 6H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 164.0, 147.8, 142.8, 135.8, 134.4, 130.32, 130.30, 128.5, 126.6, 122.6, 120.2, 42.3, 37.1, 27.8, 27.2. LCMS retention time: 3.383 min. LCMS Purity at 214 nm: 100 %. HRMS: $m/z$ calcd for C$_{20}$H$_{23}$N$_3$O$_5$S (M + H$^+$) 418.1358, found 418.1424.
containing the product were not fully pure, it was re-adsorbed to silica and purified by silica gel flash chromatography (0 - 45 % EtOAc:hexanes) to produce 26 (0.014 g, 0.033 mmol, 31 % yield). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) δ 11.24 (s, 1H), 8.28 (s, 1H), 8.11 (d, \(J = 8.8\) Hz, 2H), 7.76 (d, \(J = 8.8\) Hz, 2H), 4.29 (s, 3H), 3.00 – 2.86 (m, 4H), 1.43 – 1.28 (m, 4H), 0.80 (s, 6H). \(^{13}\)C NMR (126 MHz, DMSO-\(d_6\)) δ 156.9, 142.0, 141.1, 141.0, 130.7, 128.4, 120.5, 42.3, 37.1, 34.9, 27.8, 27.2. LCMS retention time: 2.240 min. LCMS Purity at 214 nm: 92 %. HRMS: \(m/z\) calcd for C\(_{18}\)H\(_{23}\)N\(_5\)O\(_5\)S (M + H\(^+\)) 422.1420, found 422.1408.

\(N\)-(4-(Morpholinosulfonyl)phenyl)furan-2-carboxamide (27).\)

Purchased from Chembridge Corporation. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.27 (s, 1H), 7.86 (d, \(J = 8.8\) Hz, 2H), 7.76 (d, \(J = 8.8\) Hz, 1H), 7.31 (dd, \(J = 3.5, 0.8\) Hz, 1H), 6.61 (dd, \(J = 3.5, 1.8\) Hz, 1H), 3.81 – 3.59 (m, 4H), 3.07 – 2.93 (m, 4H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 156.0, 147.1, 144.7, 141.8, 130.1, 129.3, 119.5, 116.4, 113.0, 66.1, 46.0. LCMS retention time: 2.534 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\(_{15}\)H\(_{16}\)N\(_2\)O\(_5\)S (M + H\(^+\)) 337.0780 found 337.0858.

\(N\)-(4-(Morpholinosulfonyl)phenyl)-5-methylfuran-2-carboxamide (28).\)

Prepared as described for compound 3, commercially available 4-(morpholinosulfonyl)aniline (0.13 g, 0.52 mmol), 5-methyl-2-furoyl chloride (0.090 g, 0.62 mmol) and acetonitrile (2 mL) used to produce 28 (0.083 g, 0.24 mmol, 46 % yield) as a white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.18 (s, 1H), 7.86 (d, \(J = 8.8\) Hz, 2H), 7.74 (d, \(J = 8.8\) Hz, 1H), 7.20 (dd, \(J = 3.4, 0.6\) Hz, 1H), 6.21 (dd, \(J = 3.4, 0.9\) Hz, 1H), 3.80 – 3.67 (m, 4H), 3.07 – 2.96 (m, 4H), 2.47 – 2.38 (m, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): δ 154.8, 148.5, 141.5, 130.4, 129.3, 125.6, 119.6, 118.6, 115.0, 66.1, 46.0. LCMS retention time: 2.685 min. LCMS Purity at 214 nm: 100 %. HRMS: \(m/z\) calcd for C\(_{16}\)H\(_{18}\)N\(_2\)O\(_5\)S (M + H\(^+\)) 351.0936 found 351.1009.

\(N\)-(4-(Morpholinosulfonyl)phenyl)-5-bromofuran-2-carboxamide (29).\)

Purchased from Chembridge Corporation. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.17 (s, 1H), 7.86 (d, \(J = 8.8\) Hz, 2H), 7.76 (d, \(J = 8.8\) Hz, 1H), 7.25 (d, \(J = 3.6\) Hz, 1H), 6.55 (d, \(J = 3.6\) Hz, 1H), 3.79 – 3.61 (m, 4H), 3.09 – 2.94 (m, 4H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 154.8, 148.5, 141.5, 130.4, 129.3, 125.6, 119.6, 118.6, 115.0, 66.1, 46.0. LCMS retention time: 2.834 min. LCMS Purity at 214 nm: 98.3 %. HRMS: \(m/z\) calcd for C\(_{15}\)H\(_{15}\)N\(_2\)O\(_5\)S (M + H\(^+\)) 416.9885 found 416.9936.
N-(4-(4,4-dimethylpiperidin-1-ylsulfonyl)phenyl)-5-trifluoromethylfuran-2-carboxamide (30). The same procedure was employed as with 25 but using 5-(trifluoromethyl)furan-2-carboxylic acid (0.038 g, 0.210 mmol) and thionyl chloride (0.64 mL, 8.76 mmol). After concentration the residue was added to 4-(4,4-dimethylpiperidin-1-yl)sulfonylaniline – used in the preparation of 14 - (0.047 g, 0.18 mmol) and acetonitrile (3 mL) to produce 30 (0.042 g, 0.10 mmol, 56%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.24 (s, 1H), 7.86 (d, $J$ = 8.8 Hz, 2H), 7.79 (d, $J$ = 8.8 Hz, 2H), 7.33 (dd, $J$ = 3.7, 0.9 Hz, 1H), 6.98 (dd, $J$ = 3.7, 1.1 Hz, 1H), 3.10 – 2.95 (m, 4H), 1.51 – 1.32 (m, 4H), 0.85 (s, 6H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 154.9, 148.7, 143.2 (q, $J$ = 37.7 Hz), 140.6, 132.4, 129.0, 119.8, 118.3 (q, $J$ = 264.1 Hz), 116.2, 114.0 (q, $J$ = 2.8 Hz), 42.6, 37.7, 28.2, 27.5. LCMS retention time: 3.485 min. LCMS Purity at 214 nm: 97.7 %. HRMS: m/z calcd for C$_{19}$H$_{21}$F$_3$N$_2$O$_4$S (M + H$^+$) 431.1174, found 431.1243.

Reference:

1. Hayashi, S.; Nakamoto, T.; Minoura, M.; Nakanishi, W. Evidence for effective p(Z)-pi(Ar) conjugations (Z = S, Se, and Te, as well as Z = O) in 9-(arylchalcogenyl)triptycenes: experimental and theoretical investigations. *J. Org. Chem.* **2009**, *74*, 4763-71.

Figure S1. CHOP knockout MEF cells are resistant to compound 12. Luminescent ATP-based proliferation assays performed with wildtype (wt) or CHOP knockout (ko) MEF treated with increasing doses of compound 12 for 16 hours. Data represent four independent experiments performed with triplicate samples; error bars represent standard error of the mean.
MEF cell type legend

- WT
- KO

$IC_{50}$ (µM)

- WT $4.8 \pm 1.1$
- KO $> 20$

Concentration of compound 12 (µM)

% Proliferation
Figure S2. Antiproliferation assay data for compound **12** against six head and neck squamous cell cancer cell lines.
| Panel/Cell Line | Mean Optical Densities | Percent Growth | G50 | TGI | LC50 |
|-----------------|------------------------|---------------|-----|-----|-----|
| **Leukemia**    |                        |               |     |     |     |
| CCRF-CEM        | 0.34                  | 1.21E-6       | 3.05E-8 | 0.84 | 0.59E-5 |
| HL-60(TB)       | 0.24                  | 0.9E-6        | 2.43E-8 | 0.68E-6 | 0.10E-6 |
| K-562           | 0.27                  | 1.1E-5        | 7.00E-8 | 0.34E-6 | 0.00E-6 |
| MOLT-4          | 0.32                  | 1.1E-5        | 3.17E-8 | 0.00E-6 | 0.00E-6 |
| RPMI-8226       | 0.29                  | 1.1E-5        | 2.50E-8 | 0.00E-6 | 0.00E-6 |
| **SR**          | 0.47                  | 3.9E-6        | 1.05E-8 | 0.00E-6 | 0.00E-6 |
| **Non-Small Cell Lung Cancer** |          |               |     |     |     |
| A549/BATC       | 0.38                  | 1.2E-6        | 3.30E-8 | 0.84 | 0.59E-5 |
| HOP-62          | 0.42                  | 1.3E-5        | 4.59E-8 | 0.68E-6 | 0.10E-6 |
| HOP-92          | 0.39                  | 1.3E-5        | 6.95E-8 | 0.00E-6 | 0.00E-6 |
| HCC-2766        | 0.56                  | 1.1E-5        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| HCC-324         | 0.70                  | 1.1E-5        | 1.08E-7 | 0.00E-6 | 0.00E-6 |
| HCC-460         | 0.33                  | 1.2E-6        | 9.35E-8 | 0.00E-6 | 0.00E-6 |
| HCC-822        | 0.91                  | 1.2E-5        | 5.68E-8 | 0.00E-6 | 0.00E-6 |
| **Colon Cancer**|                        |               |     |     |     |
| COLO320                 | 0.35                  | 3.1E-6        | 6.34E-8 | 0.00E-6 | 0.00E-6 |
| HCT-116         | 0.57                  | 3.5E-6        | 6.85E-8 | 0.00E-6 | 0.00E-6 |
| HCT-15          | 0.13                  | 2.7E-7        | 4.55E-8 | 0.00E-6 | 0.00E-6 |
| HT22           | 0.24                  | 2.7E-7        | 6.12E-8 | 0.00E-6 | 0.00E-6 |
| KM12           | 0.49                  | 3.6E-6        | 7.16E-8 | 0.00E-6 | 0.00E-6 |
| SW-620         | 0.27                  | 2.6E-7        | 5.80E-8 | 0.00E-6 | 0.00E-6 |
| **CNS Cancer**  |                        |               |     |     |     |
| SF-268         | 0.64                  | 5.9E-6        | 5.45E-8 | 0.00E-6 | 0.00E-6 |
| SF-291         | 0.64                  | 5.9E-6        | 4.81E-8 | 0.00E-6 | 0.00E-6 |
| SF-539        | 0.74                  | 5.9E-6        | 7.95E-8 | 0.00E-6 | 0.00E-6 |
| SNB-19        | 0.67                  | 5.9E-6        | 4.53E-8 | 0.00E-6 | 0.00E-6 |
| **Melanoma**   |                        |               |     |     |     |
| LOX(1820C)     | 0.16                  | 3.2E-7        | 4.98E-8 | 0.00E-6 | 0.00E-6 |
| MALME-3M       | 0.67                  | 5.9E-6        | 4.83E-8 | 0.00E-6 | 0.00E-6 |
| M14           | 0.45                  | 2.2E-7        | 5.56E-8 | 0.00E-6 | 0.00E-6 |
| MDA-MB-435     | 0.48                  | 2.2E-7        | 4.15E-8 | 0.00E-6 | 0.00E-6 |
| SK-MEL-2       | 0.13                  | 1.0E-5        | 5.97E-8 | 0.00E-6 | 0.00E-6 |
| SK-MEL-26      | 0.41                  | 1.0E-5        | 3.91E-8 | 0.00E-6 | 0.00E-6 |
| UACC-257       | 0.67                  | 4.2E-6        | 4.88E-8 | 0.00E-6 | 0.00E-6 |
| UACC-62       | 0.64                  | 4.2E-6        | 6.16E-8 | 0.00E-6 | 0.00E-6 |
| **Urothelial Cancer** |          |               |     |     |     |
| EGPVCI         | 0.62                  | 1.3E-6        | 5.31E-8 | 0.00E-6 | 0.00E-6 |
| OVCAR-3        | 0.54                  | 1.3E-6        | 5.52E-8 | 0.00E-6 | 0.00E-6 |
| OVCAR-4        | 0.36                  | 1.3E-6        | 2.66E-8 | 0.00E-6 | 0.00E-6 |
| OVCAR-5        | 0.51                  | 1.3E-6        | 6.82E-8 | 0.00E-6 | 0.00E-6 |
| OVCAR-8        | 0.47                  | 1.3E-6        | 4.53E-8 | 0.00E-6 | 0.00E-6 |
| OVCAR-12       | 0.44                  | 1.3E-6        | 4.91E-8 | 0.00E-6 | 0.00E-6 |
| **Renal Cancer**|                        |               |     |     |     |
| JAR5          | 0.61                  | 1.3E-6        | 2.63E-8 | 0.00E-6 | 0.00E-6 |
| **Prostate Cancer** |              |               |     |     |     |
| PC-3           | 0.48                  | 6.3E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| **Breast Cancer** |                      |               |     |     |     |
| MCF-7          | 0.52                  | 6.3E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| MXA-MB-311ATCC | 0.49                 | 6.3E-6        | 6.13E-8 | 0.00E-6 | 0.00E-6 |
| HS 578T        | 0.63                  | 2.6E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| BT-549         | 0.79                  | 2.6E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| T-47D         | 0.65                  | 5.8E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
| MDA-MB-458     | 0.56                  | 1.4E-6        | 1.00E-7 | 0.00E-6 | 0.00E-6 |
Figure S3. NCI-60 Panel results with Compound 12 – Part 1
Figure S2. Continued - NCI-60 Panel results with Compound 12 – Part 2
Table S2. *In vitro* pharmacology of compound 12

| Parameter                      | Conditions          | Results                                                                 |
|-------------------------------|---------------------|-------------------------------------------------------------------------|
| **Aqueous Solubility**        | pION’s buffer       | pH 5.0                    3.8 µg/mL, 8.7 µM 3.8 µg/mL, 9.2 µM 4.0 µg/mL, 9.7 µM |
|                               | 1x PBS<sup>a</sup>  | pH 7.4          3.9 µg/mL, 9.4 µM                                        |
| **PAMPA Permeability**        | acceptor pH: 7.4    | donor pH 5.0 291 x10<sup>6</sup> cm/s 291 x10<sup>6</sup> cm/s 245 x10<sup>6</sup> cm/s |
| **Plasma Protein Binding**    | Human               | 1 µM 99.8 % bound     |
|                               | Mouse               | 1 µM 84.5 % bound     |
|                               |                     | 10 µM 99.6 % bound     |
|                               |                     | 10 µM 84.5 % bound     |
| **Plasma Stability (37°C)**   | Human               | percent remaining at 3 hr 85.6 %                                    |
|                               | Mouse               | percent remaining after 1 hr 52.3 %                                    |
| **Hepatic Microsome Stability (37°C)** | Human | percent remaining after 1 hr 0.49 %                                    |
|                               | Mouse               | percent remaining after 1 hr 0.02 %                                    |
| **Toxicity** Fa2N-4 Immortalized Hepatocytes | Human | LC<sub>50</sub> 11.4 µM                                    |
| **Off-target Profiling**<sup>b</sup> | Assessment of compound 12 against 67 targets at 10 µM | 68% inhibition of the human dopamine transporter |

<sup>a</sup>PBS = 137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 2 mM potassium phosphate monobasic and a pH of 7.4; measurement was done at 23 °C; <sup>b</sup>Panlabs LeadProfiling Screen (formerly Ricerca), radioligand binding assay results, performed in duplicate.
Cellular Assay Protocols

High Throughput Screening (HTS)

HTS was performed with a previously described luminescent Chinese Hamster Ovary (CHO-K1) reporter assay piqued to identify small molecules that splice \textit{XBPI} or activate \textit{CHOP}.\textsuperscript{1} The assay was scaled-down to a 1536 well format using 500 CHO-CHOP-luc or CHO-XBP1-luc cells in plated in 5 \(\mu\)L F12 plus Glutamax medium supplemented with non-essential amino acids (NEAA) (Invitrogen, Grand Island, NY). When treated with 2.5 \(\mu\)g/ml tunicamycin both cell lines yielded a 6 fold increase in luciferase that consistently produced \(Z'\) values \(\geq 0.64\). The progenitor of compound “12” was identified from the HIN Molecular Libraries Small Molecule Repository of >350,000 compounds, all of which were tested at 10 \(\mu\)M. HTS screening was performed at the Conrad Prebys Center for Chemical Genomics at the Sanform|Burnham Medical Research Institute in La Jolla, CA.

Cell lines and proliferation assays

Murine embryonic fibroblasts (MEF) were cultured in Dulbecco’s Modified Eagle Medium and supplemented with penicillin-streptomycin, 10\% Fetal Bovine serum, and NEAA. Luminescent proliferation assays were performed with 30 \(\mu\)L of CellTiter-Glo\textsuperscript{®} (Promega) added to each well at the indicated time points; luminescence was measured after a 10 minute incubation. Proliferation assays were performed at least three times in triplicate 96-well plates (50 \(\mu\)L final volume) using 7500 cells/well. Oral squamous cell carcinoma proliferation assays were performed similarly using 12,500 cells. Error bars represent standard deviation of technical replicates for all proliferation assays.

PCR analysis

One microgram of Trizol (Invitrogen) harvested RNA was used for reverse transcription. cDNA pools generated with random hexamers were used for RT-PCR analysis of \textit{XBPI} using a single human-specific pair of primers that produces amplicons for un-spliced (\(XBPI_u\)) and spliced (\(XBPI_s\)). The forward primer was CCT TGT AGT TGA GAA CCA GG, and the reverse primer was GGG GCT TGG TAT ATA TGT GG.\textsuperscript{2} For quantitative RT-PCR (qRT-PCR), total RNA was harvested with a Cells-to-CT™ Kit (Ambion) in 96 well plates. RNA’s were reverse transcribed using random hexamers and amplified using SsoFast probes Supermix (Bio-Rad) and TaqMan (Life Technologies) primer probes, as previously described.\textsuperscript{3} 18s ribosomal RNA was used as for internal control and the fold changes were calculated with the delta delta CT method; error bars represent standard deviation of technical replicates. The Life Technologies primer/probe pairs were as follows: 18S (Hs99999901_s1), \textit{CHOP/DDIT3} (Hs01090850_m1), \textit{GADD34/PPP1R15} (Hs00169585_m1), spliced \textit{XBPI} (Hs03929085_g1), \textit{ERCC1} (Hs01012159_m1).

References:

2. Fribley AM, Cruz PG, Miller JR, Callaghan MU, Cai P, Narula N \textit{et al.} (2011a). Complementary cell-based high-throughput screens identify novel modulators of the unfolded protein response. \textit{Journal of biomolecular screening} 16(8):825-835.
3. Park JW, Woo KJ, Lee JT, Lim JH, Lee TJ, Kim SH \textit{et al.} (2007). Resveratrol induces pro-apoptotic endoplasmic reticulum stress in human colon cancer cells. \textit{Oncol Rep} 18(5):1269-1273.
4. Fribley AM, Miller JR, Reist TE, Callaghan MU, Kaufman RJ (2011b). Large-scale analysis of UPR-mediated apoptosis in human cells. \textit{Methods Enzymol} 491(57-71.)
**In vitro pharmacology assay protocols**

**Aqueous Solubility Protocol** - Compound solubility in aqueous solution was measured using an automated kinetic solubility method at the Sanford Burnham Medical Research Institute. The concentration of the compound in a saturated pH-buffered aqueous solution was determined by UV absorbance (250-498 nm) and compared to the spectra of a precipitation-free reference solution. Aqueous solubility was measured in phosphate buffered saline (PBS) at room temperature (23°C). PBS by definition is 137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 2 mM potassium phosphate monobasic and a pH of 7.4.

**Plasma Stability** – Stability of the compound in human plasma and mouse plasma (BioChemed Services) was determined. All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). Plasma was allowed to thaw at room temperature prior to preparing the assay solution of plasma:1X PBS (1:1). The assay solution was warmed up at 37 °C prior of adding the compound. Immediately after compounds were added, time 0 min aliquots were promptly collected and mixed with cold acetonitrile (spiked with an internal standard). The remainder of the reaction volume was incubated at 37 °C with shaking. Additional aliquots were collected 180 min after the start of the reaction and promptly quenched with cold acetonitrile (spiked with an internal standard). Samples were centrifuged at 3000 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap) and the percent of parent compound remaining after 180 min was calculated by the following formula:

\[
\text{% parent compound remaining} = \left( \frac{\text{Concentration at 180 min}}{\text{Concentration at 0 min}} \right) \times 100
\]

Results reported are the mean of each reaction duplicate, normalized to the internal standard, and expressed as a percent of compound remaining after the incubation time.

**Assay details:**
- Mouse Plasma in K3 EDTA
- Procaine and Procainamide were used as standards. Procaine is highly unstable, Procainamide is highly stable.
- Assay concentrations of standards and test compound: 1 µM
- Incubation Time: 3 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 2.5%

**Plasma Protein Binding** – Teflon® Base Plate wells were rinsed with 20% ethanol for 10 minutes. Ethanol was then removed and wells were rinsed with ultrapure water and allowed to dry. RED (rapid equilibrium dialysis) inserts from Thermo Scientific (Pierce) were placed (open end up) into the wells of the base plate. All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). The sample chambers (red ring) contained 300 µl of a mixture of plasma and compound, and the buffer chambers received 500 µl of dialysis buffer (1X PBS, pH7.4). Duplicate inserts
were made for each concentration tested. The base plate was covered with sealing tape and incubated at 37°C on an orbital shaker at 350 rpm for 4 hours. After the incubation time, equal volume from both chambers were removed and transferred to a 96 well plate containing either plasma or buffer. To precipitate proteins and release compounds, ice cold acetonitrile (with an internal standard) was added. Samples were vortexed and centrifuged at 3700 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap). The percent of free and bound compounds were calculated with the following formula:

\[
\text{% of bound parent compound} = \left( \frac{\text{amount of compound in donor} - \text{receiver}}{\text{amount of compound in donor}} \right) \times 100
\]

Results reported are the mean of each reaction duplicate, normalized to the internal standard, and expressed as a percent compound bound after the incubation time.

**Assay details:**
- Mouse in K3 EDTA
- Propranolol and Metoprolol were used as standards. Propranolol is highly bound, Metoprolol is poorly bound
- Assay concentrations of standards and test cpd: 1 µM and 10 µM
- Incubation Time: 4 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 1%

**Hepatic Microsome Stability** – Metabolic stability was assessed in the presence of mouse liver microsomes (XenoTech, P/N M1000). All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). NADPH, a required cofactor for CYP450 metabolism, was provided by the NADPH Regenerating System, Solutions A (BD Biosciences, P/N 451220) and B (BD Biosciences, P/N 451200). Compound stock solutions were initially prepared in 100% DMSO and subsequently diluted in acetonitrile for the assay. The pH of the reactions was kept at ~ 7.4 with potassium phosphate buffer (BD Biosciences, P/N 451201). The reactions were started after adding NADPH to the reaction plate containing microsomes and compounds and time 0 min aliquots were promptly collected and mixed with ice cold acetonitrile (spiked with internal standards) to quench the reactions. The remainder of the reaction volume was incubated at 37 °C with shaking. Additional aliquots were collected 60 min after the start of the reaction and promptly quenched with ice cold acetonitrile (spiked with an internal standard). Samples were centrifuged at 3000 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap) and the percent of parent compound remaining after 60 min was calculated by the following formula:

\[
\text{% parent compound remaining} = \left( \frac{\text{Concentration at 60 min}}{\text{Concentration at 0 min}} \right) \times 100
\]

All reactions were run in triplicate, except negative controls (no NADPH) which were performed as single reactions. Results reported are the mean of each reaction triplicate, normalized to the internal standard, and
expressed as a percent compound remaining after the incubation time.

**Assay details:**
- Mouse Liver Microsomes: 0.5 mg/mL protein concentration
- NADPH Regenerating System: 1.55 mM NADP+, 1.33 mM glucose-6-phosphate, 1.33 mM Magnesium chloride, and 0.4 U/mL glucose-6-phosphate dehydrogenase
- Incubation Temperature: 37 °C
- Incubation Time: 60 min
- Standards: Verapamil-HCl and Testosterone, at 20 µM and 50 µM, respectively
- Test compound at 1 µM
- Assay DMSO final concentration: ≤ 0.5%
- Assay ACN final concentration: ≤ 1.2%

**Human Hepatocyte Cytotoxicity** – Immortalized human hepatocytes, Fa2N-4 cells (XenoTech, P/N IFH15), were resuspended in MFE Plating medium (XenoTech), seeded in collagen-coated plates (VWR) at ~50,000 cells/well, and incubated in a humidified CO2 incubator at 37 °C. After 4 hrs, the medium was replaced with MFE Support medium (XenoTech). On the third day, the cells were incubated with a range of concentrations (0.01-50 µM) of the test compound. After 24 hrs, cell viability was determined by cellular ATP levels using the Luminescence ATP Detection Assay System (ATPlite 1 step, Perkin Elmer) and the Infinite M200 plate reader (Tecan).

**Assay details:**
- Cells used: Fa2N-4, immortalized human hepatocytes
- Media used for Fa2N-4 cells: MFE Plating and MFE Support (with 1% Penicillin, Streptomycin, and Amphotericin mixture)
- Assay DMSO final concentration = 0.5%
- Treatment time: 24 hrs
- Camptothecin and Terfenadine were used as standards. Camptothecin is highly toxic and Terfenadine is highly non-toxic.
Table S3. Panlabs Profiling Results with Compound 12

| Cat #   | Assay Name                    | Batch* | Spec. | Rep. | Conc. | % Inh. |
|---------|-------------------------------|--------|-------|------|-------|--------|
| 200510  | Adenosine A<sub>1</sub>       | 310992 | hum   | 2    | 10 µM | 12     |
| 200610  | Adenosine A<sub>2A</sub>      | 310993 | hum   | 2    | 10 µM | 8      |
| 200720  | Adenosine A<sub>3</sub>       | 310976 | hum   | 2    | 10 µM | 6      |
| 203100  | Adrenergic α<sub>1A</sub>     | 310901 | rat   | 2    | 10 µM | 19     |
| 203200  | Adrenergic α<sub>1B</sub>     | 310902 | rat   | 2    | 10 µM | 13     |
| 203400  | Adrenergic α<sub>1D</sub>     | 310903 | hum   | 2    | 10 µM | 8      |
| 203620  | Adrenergic α<sub>2A</sub>     | 310904 | hum   | 2    | 10 µM | 11     |
| 204010  | Adrenergic β<sub>1</sub>      | 310974 | hum   | 2    | 10 µM | 1      |
| 204110  | Adrenergic β<sub>2</sub>      | 310983 | hum   | 2    | 10 µM | 17     |
| 285010  | Androgen (Testosterone) AR    | 311022 | rat   | 2    | 10 µM | 8      |
| 212510  | Bradykinin B<sub>1</sub>      | 311038 | hum   | 2    | 10 µM | 11     |
| 212620  | Bradykinin B<sub>2</sub>      | 311061 | hum   | 2    | 10 µM | 4      |
| 214510  | Calcium Channel L-Type, Benzothiazepine | 310999 | rat   | 2    | 10 µM | 6      |
| 214600  | Calcium Channel L-Type, Dihydropyridine | 310998 | rat   | 2    | 10 µM | 24     |
| 216000  | Calcium Channel N-Type        | 310899 | rat   | 2    | 10 µM | 18     |
| 217030  | Cannabinoid CB<sub>1</sub>    | 311000 | hum   | 2    | 10 µM | 20     |
| 219500  | Dopamine D<sub>1</sub>        | 310984 | hum   | 2    | 10 µM | -2     |
| 219700  | Dopamine D<sub>2S</sub>       | 310985 | hum   | 2    | 10 µM | 21     |
| 219800  | Dopamine D<sub>3</sub>        | 311010 | hum   | 2    | 10 µM | 9      |
| 219900  | Dopamine D<sub>4</sub>        | 310959 | hum   | 2    | 10 µM | 1      |
| 224010  | Endothelin ET<sub>A</sub>     | 311036 | hum   | 2    | 10 µM | -10    |
| 224110  | Endothelin ET<sub>B</sub>     | 311037 | hum   | 2    | 10 µM | 3      |
| 225510  | Epidermal Growth Factor (EGF) | 310884 | hum   | 2    | 10 µM | -5     |
| 226010  | Estrogen ER<sub>α</sub>       | 310897 | hum   | 2    | 10 µM | 0      |
| 226600  | GABA<sub>A</sub>, Flunitrazepam, Central | 310986 | rat   | 2    | 10 µM | 21     |
| 226500  | GABA<sub>A</sub>, Muscimol, Central | 311011 | rat   | 2    | 10 µM | 21     |
| 228610  | GABA<sub>B1A</sub>           | 310896 | hum   | 2    | 10 µM | 5      |
| 232030  | Glucocorticoid                | 311064 | hum   | 2    | 10 µM | 19     |
| 232700  | Glutamate, Kainate            | 311034 | rat   | 2    | 10 µM | -9     |
| 232810  | Glutamate, NMDA, Agonism      | 311035 | rat   | 2    | 10 µM | 4      |
| 232910  | Glutamate, NMDA, Glycine      | 310895 | rat   | 2    | 10 µM | 2      |
| 233000  | Glutamate, NMDA, Phencyclidine| 311003 | rat   | 2    | 10 µM | -3     |
| 239610  | Histamine H<sub>1</sub>       | 310934 | hum   | 2    | 10 µM | -2     |
| 239710  | Histamine H<sub>2</sub>       | 310975 | hum   | 2    | 10 µM | 10     |

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
R=See Remarks (if any) at end of this section.
ham=Hamster; hum=Human
| Cat #   | Assay Name                  | Batch* | Spec. | Rep. | Conc. | % Inh. |
|--------|-----------------------------|--------|-------|------|-------|--------|
| 239820 | Histamine H₃                | 311012 | hum   | 2    | 10 µM | -5     |
| 241000 | Imidazoline I₂, Central     | 311006 | rat   | 2    | 10 µM | -3     |
| 243520 | Interleukin IL-1            | 311266 | mouse | 2    | 10 µM | -4     |
| 250460 | Leukotriene, Cysteinyl CysLT₁ | 311007 | hum   | 2    | 10 µM | 11     |
| 251600 | Melatonin MT₁              | 310969 | hum   | 2    | 10 µM | 8      |
| 252610 | Muscarinic M₁              | 311151 | hum   | 2    | 10 µM | 2      |
| 252710 | Muscarinic M₂              | 310988 | hum   | 2    | 10 µM | 10     |
| 252810 | Muscarinic M₃              | 310989 | hum   | 2    | 10 µM | 13     |
| 257010 | Neuropeptide Y Y₁          | 310893 | hum   | 2    | 10 µM | 7      |
| 257110 | Neuropeptide Y Y₂          | 310894 | hum   | 2    | 10 µM | 4      |
| 258590 | Nicotinic Acetylcholine     | 310971 | hum   | 2    | 10 µM | -1     |
| 258700 | Nicotinic Acetylcholine α, Bungarotoxin | 31097 | hum   | 2    | 10 µM | -1     |
| 260130 | Opiate δ₁ (OP1, DOP)       | 310879 | hum   | 2    | 10 µM | -13    |
| 260210 | Opiate κ(OP2, KOP)         | 310880 | hum   | 2    | 10 µM | 7      |
| 260410 | Opiate μ(OP3, MOP)         | 310881 | hum   | 2    | 10 µM | 1      |
| 264500 | Phorbol Ester              | 310990 | mouse | 2    | 10 µM | 9      |
| 265010 | Platelet Activating Factor (PAF) | 310876 | hum   | 2    | 10 µM | 39     |
| 265600 | Potassium Channel [KATP]   | 310991 | ham   | 2    | 10 µM | 11     |
| 265900 | Potassium Channel hERG     | 310905 | hum   | 2    | 10 µM | 32     |
| 268420 | Prostanoid EP₄             | 311013 | hum   | 2    | 10 µM | -2     |
| 268700 | Purinergic P₂X             | 311014 | rabbit| 2    | 10 µM | -12    |
| 268810 | Purinergic P₂Y             | 311015 | rat   | 2    | 10 µM | 9      |
| 270000 | Rilpimpr                   | 311177 | rat   | 2    | 10 µM | 12     |
| 271110 | Serotonin (5-Hydroxytryptamine) 5-HT₁A | 311017 | hum   | 2    | 10 µM | 4      |
| 271700 | Serotonin (5-Hydroxytryptamine) 5-HT₂B | 311019 | hum   | 2    | 10 µM | 8      |
| 271910 | Serotonin (5-Hydroxytryptamine) 5-HT₃ | 310891 | hum   | 2    | 10 µM | -13    |
| 278110 | Sigma σ₁                   | 311021 | hum   | 2    | 10 µM | 38     |
| 255520 | Tachykinin NK₁             | 311044 | hum   | 2    | 10 µM | 3      |
| 258900 | Thyroid Hormone            | 310909 | rat   | 2    | 10 µM | 7      |
| 220320 | Transporter, Dopamine (DAT) | 310996 | hum   | 2    | 10 µM | 68     |
| 222400 | Transporter, GABA          | 311002 | rat   | 2    | 10 µM | 17     |
| 204410 | Transporter, Norepinephrine (NET) | 310995 | hum   | 2    | 10 µM | 28     |
| 274030 | Transporter, Serotonin (5-Hydroxytryptamine) (SERT) | 311020 | hum   | 2    | 10 µM | 5      |

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
R=See Remarks (if any) at end of this section.
ham=Hamster; hum=Human
## Panlabs Methods

**200510  Adenosine A₁**

| Source:       | Human recombinant CHO cells |
|---------------|----------------------------|
| Vehicle:      | 1% DMSO                     |
| Incubation Time/Temp: | 90 minutes @ 25°C |
| Incubation Buffer: | 20 mM HEPES, pH 7.4, 10 mM MgCl₂, 100 mM NaCl |
| Kd:           | 1.40 nM *                   |
| Ligand:       | 1.0 nM [³H] DPCPX            |
| Non-Specific Ligand: | 100 µM R(−)-PIA |
| Specific Binding: | 85% *                      |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 2.70 pmole/mg Protein *     |

**200610  Adenosine A₂A**

| Source:       | Human recombinant HEK-293 cells |
|---------------|---------------------------------|
| Vehicle:      | 1% DMSO                         |
| Incubation Time/Temp: | 90 minutes @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM EDTA, 2 U/mL Adenosine Deaminase |
| Kd:           | 0.064 µM *                     |
| Ligand:       | 0.050 µM [³H] CGS-21680        |
| Non-Specific Ligand: | 50.0 µM NECA |
| Specific Binding: | 85% *                      |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 7.0 pmole/mg Protein *         |

**200720  Adenosine A₃**

| Source:       | Human recombinant CHO-K1 cells |
|---------------|--------------------------------|
| Vehicle:      | 1% DMSO                        |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 25 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.1% BSA |
| Kd:           | 5.90 nM *                      |
| Ligand:       | 0.50 nM [¹²⁵I] AB-MECA         |
| Non-Specific Ligand: | 1.0 µM IB-MECA            |
| Specific Binding: | 83% *                      |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 1.80 pmole/mg Protein *       |

**203100  Adrenergic α₁A**

| Source:       | Wistar Rat submaxillary gland |
|---------------|--------------------------------|
| Vehicle:      | 1% DMSO                        |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA |
| Kd:           | 0.17 nM *                      |
| Ligand:       | 0.25 nM [³H] Prazosin          |
| Non-Specific Ligand: | 10.0 µM Phentolamine |
| Specific Binding: | 90% *                      |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 0.18 pmole/mg Protein *       |

* Historical Values
## Panlabs Methods

| **203200** | Adrenergic \( \alpha_{1B} \) |
|---|---|---|---|---|
| **Source:** | Wistar Rat liver | **Ligand:** | 0.25 nM \([\text{H}]\) Prazosin |
| **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10.0 µM Phentolamine |
| **Incubation Time/Temp:** | 60 minutes @ 25°C | **Specific Binding:** | 90% * |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA | **Quantitation Method:** | Radioligand Binding |
| **Kd:** | 0.31 nM * | **Significance Criteria:** | \( \geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | | | 0.18 pmole/mg Protein * |

| **203400** | Adrenergic \( \alpha_{1D} \) |
|---|---|---|---|---|
| **Source:** | Human recombinant HEK-293 cells | **Ligand:** | 0.60 nM \([\text{H}]\) Prazosin |
| **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10.0 µM Phentolamine |
| **Incubation Time/Temp:** | 60 minutes @ 25°C | **Specific Binding:** | 80% * |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4 | **Quantitation Method:** | Radioligand Binding |
| **Kd:** | 0.58 nM * | **Significance Criteria:** | \( \geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | | | 0.17 pmole/mg Protein * |

| **203620** | Adrenergic \( \alpha_{2A} \) |
|---|---|---|---|---|
| **Source:** | Human recombinant insect Sf9 cells | **Ligand:** | 1.0 nM \([\text{H}]\) MK-912 |
| **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10.0 µM WB-4101 |
| **Incubation Time/Temp:** | 60 minutes @ 25°C | **Specific Binding:** | 95% * |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 12.5 mM MgCl\(_2\), 2 mM EDTA | **Quantitation Method:** | Radioligand Binding |
| **Kd:** | 0.60 nM * | **Significance Criteria:** | \( \geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | | | 4.60 pmole/mg Protein * |

| **204010** | Adrenergic \( \beta_{1} \) |
|---|---|---|---|---|
| **Source:** | Human recombinant CHO-K1 cells | **Ligand:** | 0.030 nM \([^{125}\text{I}]\) Cyanopindolol |
| **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 100 µM S(-)-Propranolol |
| **Incubation Time/Temp:** | 2 hours @ 25°C | **Specific Binding:** | 95% * |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 5 mM EDTA, 1.5 mM CaCl\(_2\), 120 mM NaCl | **Quantitation Method:** | Radioligand Binding |
| **Kd:** | 0.041 nM * | **Significance Criteria:** | \( \geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | | | 0.072 pmole/mg Protein * |

* Historical Values
Panlabs Methods

**204110 Adrenergic β₂**

| Source: | Human recombinant CHO cells |
|---------|-----------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 5.0 mM MgCl₂, 120 mM NaCl |
| Kd: | 0.44 nM * |
| Ligand: | 0.20 nM [³H] CGP-12177 |
| Non-Specific Ligand: | 10.0 µM ICI-l118551 |
| Specific Binding: | 95% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 0.44 pmole/mg Protein * |

**285010 Androgen (Testosterone) AR**

| Source: | Rat recombinant E. coli |
|---------|-------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 4 hours @ 4°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 0.8 M NaCl, 10% Glycerol, 2 mM Dithiothreitol, 0.1% BSA, 2% EtOH |
| Kd: | 3.0 nM * |
| Ligand: | 1.50 nM [³H] Mibolerone |
| Non-Specific Ligand: | 10.0 µM Mibolerone |
| Specific Binding: | 90% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 930 pmole/mg Protein * |

**212510 Bradykinin B₁**

| Source: | Human IMR-90 cells |
|---------|-------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 20 mM HEPES, pH 7.4, 125 mM N-Methyl-D-glucamine, 5 mM KCl, 1 mM 1,10-Phenanthroline, 140 µg/ml Bacitracin |
| Kd: | 0.17 nM * |
| Ligand: | 0.50 nM [³H] (Des-Arg¹⁰)-Kallidin |
| Non-Specific Ligand: | 10.0 µM (Des-Arg⁹, Leu⁸)-Bradykinin |
| Specific Binding: | 80% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 0.55 pmole/mg Protein * |

**212620 Bradykinin B₂**

| Source: | Human recombinant Chem-1 cells |
|---------|-------------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 50 mM HEPES, pH 7.4, 0.2% BSA, 1 mM CaCl₂, 5 mM MgCl₂ |
| Kd: | 0.85 nM * |
| Ligand: | 0.50 nM [³H] Bradykinin |
| Non-Specific Ligand: | 5.0 µM Bradykinin |
| Specific Binding: | 90% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 9.40 pmole/mg Protein * |

* Historical Values
## Panlabs Methods

| Experiment | Source | Ligand | Non-Specific Ligand | Specific Binding | Quantitation Method | Significance Criteria |
|------------|--------|--------|---------------------|------------------|---------------------|-----------------------|
| 214510     | Wistar Rat brain | 2.0 nM $[^3]H$ Diltiazem | 10.0 µM Diltiazem | 73% * | Radioligand Binding | ≥50% of max stimulation or inhibition |
|            |        | Kd: 0.016 µM * | | | | |
| 214600     | Wistar Rat cerebral cortex | 0.10 nM $[^3]H$ Nitrendipine | 1.0 µM Nifedipine | 91% * | Radioligand Binding | ≥50% of max stimulation or inhibition |
|            |        | Kd: 0.18 nM * | | | | |
| 216000     | Wistar Rat frontal brain | 10 pM $[^{125}]$ ω-Conotoxin GVIA | 0.10 µM ω-Conotoxin GVIA | 96% * | Radioligand Binding | ≥50% of max stimulation or inhibition |
|            |        | Kd: 0.051 nM * | | | | |
| 217030     | Human recombinant Chem-1 cells | 2.0 nM $[^3]H$ SR141716A | 10.0 µM R(+)WIN-55,212-2 | 70% * | Radioligand Binding | ≥50% of max stimulation or inhibition |
|            |        | Kd: 5.90 nM * | | | | |

* Historical Values
|                | Dopamine D<sub>1</sub>                                                                 | Ligand: 1.40 nM [³H] SCH-23990 | Non-Specific Ligand: 10.0 µM (+)-Butaclamol | Specific Binding: 90% * | Quantitation Method: Radioligand Binding |
|----------------|----------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------|------------------------|----------------------------------------|
| Source:        | Human recombinant CHO cells                                                               |                                  |                                             |                        |                                        |
| Vehicle:       | 1% DMSO                                                                                 |                                  |                                             |                        |                                        |
| Incubation Time/Temp:       | 2 hours @ 37°C                                                                            |                                  |                                             |                        |                                        |
| Incubation Buffer:       | 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl                  |                                  |                                             |                        |                                        |
| Kd:             | 1.40 nM *                                                                               |                                  |                                             |                        |                                        |

|                | Dopamine D<sub>2S</sub>                                                                  | Ligand: 0.16 nM [³H] Spiperone   | Non-Specific Ligand: 10.0 µM Haloperidol     | Specific Binding: 90% * | Quantitation Method: Radioligand Binding |
|----------------|----------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------|------------------------|----------------------------------------|
| Source:        | Human recombinant CHO cells                                                               |                                  |                                             |                        |                                        |
| Vehicle:       | 1% DMSO                                                                                 |                                  |                                             |                        |                                        |
| Incubation Time/Temp:       | 2 hours @ 25°C                                                                            |                                  |                                             |                        |                                        |
| Incubation Buffer:       | 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl                  |                                  |                                             |                        |                                        |
| Kd:             | 0.090 nM *                                                                              |                                  |                                             |                        |                                        |

|                | Dopamine D<sub>3</sub>                                                                  | Ligand: 0.70 nM [³H] Spiperone   | Non-Specific Ligand: 25.0 µM S(-)-Sulpiride | Specific Binding: 85% * | Quantitation Method: Radioligand Binding |
|----------------|----------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------|------------------------|----------------------------------------|
| Source:        | Human recombinant CHO cells                                                               |                                  |                                             |                        |                                        |
| Vehicle:       | 1% DMSO                                                                                 |                                  |                                             |                        |                                        |
| Incubation Time/Temp:       | 2 hours @ 37°C                                                                            |                                  |                                             |                        |                                        |
| Incubation Buffer:       | 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl                  |                                  |                                             |                        |                                        |
| Kd:             | 0.36 nM *                                                                               |                                  |                                             |                        |                                        |

|                | Dopamine D<sub>4.2</sub>                                                                | Ligand: 0.50 nM [³H] Spiperone   | Non-Specific Ligand: 10.0 µM Haloperidol     | Specific Binding: 90% * | Quantitation Method: Radioligand Binding |
|----------------|----------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------|------------------------|----------------------------------------|
| Source:        | Human recombinant CHO-K1 cells                                                          |                                  |                                             |                        |                                        |
| Vehicle:       | 1% DMSO                                                                                 |                                  |                                             |                        |                                        |
| Incubation Time/Temp:       | 2 hours @ 25°C                                                                            |                                  |                                             |                        |                                        |
| Incubation Buffer:       | 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl                  |                                  |                                             |                        |                                        |
| Kd:             | 0.32 nM *                                                                               |                                  |                                             |                        |                                        |

* Historical Values
### Panlabs Methods

#### 224010 Endothelin ETA

| Source: | Human recombinant CHO-K1 cells |
|---------|---------------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 2 hours @ 37°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 0.1% BSA, 0.5 mM CaCl₂, 0.05% Tween-20 |
| Kd: | 0.048 nM * |
| Ligand: | 0.030 nM [¹²⁵I] Endothelin-1 |
| Non-Specific Ligand: | 0.10 µM Endothelin-1 |
| Specific Binding: | 90% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 0.35 pmole/mg Protein * |

#### 224110 Endothelin ETB

| Source: | Human recombinant CHO-K1 cells |
|---------|---------------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 2 hours @ 25°C |
| Incubation Buffer: | 50 mM HEPES, pH 7.4, 1 mM CaCl₂, 5 mM MgCl₂, 0.5% BSA |
| Kd: | 0.085 nM * |
| Ligand: | 0.10 nM [¹²⁵I] Endothelin-1 |
| Non-Specific Ligand: | 0.10 µM Endothelin-1 |
| Specific Binding: | 75% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 4.30 pmole/mg Protein * |

#### 225510 Epidermal Growth Factor (EGF)

| Source: | Human A431 cells |
|---------|------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 60 minutes @ 25°C |
| Incubation Buffer: | 50 mM HEPES, pH 7.7, 0.1% BSA, 1.2 mM CaCl₂, 5 mM KCl, 1.2 mM MgSO₄, 138 mM NaCl |
| Kd: | 0.17 nM * |
| Ligand: | 0.080 nM [¹²⁵I] EGF (human) |
| Non-Specific Ligand: | 0.10 µM EGF (human) |
| Specific Binding: | 90% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 5.50 pmole/mg Protein * |

#### 226010 Estrogen ERα

| Source: | Human recombinant insect Sf9 cells |
|---------|-----------------------------------|
| Vehicle: | 1% DMSO |
| Incubation Time/Temp: | 2 hours @ 25°C |
| Incubation Buffer: | 10 mM Tris-HCl, pH 7.4, 0.1% BSA, 10% Glycerol, 1 mM DTT |
| Kd: | 0.20 nM * |
| Ligand: | 0.50 nM [³H] Estradiol |
| Non-Specific Ligand: | 1.0 µM Diethylstilbestrol |
| Specific Binding: | 85% * |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax: | 1400 pmole/mg Protein * |

* Historical Values
### Panlabs Methods

| Experiment ID | Source | Ligand | Vehicle | Incubation Time/Temp | Specific Binding | Non-Specific Ligand | Kd | Significance Criteria | Bmax |
|---------------|--------|--------|---------|----------------------|-----------------|---------------------|----|----------------------|------|
| 226600        | GABA<sub>‡</sub>, Flunitrazepam, Central | 1.0 nM [³H] Flunitrazepam | 1% DMSO | 60 minutes @ 25°C | 91% * | 10.0 µM Diazepam | 4.40 nM * | ≥50% of max stimulation or inhibition | 1.20 pmole/mg Protein * |
| 226500        | GABA<sub>‡</sub>, Muscimol, Central | 1.0 nM [³H] Muscimol | 1% DMSO | 10 minutes @ 4°C | 90% * | 0.10 µM Muscimol | 3.80 nM * | ≥50% of max stimulation or inhibition | 1.80 pmole/mg Protein * |
| 228610        | GABA<sub>B1A</sub> | 4.0 nM [³H] CGP-54626 | 1% DMSO | 3 hours @ 25°C | 90% * | 3.0 mM GABA | 3.30 nM * | ≥50% of max stimulation or inhibition | 48.0 pmole/mg Protein * |
| 232030        | Glucocorticoid | 5.0 nM [³H] Dexamethasone | 1% DMSO | 1 day @ 4°C | 97% * | 10.0 µM Dexamethasone | 4.60 nM * | ≥50% of max stimulation or inhibition | 1.0 pmole/mg * |

* Historical Values
Panlabs Methods

■ 232700  Glutamate, Kainate

Source: Wistar Rat brain (minus cerebellum)  
Vehicle: 1% DMSO  
Incubation Time/Temp: 60 minutes @ 4°C  
Incubation Buffer: 50 mM Tris-HCl, pH 7.4  
Kd: 0.012 µM *  
Ligand: 5.0 nM [3H] Kainic acid  
Non-Specific Ligand: 1.0 mM L-Glutamic acid  
Specific Binding: 80% *  
Quantiation Method: Radioligand Binding  
Significance Criteria: ≥50% of max stimulation or inhibition  
Bmax: 0.35 pmole/mg Protein *

■ 232810  Glutamate, NMDA, Agonism

Source: Wistar Rat cerebral cortex  
Vehicle: 1% DMSO  
Incubation Time/Temp: 20 minutes @ 4°C  
Incubation Buffer: 50 mM Tris-HCl, pH 7.4  
Kd: 0.019 µM *  
Ligand: 2.0 nM [3H] CGP-39653  
Non-Specific Ligand: 1.0 mM L-Glutamic acid  
Specific Binding: 70% *  
Quantiation Method: Radioligand Binding  
Significance Criteria: ≥50% of max stimulation or inhibition  
Bmax: 2.30 pmole/mg Protein *

■ 232910  Glutamate, NMDA, Glycine

Source: Wistar Rat cerebral cortex  
Vehicle: 1% DMSO  
Incubation Time/Temp: 30 minutes @ 4°C  
Incubation Buffer: 50 mM HEPES, pH 7.7  
Kd: 6.0 nM *  
Ligand: 0.33 nM [3H] MDL 105,519  
Non-Specific Ligand: 10.0 µM MDL 105,519  
Specific Binding: 85% *  
Quantiation Method: Radioligand Binding  
Significance Criteria: ≥50% of max stimulation or inhibition  
Bmax: 3.70 pmole/mg Protein *

■ 233000  Glutamate, NMDA, Phencyclidine

Source: Wistar Rat cerebral cortex  
Vehicle: 1% DMSO  
Incubation Time/Temp: 45 minutes @ 25°C  
Incubation Buffer: 10 mM Tris-HCl, pH 7.4  
Kd: 8.40 nM *  
Ligand: 4.0 nM [3H] TCP  
Non-Specific Ligand: 1.0 µM Dizocilipine ((+)-MK-801)  
Specific Binding: 94% *  
Quantiation Method: Radioligand Binding  
Significance Criteria: ≥50% of max stimulation or inhibition  
Bmax: 0.78 pmole/mg Protein *

* Historical Values
# Panlabs Methods

## 239610 Histamine H₁

| Source:        | Human recombinant CHO-K1 cells |
|---------------|-------------------------------|
| Vehicle:      | 1% DMSO                       |
| Incubation Time/Temp: | 3 hours @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 2 mM MgCl₂, 100 mM NaCl, 250 mM Sucrose |
| Kd:           | 1.10 nM *                     |
| Ligand:       | 1.20 nM [³H] Pyrilamine       |
| Non-Specific Ligand: | 1.0 µM Pyrilamine |
| Specific Binding: | 94% *                        |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 6.70 pmole/mg Protein *       |

## 239710 Histamine H₂

| Source:        | Human recombinant CHO-K1 cells |
|---------------|-------------------------------|
| Vehicle:      | 1% DMSO                       |
| Incubation Time/Temp: | 2 hours @ 25°C |
| Incubation Buffer: | 50 mM Phosphate, pH 7.4 |
| Kd:           | 0.45 nM *                     |
| Ligand:       | 0.10 nM [¹²⁵I] Aminopotentidine |
| Non-Specific Ligand: | 3.0 µM Tiotidine |
| Specific Binding: | 90% *                        |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 6.90 pmole/mg Protein *       |

## 239820 Histamine H₃

| Source:        | Human recombinant CHO-K1 cells |
|---------------|-------------------------------|
| Vehicle:      | 1% DMSO                       |
| Incubation Time/Temp: | 2 hours @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.1% BSA |
| Kd:           | 0.38 nM *                     |
| Ligand:       | 0.40 nM [³H] N-α-Methylhistamine (NAMH) |
| Non-Specific Ligand: | 1.0 µM R(−)-α-Methylhistamine (RAMH) |
| Specific Binding: | 90% *                        |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 2.0 pmole/mg Protein *        |

## 241000 Imidazoline I₂, Central

| Source:        | Wistar Rat cerebral cortex |
|---------------|----------------------------|
| Vehicle:      | 1% DMSO                     |
| Incubation Time/Temp: | 30 minutes @ 25°C |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA |
| Kd:           | 4.0 nM *                    |
| Ligand:       | 2.0 nM [³H] Idazoxan        |
| Non-Specific Ligand: | 1.0 µM Idazoxan |
| Specific Binding: | 85% *                        |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:         | 0.14 pmole/mg Protein *     |

* Historical Values
## Panlabs Methods

### 243520  Interleukin IL-1

| Source               | Mouse 3T3-SWISS cells                               | Ligand                  | 0.10 nM $[^{25}I]$ Interleukin-1β |
|----------------------|-----------------------------------------------------|--------------------------|-----------------------------------|
| Vehicle              | 1% DMSO                                             | Non-Specific Ligand      | 10.0 µM Interleukin-1β            |
| Incubation Time/Temp | 2 hours @ 37°C                                      | Specific Binding         | 80% *                             |
| Incubation Buffer    | RPMI 1640, 20 mM HEPES, pH 7.4, 0.1% Sodium Azide, 1% BSA | Quantiation Method       | Radioligand Binding               |
| $K_d$                | 0.25 nM *                                           | Significance Criteria    | ≥50% of max stimulation or inhibition |

### 250460  Leukotriene, Cysteinyll CysLT$_1$

| Source               | Human recombinant CHO-K1 cells                      | Ligand                  | 0.30 nM $[^{3}H]$ LTD$_4$ |
|----------------------|-----------------------------------------------------|--------------------------|---------------------------|
| Vehicle              | 1% DMSO                                             | Non-Specific Ligand      | 0.30 µM LTD$_4$           |
| Incubation Time/Temp | 30 minutes @ 25°C                                   | Specific Binding         | 93% *                     |
| Incubation Buffer    | 50 mM Tris-HCl, pH 7.4, 5 mM CaCl$_2$, 5 mM MgCl$_2$, 100 µg/ml Bacitracin, 1 mM Benazamidine, 0.1 mM PMSF | Quantification Method    | Radioligand Binding       |
| $K_d$                | 0.21 nM *                                           | Significance Criteria    | ≥50% of max stimulation or inhibition |

### 251600  Melatonin MT$_1$

| Source               | Human recombinant CHO-K1 cells                      | Ligand                  | 0.050 nM $[^{125}I]$ 2-Iodomelatonin |
|----------------------|-----------------------------------------------------|--------------------------|---------------------------------------|
| Vehicle              | 1% DMSO                                             | Non-Specific Ligand      | 1.0 µM 6-Chloromelatonin              |
| Incubation Time/Temp | 3 hours @ 25°C                                      | Specific Binding         | 97% *                                 |
| Incubation Buffer    | 25 mM HEPES, pH 7.4, 5 mM MgCl$_2$, 1 mM CaCl$_2$, 0.5% BSA | Quantification Method    | Radioligand Binding                   |
| $K_d$                | 0.054 nM *                                          | Significance Criteria    | ≥50% of max stimulation or inhibition |

### 252610  Muscarinic M$_1$

| Source               | Human recombinant CHO-K1 cells                      | Ligand                  | 0.80 nM $[^{3}H]$ N-Methylscopolamine |
|----------------------|-----------------------------------------------------|--------------------------|---------------------------------------|
| Vehicle              | 1% DMSO                                             | Non-Specific Ligand      | 1.0 µM Atropine                       |
| Incubation Time/Temp | 2 hours @ 25°C                                      | Specific Binding         | 95% *                                 |
| Incubation Buffer    | 50 mM Tris-HCl, pH 7.4, 10 mM MgCl$_2$, 1 mM EDTA | Quantification Method    | Radioligand Binding                   |
| $K_d$                | 0.26 nM *                                           | Significance Criteria    | ≥50% of max stimulation or inhibition |

* Historical Values
## Panlabs Methods

| 252710  | Muscarinic M\(_2\) |
|----------|---------------------|
| **Source:** | Human recombinant CHO-K1 cells |
| **Vehicle:** | 1% DMSO |
| **Incubation Time/Temp:** | 2 hours @ 25°C |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 10 mM MgCl\(_2\), 1 mM EDTA |
| **Ligand:** | 0.80 nM \(^{3}H\) N-Methylscopolamine |
| **Non-Specific Ligand:** | 1.0 µM Atropine |
| **Specific Binding:** | 95% * |
| **Quantitation Method:** | Radioligand Binding |
| **Significance Criteria:** | \(\geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | 5.10 pmole/mg Protein * |

| 252810  | Muscarinic M\(_3\) |
|----------|---------------------|
| **Source:** | Human recombinant CHO-K1 cells |
| **Vehicle:** | 1% DMSO |
| **Incubation Time/Temp:** | 2 hours @ 25°C |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 10 mM MgCl\(_2\), 1 mM EDTA |
| **Ligand:** | 0.80 nM \(^{3}H\) N-Methylscopolamine |
| **Non-Specific Ligand:** | 1.0 µM Atropine |
| **Specific Binding:** | 95% * |
| **Quantitation Method:** | Radioligand Binding |
| **Significance Criteria:** | \(\geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | 5.40 pmole/mg Protein * |

| 257010  | Neuropeptide Y Y\(_1\) |
|----------|---------------------|
| **Source:** | Human SK-N-MC cells |
| **Vehicle:** | 1% DMSO |
| **Incubation Time/Temp:** | 60 minutes @ 37°C |
| **Incubation Buffer:** | 25 mM HEPES, pH 7.4, 1 mM MgCl\(_2\), 2.5 mM CaCl\(_2\), 0.1% BSA, 0.01% Bacitracin |
| **Ligand:** | 0.015 nM \(^{125}\)I Peptide YY |
| **Non-Specific Ligand:** | 1.0 µM Neuropeptide Y (human, rat) |
| **Specific Binding:** | 80% * |
| **Quantitation Method:** | Radioligand Binding |
| **Significance Criteria:** | \(\geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | 0.58 pmole/mg protein * |

| 257110  | Neuropeptide Y Y\(_2\) |
|----------|---------------------|
| **Source:** | Human KAN-TS cells |
| **Vehicle:** | 1% DMSO |
| **Incubation Time/Temp:** | 2 hours @ 37°C |
| **Incubation Buffer:** | 25 mM HEPES, pH 7.4, 2.5 mM CaCl\(_2\), 1 mM MgCl\(_2\), 0.1% Bacitracin |
| **Ligand:** | 10 pM \(^{125}\)I Peptide YY |
| **Non-Specific Ligand:** | 1.0 µM Neuropeptide Y (13-36) (porcine) |
| **Specific Binding:** | 90% * |
| **Quantitation Method:** | Radioligand Binding |
| **Significance Criteria:** | \(\geq 50\% \) of max stimulation or inhibition |
| **Bmax:** | 0.5 pmole/mg Protein * |

* Historical Values
## Panlabs Methods

### 258590 Nicotinic Acetylcholine

| Source | Human IMR-32 cells |
|--------|-------------------|
| Vehicle | 1% DMSO |
| Incubation Time/Temp | 60 minutes @ 25°C |
| Incubation Buffer | 20 mM HEPES, pH 7.5, 150 mM NaCl, 1.5 mM KCl, 2 mM CaCl₂, 1 mM MgSO₄. |
| Kd | 0.22 nM * |
| Ligand | 0.10 nM [¹²⁵I] Epibatidine |
| Non-Specific Ligand | 300 µM (-)-Nicotine |
| Specific Binding | 97% * |
| Significance Criteria | ≥50% of max stimulation or inhibition |
| Bmax | 0.46 pmole/mg Protein * |
| Quantiation Method | Radioligand Binding |

### 258700 Nicotinic Acetylcholine α, Bungarotoxin

| Source | Human RD cells |
|--------|----------------|
| Vehicle | 1% DMSO |
| Incubation Time/Temp | 2 hours @ 25°C |
| Incubation Buffer | 150 mM NaCl, 4 mM KCl, 2.3 mM CaCl₂ |
| Kd | 1.10 nM * |
| Ligand | 0.60 nM [¹²⁵I] α-Bungarotoxin |
| Non-Specific Ligand | 1.0 µM α-Bungarotoxin |
| Specific Binding | 85% * |
| Significance Criteria | ≥50% of max stimulation or inhibition |
| Bmax | 1.0 pmole/mg Protein * |
| Quantiation Method | Radioligand Binding |

### 260130 Opiate δ₁ (OP1, DOP)

| Source | Human recombinant HEK-293 cells |
|--------|---------------------------------|
| Vehicle | 1% DMSO |
| Incubation Time/Temp | 60 minutes @ 25°C |
| Incubation Buffer | 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 mM MgCl₂ |
| Kd | 0.27 nM * |
| Ligand | 1.30 nM [³H] Naltrindole |
| Non-Specific Ligand | 1.0 µM Naltrindole |
| Specific Binding | 95% * |
| Significance Criteria | ≥50% of max stimulation or inhibition |
| Bmax | 7.60 pmole/mg Protein * |
| Quantiation Method | Radioligand Binding |

### 260210 Opiate κ(OP2, KOP)

| Source | Human recombinant HEK-293 cells |
|--------|---------------------------------|
| Vehicle | 1% DMSO |
| Incubation Time/Temp | 60 minutes @ 25°C |
| Incubation Buffer | 50 mM Tris-HCl, pH 7.4 |
| Kd | 0.40 nM * |
| Ligand | 0.60 nM [³H] Diprenorphine |
| Non-Specific Ligand | 10.0 µM Naloxone |
| Specific Binding | 90% * |
| Significance Criteria | ≥50% of max stimulation or inhibition |
| Bmax | 1.10 pmole/mg Protein * |
| Quantiation Method | Radioligand Binding |

* Historical Values
### Panlabs Methods

**260410 Opiate µ(OP3, MOP)**

| Source                  | Human recombinant CHO-K1 cells |
|-------------------------|-------------------------------|
| Vehicle                 | 1% DMSO                       |
| **Incubation Time/Temp:**| 60 minutes @ 25°C             |
| Incubation Buffer       | 50 mM Tris-HCl, pH 7.4        |
| **Kd:**                 | 0.41 nM *                     |
| **Ligand:**             | 0.60 nM [3H] Diprenorphine    |
| **Non-Specific Ligand:**| 10.0 µM Naloxone              |
| **Specific Binding:**   | 90% *                         |
| **Quantiation Method:** | Radioligand Binding           |
| **Significance Criteria:**| ≥50% of max stimulation or inhibition |
| **Bmax:**               | 3.80 pmole/mg Protein *       |

**264500 Phorbol Ester**

| Source                  | ICR Mouse brain              |
|-------------------------|-------------------------------|
| Vehicle                 | 1% DMSO                       |
| **Incubation Time/Temp:**| 60 minutes @ 25°C             |
| Incubation Buffer       | 20 mM Tris-HCl, pH 7.4, 5 mM CaCl₂ |
| **Kd:**                 | 8.70 nM *                     |
| **Ligand:**             | 3.0 nM [3H] PDBu              |
| **Non-Specific Ligand:**| 1.0 µM PDBu                   |
| **Specific Binding:**   | 80% *                         |
| **Quantiation Method:** | Radioligand Binding           |
| **Significance Criteria:**| ≥50% of max stimulation or inhibition |
| **Bmax:**               | 26.0 pmole/mg Protein *       |

**265010 Platelet Activating Factor (PAF)**

| Source                  | Human platelets              |
|-------------------------|-------------------------------|
| Vehicle                 | 1% DMSO                       |
| **Incubation Time/Temp:**| 3 hours @ 25°C                |
| Incubation Buffer       | 50 mM Tris-HCl, pH 7.4, 100 mM KCl, 5 mM EDTA, 5 mM MgCl₂, 0.25% BSA |
| **Kd:**                 | 0.13 nM *                     |
| **Ligand:**             | 0.12 nM [3H] PAF              |
| **Non-Specific Ligand:**| 1.0 µM PAF                    |
| **Specific Binding:**   | 90% *                         |
| **Quantiation Method:** | Radioligand Binding           |
| **Significance Criteria:**| ≥50% of max stimulation or inhibition |
| **Bmax:**               | 120 R/cell *                  |

**265600 Potassium Channel [K<sub>ATP</sub>]**

| Source                  | Hamster pancreatic HIT-T15 beta cells |
|-------------------------|---------------------------------------|
| Vehicle                 | 1% DMSO                               |
| **Incubation Time/Temp:**| 2 hours @ 25°C                        |
| Incubation Buffer       | 50 mM MOPS, pH 7.4, 0.1 mM CaCl₂      |
| **Kd:**                 | 0.64 nM *                             |
| **Ligand:**             | 5.0 nM [3H] Glyburide                |
| **Non-Specific Ligand:**| 1.0 µM Glyburide                      |
| **Specific Binding:**   | 90% *                                 |
| **Quantiation Method:** | Radioligand Binding                  |
| **Significance Criteria:**| ≥50% of max stimulation or inhibition |
| **Bmax:**               | 1.0 pmole/mg Protein *                |

* Historical Values
## Panlabs Methods

### 265900  Potassium Channel hERG

| Source:             | Human recombinant HEK-293 cells |
|---------------------|---------------------------------|
| Vehicle:            | 1% DMSO                         |
| Incubation Time/Temp: | 60 minutes @ 25°C               |
| Incubation Buffer:  | 10 mM HEPES, pH 7.4, 0.1% BSA, 5 mM KCl, 0.8 mM MgCl₂, 130 mM NaCl, 1 mM EGTA, 10 mM Glucose |
| Kd:                 | 6.80 nM *                       |
| Ligand:             | 1.50 nM [³H] Astemizole         |
| Non-Specific Ligand:| 10.0 µM Astemizole              |
| Specific Binding:   | 90% *                           |
| Quantitation Method:| Radioligand Binding             |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:               | 6.30 pmole/mg Protein *         |

### 268420  Prostanoid EP₄

| Source:             | Human recombinant Chem-1 cells |
|---------------------|--------------------------------|
| Vehicle:            | 1% DMSO                         |
| Incubation Time/Temp: | 2 hours @ 25°C                 |
| Incubation Buffer:  | 10 mM MES, pH 6.0, 1 mM EDTA, 10 mM MgCl₂ |
| Kd:                 | 0.69 nM *                       |
| Ligand:             | 1.0 nM [³H] Prostaglandin E₂ (PGE₂) |
| Non-Specific Ligand:| 10.0 µM Prostaglandin E₂ (PGE₂) |
| Specific Binding:   | 90% *                           |
| Quantitation Method:| Radioligand Binding             |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:               | 4.30 pmole/mg Protein *         |

### 268700  Purinergic P₂X

| Source:             | New Zealand derived albino Rabbit urinary bladder |
|---------------------|---------------------------------------------------|
| Vehicle:            | 1% DMSO                                           |
| Incubation Time/Temp: | 30 minutes @ 25°C               |
| Incubation Buffer:  | 50 mM Tris-HCl, pH 7.4               |
| Kd1:                | 2.20 nM *                                     |
| Kd2:                | 2.20 µM *                                      |
| Ligand:             | 8.0 nM [³H] α, β-Methylene-ATP                 |
| Non-Specific Ligand:| 100 µM β, γ-Methylene ATP                   |
| Specific Binding:   | 80% *                                           |
| Quantitation Method:| Radioligand Binding                          |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax1:              | 2.0 pmole/mg Protein *                        |
| Bmax2:              | 790 pmole/mg Protein *                       |

### 268810  Purinergic P₂Y

| Source:             | Wistar Rat brain                             |
|---------------------|----------------------------------------------|
| Vehicle:            | 1% DMSO                                      |
| Incubation Time/Temp: | 60 minutes @ 25°C               |
| Incubation Buffer:  | 50 mM Tris-HCl, pH 7.4               |
| Kd:                 | 0.015 µM *                               |
| Ligand:             | 0.10 nM [³⁵S] ATP-αS                        |
| Non-Specific Ligand:| 10.0 µM ADP-βS                           |
| Specific Binding:   | 87% *                                       |
| Quantitation Method:| Radioligand Binding                          |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:               | 16.0 pmole/mg Protein *                     |

* Historical Values
# Panlabs Methods

| ID   | Source                        | Ligand                                      | Specific Binding | Kd       | Bmax        |
|------|-------------------------------|---------------------------------------------|------------------|----------|-------------|
| 270000 | Wistar Rat brain             | 1.80 nM [³H] Rolipram                       | 90% *            | 1.0 nM * | 0.31 pmole/mg Protein * |
|      |                               | Non-Specific Ligand: 10.0 μM Rolipram       |                  |          |             |
|      |                               | Quantification Method: Radioligand Binding  |                  |          |             |
|      |                               | Significance Criteria: ≥50% of max stimulation or inhibition | |          |             |
|      | Vehicle: 1% DMSO              |                                             |                  |          |             |
|      | Incubation Time/Temp: 60 minutes @ 4°C |                                             |                  |          |             |
|      | Incubation Buffer: 50 mM Tris-HCl, pH 7.4 |                                             |                  |          |             |

| ID   | Source                        | Ligand                                      | Specific Binding | Kd       | Bmax        |
|------|-------------------------------|---------------------------------------------|------------------|----------|-------------|
| 271110 | Human recombinant CHO-K1 cells | 1.50 nM [³H] 8-OH-DPAT                     | 75% *            | 2.0 nM * | 1.30 pmole/mg Protein * |
|      |                               | Non-Specific Ligand: 10.0 μM Metergoline    |                  |          |             |
|      |                               | Quantification Method: Radioligand Binding  |                  |          |             |
|      |                               | Significance Criteria: ≥50% of max stimulation or inhibition | |          |             |
|      | Vehicle: 1% DMSO              |                                             |                  |          |             |
|      | Incubation Time/Temp: 60 minutes @ 25°C |                                             |                  |          |             |
|      | Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | 0.1% Ascorbic Acid, 0.5 mM EDTA, 10 mM MgSO₄ |                  |          |             |

| ID   | Source                        | Ligand                                      | Specific Binding | Kd       | Bmax        |
|------|-------------------------------|---------------------------------------------|------------------|----------|-------------|
| 271700 | Human recombinant CHO-K1 cells | 1.20 nM [³H] Lysergic acid diethylamide (LSD) | 80% *            | 2.10 nM *| 1.10 pmole/mg Protein * |
|      |                               | Non-Specific Ligand: 10.0 μM Serotonin (5-HT) |                  |          |             |
|      |                               | Quantification Method: Radioligand Binding  |                  |          |             |
|      |                               | Significance Criteria: ≥50% of max stimulation or inhibition | |          |             |
|      | Vehicle: 1% DMSO              |                                             |                  |          |             |
|      | Incubation Time/Temp: 60 minutes @ 37°C |                                             |                  |          |             |
|      | Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | 4 mM CaCl₂, 0.1% Ascorbic Acid |                  |          |             |

| ID   | Source                        | Ligand                                      | Specific Binding | Kd       | Bmax        |
|------|-------------------------------|---------------------------------------------|------------------|----------|-------------|
| 271910 | Human recombinant HEK-293 cells | 0.69 nM [³H] GR-65630                      | 90% *            | 0.20 nM *| 11.0 pmole/mg Protein * |
|      |                               | Non-Specific Ligand: 10.0 μM MDL 72222      |                  |          |             |
|      |                               | Quantification Method: Radioligand Binding  |                  |          |             |
|      |                               | Significance Criteria: ≥50% of max stimulation or inhibition | |          |             |
|      | Vehicle: 1% DMSO              |                                             |                  |          |             |
|      | Incubation Time/Temp: 60 minutes @ 25°C |                                             |                  |          |             |
|      | Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | 1 mM EDTA, 5 mM MgCl₂ |                  |          |             |

* Historical Values
**Panlabs Methods**

| **278110** Sigma σ₁ | **Ligand:** 8.0 nM [³H] Haloperidol |
|---------------------|-------------------------------------|
| **Source:** Human Jurkat cells | **Non-Specific Ligand:** 10.0 µM Haloperidol |
| **Vehicle:** 1% DMSO | **Specific Binding:** 80% * |
| **Incubation Time/Temp:** 4 hours @ 25°C | **Quantitation Method:** Radioligand Binding |
| **Incubation Buffer:** 5 mM Potassium Phosphate, pH 7.5 | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Kd:** 5.80 nM * | **Bmax:** 0.71 pmole/mg Protein * |

| **255520** Tachykinin NK₁ | **Ligand:** 0.80 nM [³H] Substance P |
|---------------------------|-------------------------------------|
| **Source:** Human recombinant CHO cells | **Non-Specific Ligand:** 10.0 µM L-703,606 |
| **Vehicle:** 1% DMSO | **Specific Binding:** 90% * |
| **Incubation Time/Temp:** 90 minutes @ 4°C | **Quantitation Method:** Radioligand Binding |
| **Incubation Buffer:** 20 mM HEPES, pH 7.4, 1 mM MnCl₂, 0.1% BSA | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Kd:** 2.10 nM * | **Bmax:** 1.70 pmole/mg Protein * |

| **285900** Thyroid Hormone | **Ligand:** 0.030 nM [¹²³I] Triiodothyronine |
|---------------------------|-------------------------------------|
| **Source:** Wistar Rat liver | **Non-Specific Ligand:** 1.0 µM Triiodothyronine |
| **Vehicle:** 1% DMSO | **Specific Binding:** 77% * |
| **Incubation Time/Temp:** 18 hours @ 4°C | **Quantitation Method:** Radioligand Binding |
| **Incubation Buffer:** 20 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10% Glycerol, 2 mM EDTA, 5 mM DTT | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Kd:** 0.034 nM * | **Bmax:** 0.16 pmole/mg Protein * |

| **220320** Transporter, Dopamine (DAT) | **Ligand:** 0.15 nM [¹²⁵] RTI-55 |
|-----------------------------|-------------------------------------|
| **Source:** Human recombinant CHO-K1 cells | **Non-Specific Ligand:** 10.0 µM Nomifensine |
| **Vehicle:** 1% DMSO | **Specific Binding:** 90% * |
| **Incubation Time/Temp:** 3 hours @ 4°C | **Quantitation Method:** Radioligand Binding |
| **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 µM Leupeptin, 10 µM PMSF | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Kd:** 0.58 nM * | **Bmax:** 0.047 pmole/mg Protein * |

* Historical Values
Panlabs Methods

**226400**  Transporter, GABA

| Source:       | Wistar Rat cerebral cortex |
|--------------|-----------------------------|
| Vehicle:     | 1% DMSO                     |
| Incubation Time/Temp: | 20 minutes @ 25°C |
| Incubation Buffer: | 10 mM HEPES, pH 7.5, 120 mM NaCl, 4 mM Ca(CH3COO)2, 10 µM Isoguvacine, 10 µM S(-)-Baclofen |
| Kd:          | 0.30 µM *                   |
| Ligand:      | 6.0 nM [3H] GABA            |
| Non-Specific Ligand: | 10.0 µM NO-711 |
| Specific Binding: | 80% *                      |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:        | 60.0 pmole/mg Protein *     |

**204410**  Transporter, Norepinephrine (NET)

| Source:       | Human recombinant MDCK cells |
|--------------|------------------------------|
| Vehicle:     | 1% DMSO                      |
| Incubation Time/Temp: | 3 hours @ 4°C               |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 µM Leupeptin, 10 µM PMSF |
| Kd:          | 0.024 µM *                   |
| Ligand:      | 0.20 nM [125I] RTI-55        |
| Non-Specific Ligand: | 10.0 µM Desipramine |
| Specific Binding: | 75% *                       |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:        | 2.50 pmole/mg Protein *      |

**274030**  Transporter, Serotonin (5-Hydroxytryptamine) (SERT)

| Source:       | Human recombinant HEK-293 cells |
|--------------|---------------------------------|
| Vehicle:     | 1% DMSO                         |
| Incubation Time/Temp: | 60 minutes @ 25°C              |
| Incubation Buffer: | 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl |
| Kd:          | 0.078 nM *                      |
| Ligand:      | 0.40 nM [3H] Paroxetine         |
| Non-Specific Ligand: | 10.0 µM Imipramine |
| Specific Binding: | 95% *                        |
| Quantiation Method: | Radioligand Binding |
| Significance Criteria: | ≥50% of max stimulation or inhibition |
| Bmax:        | 4.40 pmole/mg Protein *         |

* Historical Values
| Cat #  | Assay Name          | Reference Compound                  | IC_{50}* | K_{i}  | n_H | Batch * | IC_{50}* |
|-------|---------------------|-------------------------------------|----------|-------|-----|---------|----------|
| 200510| Adenosine A_{1}     | R(-)-PIA                            | 0.83 µM  | 0.49 µM| 0.90| 310992  | 0.33 µM  |
| 200610| Adenosine A_{2A}    | CGS-21680                           | 0.13 µM  | 0.079 µM| 1.0 | 310993  | 0.13 µM  |
| 200720| Adenosine A_{3}     | IB-MECA                             | 0.78 nM  | 0.72 nM| 0.80| 310976  | 0.85 nM  |
| 203100| Adrenergic α_{1A}   | Prazosin                            | 0.69 nM  | 0.28 nM| 0.90| 310901  | 0.24 nM  |
| 203200| Adrenergic α_{1B}   | Prazosin                            | 0.27 nM  | 0.15 nM| 1.0 | 310902  | 0.23 nM  |
| 203400| Adrenergic α_{2D}   | Prazosin                            | 0.88 nM  | 0.43 nM| 0.70| 310903  | 0.75 nM  |
| 203620| Adrenergic α_{2A}   | Yohimbine                           | 8.40 nM  | 3.10 nM| 0.90| 310904  | 4.75 nM  |
| 204010| Adrenergic β_{1}    | S(-)-Propranolol                    | 2.50 nM  | 1.40 nM| 0.80| 310974  | 0.81 nM  |
| 204110| Adrenergic β_{2}    | S(-)-Propranolol                    | 0.78 nM  | 0.54 nM| 1.20| 310983  | 0.42 nM  |
| 285010| Androgen (Testosterone) AR | Testosterone                        | 6.50 nM  | 4.30 nM| 1.0 | 311022  | 4.15 nM  |
| 212510| Bradykinin B_{1}    | (Des-LArg^{10})-Kallidin            | 0.87 nM  | 0.22 nM| 1.10| 311038  | 0.31 nM  |
| 212620| Bradykinin B_{2}    | Bradykinin                          | 1.80 nM  | 1.10 nM| 1.0 | 311061  | 1.52 nM  |
| 214510| Calcium Channel L-Type, Benzothiazepine | Diltiazem                        | 0.036 µM | 0.032 µM| 0.90| 310999  | 0.021 µM |
| 214600| Calcium Channel L-Type, Dihydropyridine | Nitrendipine                 | 0.72 nM  | 0.46 nM| 0.90| 310998  | 0.31 nM  |
| 216000| Calcium Channel N-Type | ω-Conotoxin GVIA                   | 0.034 nM | 0.028 nM| 1.60| 310899  | 0.038 nM |
| 217030| Cannabinoid CB_{1}  | R(+)-WIN-55,212-2                   | 0.20 µM  | 0.15 µM| 0.70| 311000  | 0.11 µM  |
| 219500| Dopamine D_{1}      | R(+)-SCH-23390                     | 1.40 nM  | 0.70 nM| 0.90| 310984  | 0.84 nM  |
| 219700| Dopamine D_{2S}     | Spiperone                           | 0.25 nM  | 0.089 nM| 1.0 | 310985  | 0.21 nM  |
| 219800| Dopamine D_{3}      | Spiperone                           | 0.36 nM  | 0.12 nM| 0.90| 311010  | 0.43 nM  |
| 219900| Dopamine D_{4,2}    | Spiperone                           | 0.50 nM  | 0.20 nM| 0.90| 310959  | 0.37 nM  |
| 224010| Endothelin ET_{A}   | Endothelin-1                        | 0.23 nM  | 0.14 nM| 1.10| 311036  | 0.11 nM  |
| 224110| Endothelin ET_{B}   | Endothelin-1                        | 0.13 nM  | 0.060 nM| 0.90| 311037  | 0.056 nM |
| 225510| Epidermal Growth Factor (EGF) | EGF (human)                    | 1.60 nM  | 1.10 nM| 1.10| 310884  | 2.65 nM  |
| 226010| Estrogen ERα        | Diethylstilbestrol                  | 0.77 nM  | 0.22 nM| 1.0 | 310897  | 0.30 nM  |
| 226600| GABA_{A}, Flunitrazepam, Central | Diazepam                         | 0.016 µM | 0.013 µM| 0.80| 310986  | 0.028 µM |
| 226500| GABA_{A}, Muscimol, Central | GABA                                | 0.032 µM | 0.026 µM| 0.90| 311011  | 0.071 µM |
| 228610| GABA_{B_{1A}}      | CGP-54626                           | 6.40 nM  | 2.90 nM| 1.0 | 310896  | 3.97 nM  |
| 232030| Glucocorticoid     | Dexamethasone                       | 3.80 nM  | 1.80 nM| 0.90| 311064  | 4.14 nM  |
| 232700| Glutamate, Kainate  | L-Glutamic acid                     | 0.24 µM  | 0.17 µM| 0.80| 311034  | 0.14 µM  |
| 232810| Glutamate, NMDA, Agonism | L-Glutamic acid                | 0.41 µM  | 0.37 µM| 0.90| 311035  | 0.23 µM  |
| 232910| Glutamate, NMDA, Glycine | MDL 105,519                    | 0.022 µM | 0.021 µM| 0.60| 310895  | 0.012 µM |
| 233000| Glutamate, NMDA, Phencyclidine | Dizocilpine ((+)-MK-801)         | 5.10 nM  | 3.40 nM| 0.70| 311003  | 4.75 nM  |
| 239610| Histamine H_{1}     | Pyrilamine                          | 3.30 nM  | 1.60 nM| 1.0 | 310934  | 1.90 nM  |
| 239710| Histamine H_{2}     | Tiotidine                           | 0.022 µM | 0.018 µM| 1.10| 310975  | 0.021 µM |
| 239820| Histamine H_{3}     | R-(+)-α-Methylhistamine (RAMH)     | 2.30 nM  | 1.10 nM| 1.10| 311012  | 1.56 nM  |
| 241000| Imidazoline I_{2}, Central | Idazoxan                          | 0.012 µM | 8.0 nM | 1.0  | 311006  | 6.96 nM  |
| 243520| Interleukin IL-1    | IL-1β                               | 0.19 nM  | 0.14 nM| 1.30| 311266  | 0.25 nM  |
| Cat #  | Assay Name                     | Reference Compound                  | IC₅₀* | KH | nH | Batch * | Concurrent IC₅₀* |
|-------|--------------------------------|-------------------------------------|------|----|----|---------|-----------------|
| 250460| Leukotriene, Cysteinyl CysLT₁  | LTD₄                                | 0.70 nM | 0.29 nM | 1.0 | 311007 | 1.10 nM         |
| 251600| Melatonin MT₁                   | Melatonin                           | 0.21 nM | 0.11 nM | 0.70 | 310969 | 0.12 nM         |
| 252610| Muscarinic M₁                   | 4-DAMP                             | 4.50 nM | 1.10 nM | 1.0 | 311151 | 5.27 nM         |
| 252710| Muscarinic M₂                   | 4-DAMP                             | 0.055 µM | 0.023 µM | 1.0 | 310988 | 0.028 µM        |
| 257010| Neuropeptide Y Y₁               | Neuropeptide Y (human, rat)        | 0.22 nM | 0.21 nM | 1.10 | 310893 | 0.48 nM         |
| 257110| Neuropeptide Y Y₂               | Neuropeptide Y (13-36) (porcine)   | 0.21 nM | 0.12 nM | 0.90 | 310894 | 0.56 nM         |
| 258590| Nicotinic Acetylcholine         | Epibatidine                         | 0.076 nM | 0.052 nM | 0.90 | 310971 | 0.20 nM         |
| 258700| Nicotinic Acetylcholine α, Bungarotoxin | α-Bungarotoxin                  | 1.10 nM | 0.72 nM | 1.10 | 310972 | 1.43 nM         |
| 260130| Opiate δ₁ (OP1, DOP)            | Naltrindole                         | 0.91 nM | 0.16 nM | 1.0 | 310879 | 1.26 nM         |
| 260210| Opiate κ(OP2, KOP)              | U-69593                             | 0.016 µM | 6.40 nM | 0.5 | 310880 | 0.026 µM        |
| 260410| Opiate μ(OP3, MOP)              | DAMGO                               | 0.020 µM | 8.10 nM | 0.60 | 310881 | 0.060 µM        |
| 264500| Phorbol Ester                   | PMA                                 | 0.79 nM | 0.59 nM | 1.0 | 310990 | 1.07 nM         |
| 265010| Platelet Activating Factor (PAF)| PAF                                 | 0.28 nM | 0.15 nM | 0.90 | 310876 | 0.31 nM         |
| 265600| Potassium Channel [K⁺ATP]       | Glyburide                           | 5.70 nM | 0.65 nM | 0.80 | 310991 | 3.85 nM         |
| 265900| Potassium Channel hERG         | Astemizole                          | 2.60 nM | 2.10 nM | 1.10 | 310905 | 5.55 nM         |
| 268420| Prostanoid EP₄                  | Prostaglandin E₂ (PGE₂)            | 1.10 nM | 0.45 nM | 0.90 | 311013 | 1.40 nM         |
| 268700| Purinergic P₂X                  | α, β-Methylene ATP                  | 0.082 µM | 0.018 µM | 1.10 | 311014 | 0.028 µM        |
| 268810| Purinergic P₂Y                  | ATP                                 | 0.018 µM | 0.018 µM | 0.90 | 311015 | 0.015 µM        |
| 270000| Rolipram                        | Rolipram                            | 5.70 nM | 2.10 nM | 1.0 | 311177 | 1.90 nM         |
| 271110| Serotonin (5-Hydroxytryptamine) | Metergoline                         | 4.10 nM | 2.30 nM | 0.90 | 311017 | 2.92 nM         |
| 271700| Serotonin (5-Hydroxytryptamine) | 5-HT₁α                              | 0.29 µM | 0.18 µM | 0.60 | 311019 | 0.62 µM         |
| 271910| Serotonin (5-Hydroxytryptamine) | 5-HT₁β                              | 0.011 µM | 2.50 nM | 0.80 | 310891 | 0.019 µM        |
| 278110| Sigma σ₁                        | Haloperidol                         | 0.021 µM | 8.80 nM | 0.90 | 311021 | 7.82 nM         |
| 255520| Tachykinin NK₁                  | L-703,606                           | 3.60 nM | 2.60 nM | 1.0 | 311044 | 2.62 nM         |
| 285900| Thyroid Hormone                 | Triiodothyronine                    | 0.034 nM | 0.018 nM | 1.0 | 310909 | 0.044 nM        |
| 220320| Transporter, Dopamine (DAT)     | GBR-12909                           | 1.70 nM | 1.30 nM | 0.90 | 310996 | 0.61 nM         |
| 226400| Transporter, GABA                | NO-711                              | 0.20 µM | 0.20 µM | 1.10 | 311002 | 0.39 µM         |
| 204410| Transporter, Norepinephrine (NET)| Desipramine                       | 0.93 nM | 0.92 nM | 0.60 | 310995 | 0.55 nM         |
| 274030| Transporter, Serotonin (5-Hydroxytryptamine) (SERT)| Fluoxetine | 8.60 nM | 1.40 nM | 0.90 | 311020 | 5.98 nM         |

* Batch: Represents compounds tested concurrently in the same assay(s).