Supporting Information

Development of (E)-2-((1,4-dimethylpiperazin-2-ylidene)amino)-5-nitro-N-phenylbenzamide, ML336: novel 2-amidinophenylbenzamides as potent inhibitors of Venezuelan Equine Encephalitis Virus

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Experimental and analytical details for synthetic intermediates:

![Chemical structure of 2-(Chloromethyl)-6-nitro-3-phenylquinazolin-4(3H)-one](image)

**2-(Chloromethyl)-6-nitro-3-phenylquinazolin-4(3H)-one.** Following the same procedure used to synthesize 1 (*step 2*), 2-(2-chloroacetamido)-5-nitrobenzoic acid (1.47 g, 5.7 mmol) and aniline (0.73 mL, 8.0 mmol) were used to produce the title compound (1.24 g, 69%) as a burnt-orange solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.13 (d, $J = 2.6$ Hz, 1H), 8.59 (dd, $J = 8.9, 2.6$ Hz, 1H), 7.91 (d, $J = 9.0$ Hz, 1H), 7.65–7.58 (m, 3H), 7.39–7.35 (m, 2H), 4.28 (s, 2H).

![Chemical structure of Methyl 2-amino-5-cyanobenzoate](image)

**Methyl 2-amino-5-cyanobenzoate.** To a solution of 4-amino-3-iodobenzoic acid (0.68 g, 2.8 mmol) in MeCN (15 mL) and MeOH (7.5 mL) were added Pd(OAc)$_2$ (0.063 g, 0.28 mmol), DPPF (0.16 g, 0.28 mmol), Et$_3$N (0.28 g, 2.8 mmol) and K$_2$CO$_3$ (1.16 g, 8.4 mmol). The reaction mixture was purged with CO for 5 min, followed by stirring under 1 atm of CO (balloon) at 75 °C for 12 hours. The reaction mixture was diluted with EtOAc (30 mL), washed with H$_2$O and brine, and the separated organic extracts were dried with MgSO$_4$. After filtration and concentration, the crude product was purified by flash chromatography (EtOAc/hexanes) to give the title compound (0.27 g, 54%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.17 (d, $J = 2.0$ Hz, 1H), 7.43 (dd, $J = 8.7, 2.0$ Hz, 1H), 6.67 (d, $J = 8.6$ Hz, 1H), 6.33 (s, 2H), 3.88 (s, 3H).
2-Amino-5-cyanobenzoic acid. To a solution of methyl 2-amino-5-cyanobenzoate (120 mg, 0.68 mmol) in THF (3.5 mL) and water (3.5 mL), was added LiOH (33 mg, 1.38 mmol). The mixture was stirred at rt for 3 hours and concentrated. The remaining residue was dissolved in water (5 mL), and acidified to pH 3 with 1 M HCl. The precipitate was collected by filtration, washed with water (5 mL) and dried under air. The title compound (105 mg, 95%) was obtained as a white solid and used in the next step without further purification.

\[\text{O} \quad \begin{array}{c}
\text{NC} \\
\text{NH} \\
\text{O} \\
\text{Cl}
\end{array} \]

2-(2-Chloroacetamido)-5-cyanobenzoic acid. Following the same procedure used to synthesize 3, 2-amino-5-cyanobenzoic acid (100 mg, 0.62 mmol) was used to produce the title compound (145 mg, 98%) as a pale-orange solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.07 (s, 1H), 8.68 (d, \(J = 8.8\) Hz, 1H), 8.38 (d, \(J = 2.1\) Hz, 1H), 8.08 (dd, \(J = 8.8, 2.1\) Hz, 1H), 4.51 (s, 2H).

\[\text{O} \quad \begin{array}{c}
\text{NC} \\
\text{N} \\
\text{Cl}
\end{array} \]

2-(Chloromethyl)-4-oxo-3-phenyl-3,4-dihydroquinazoline-6-carbonitrile. Following the same procedure used to synthesize 1 (step 2), 2-(2-chloroacetamido)-5-cyanobenzoic acid (140 mg, 0.59 mmol) was used to produce the title compound (138 mg, 80%) as a red solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.60 (dd, \(J = 2.0, 0.6\) Hz, 1H), 8.00 (dd, \(J = 8.5, 1.9\) Hz, 1H), 7.86 (dd, \(J = 8.5, 0.6\) Hz, 1H), 7.65–7.56 (m, 3H), 7.37–7.32 (m, 2H), 4.27 (s, 2H).

\[\text{O}_2\text{N} \quad \begin{array}{c}
\text{NC} \\
\text{N} \\
\text{Cl}
\end{array} \]

3
**2-(Chloromethyl)-3-(3-fluorophenyl)-6-nitroquinazolin-4(3H)-one.** Following the same procedure used to synthesize 1 (step 2), 2-(2-chloroacetamido)-5-nitrobenzoic acid (193 mg, 0.75 mmol) and 3-fluoroaniline (0.10 mL, 1.04 mmol) were used to produce the title compound (164 mg, 66%) as a burnt-orange solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.13 (d, $J = 2.6$ Hz, 1H), 8.60 (dd, $J = 8.9, 2.6$ Hz, 1H), 7.92 (d, $J = 8.9$ Hz, 1H), 7.65–7.56 (m, 1H), 7.38–7.29 (m, 1H), 7.22–7.12 (m, 2H), 4.30 (s, 2H).

![Structure of 2-(Chloromethyl)-3-(3-fluorophenyl)-6-nitroquinazolin-4(3H)-one](image)

**2-(Chloromethyl)-3-(4-fluorophenyl)-6-nitroquinazolin-4(3H)-one.** Following the same procedure used to synthesize 1 (step 2), 2-(2-chloroacetamido)-5-nitrobenzoic acid (193 mg, 0.75 mmol) and 4-fluoroaniline (0.10 mL, 1.06 mmol) were used to produce the title compound (173 mg, 70%) as a burnt-orange solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.13 (d, $J = 2.6$ Hz, 1H), 8.60 (dd, $J = 8.9, 2.6$ Hz, 1H), 7.91 (d, $J = 8.9$ Hz, 1H), 7.40–7.27 (m, 4H), 4.29 (s, 2H).

![Structure of 2-(Chloromethyl)-3-(4-fluorophenyl)-6-nitroquinazolin-4(3H)-one](image)

**tert-Butyl-4-((6-nitro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)piperazine-1-carboxylate.** Following the same procedure used to synthesize 1 except the product was purified by flash chromatography with a mobile phase gradient of 0-50% EtOAc/hexanes, 2-(chloromethyl)-6-nitro-3-phenylquinazolin-4(3H)-one (253 mg, 0.80 mmol) and 1-BOC-piperazine (224 mg, 1.20 mmol) were used to produce the title compound (103 mg, 28%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.13 (d, $J = 2.5$ Hz, 1H), 8.56 (dd, $J = 9.0, 2.7$ Hz, 1H), 7.87 (d, $J = 8.9$ Hz, 1H), 7.60–7.51 (m, 3H), 7.35–7.29 (m, 2H), 3.33–3.27 (m, 6H), 2.30–2.24 (m, 4H), 1.43 (s, 9H).

![Structure of tert-Butyl-4-((6-nitro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)piperazine-1-carboxylate](image)
2-(Chloromethyl)-3-(4-methoxyphenyl)-6-nitroquinazolin-4(3H)-one. Following the same procedure used to synthesize 1 (step 2), 2-(2-chloroacetamido)-5-nitrobenzoic acid (241 mg, 0.93 mmol) and p-anisidine (154 mg, 1.25 mmol) were used to produce the title compound (220 mg, 68%) as a burnt-orange solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.13 (d, $J = 2.5$ Hz, 1H), 8.58 (dd, $J = 8.9$, 2.6 Hz, 1H), 7.90 (d, $J = 8.9$ Hz, 1H), 7.29–7.24 (m, 2H), 7.11–7.06 (m, 2H), 4.30 (s, 2H), 3.90 (s, 3H).

**tert-Butyl-4-((3-(4-methoxyphenyl)-6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazine-1-carboxylate.** Following the same procedure used to synthesize 1, 2-(chloromethyl)-3-(4-methoxyphenyl)-6-nitroquinazolin-4(3H)-one (212 mg, 0.61 mmol) and 1-BOC-piperazine (172 mg, 0.92 mmol) were used to produce the title compound (210 mg, 69%) as a pale-yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.13 (d, $J = 2.3$ Hz, 1H), 8.55 (dd, $J = 9.0$, 2.7 Hz, 1H), 7.86 (d, $J = 8.7$ Hz, 1H), 7.25–7.20 (m, 2H), 7.07–7.02 (m, 2H), 3.88 (s, 3H), 3.36–3.31 (m, 4H), 3.30 (s, 2H), 2.35–2.28 (m, 4H), 1.43 (s, 9H).

2-(Chloromethyl)-6-nitro-3-(thiophen-3-yl)quinazolin-4(3H)-one. The free amine of thiophen-3-amine was produced from thiophen-3-amine oxalate (Combi-Blocks, ST-4087) by treating it with
saturated aq. NaHCO₃, extracting with CH₂Cl₂, drying with Na₂SO₄, and then using the resulting clear, light-brown oil in a timely fashion. Following the same procedure used to synthesize 1 (step 2), 2-(2-chloroacetamido)-5-nitrobenzoic acid (241 mg, 0.93 mmol) and thiophen-3-amine (130 mg, 1.31 mmol) were used to produce the title compound (170 mg, 57%) as a black solid. ¹H NMR (400 MHz, CDCl₃) δ 9.13 (d, J = 2.5 Hz, 1H), 8.59 (dd, J = 8.9, 2.6 Hz, 1H), 7.90 (d, J = 8.9 Hz, 1H), 7.57 (dd, J = 5.1, 3.2 Hz, 1H), 7.51 (dd, J = 3.2, 1.4 Hz, 1H), 7.11 (dd, J = 5.1, 1.4 Hz, 1H), 4.34 (br s, 2H).

**tert-Butyl-4-((6-nitro-4-oxo-3-(thiophen-3-yl)-3,4-dihydroquinazolin-2-yl)methyl)piperazine-1-carboxylate.** Following the same procedure used to synthesize 1, 2-(chloromethyl)-6-nitro-3-(thiophen-3-yl)quinazolin-4(3H)-one (164 mg, 0.51 mmol) and 1-BOC-piperazine (143 mg, 0.77 mmol) were used to produce the title compound (177 mg, 74%) as a brown solid. ¹H NMR (400 MHz, CDCl₃) δ 8.97 (d, J = 2.6 Hz, 1H), 8.47 (dd, J = 8.9, 2.7 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.45 (dd, J = 5.1, 3.2 Hz, 1H), 7.38 (dd, J = 3.2, 1.4 Hz, 1H), 7.05 (dd, J = 5.1, 1.4 Hz, 1H), 3.39–3.23 (m, 6H), 2.36–2.25 (m, 4H), 1.40 (s, 9H).

**tert-Butyl-ethyl(2-(methylamino)ethyl)carbamate.** Benzaldehyde (0.26 mL, 2.56 mmol) was added dropwise to a solution of (2-aminoethyl)ethyl carbamic acid tert-butyl ester (377 mg, 2.00 mmol) in dry toluene (5 mL) at rt. The reaction flask was equipped with a Dean-Stark trap and a reflux condenser. The mixture was heated at 150 °C for 1.5 hours. After cooling to rt, the Dean-Stark trap was removed and a solution of methyl p-toluenesulfonate (0.31 mL, 2.05 mmol) in dry toluene (0.5 mL) was added dropwise. The mixture was heated to gentle reflux at 125 °C for 15 hours. After cooling to rt, water (2 mL) was added and the mixture was heated at 80 °C for 30 min.
After cooling to rt, the biphasic layers were separated and 2 M aq. KOH (4 mL) was added to the aq. layer. The product was extracted from the aq. layer with CH$_2$Cl$_2$ (3 x 15 mL), filtered and organic extracts were dried (Na$_2$SO$_4$) to give the title compound (171 mg, 42%) as a clear, pale-yellow oil, which was used in the next step without further purification.

tert-Butyl-ethyl(2-(methyl((6-nitro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)amino)ethyl)carbamate. Following the same procedure used to synthesize 1, 2-(chloromethyl)-6-nitro-3-phenylquinazolin-4(3H)-one (177 mg, 0.56 mmol) and tert-butyl ethyl(2-(methylamino)ethyl)carbamate (161 mg, 0.80 mmol) were used to produce the title compound (88 mg, 33%) as a clear, dark-red oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.07 (d, $J = 2.7$ Hz, 1H), 8.53 (dd, $J = 8.9$, 2.6 Hz, 1H), 7.88 (d, $J = 8.9$ Hz, 1H), 7.59–7.52 (m, 3H), 7.33–7.29 (m, 2H), 3.37 (s, 2H), 3.20–3.00 (m, 4H), 2.52–2.42 (m, 2H), 2.22 (s, 3H), 1.40 (br s, 9H), 1.02 (t, $J = 7.2$ Hz, 3H).
**Solubility Assessment Protocol**

Compound solubility in aqueous solution was measured using an automated kinetic solubility method at the Sanford Burnham Medical Research Institute at Lake Nona. 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. Solubility in the TC-83 VEEV CPE assay medium, consisting of high glucose DMEM (Dulbecco's Modified Eagle's Medium) with 10% FBS and 1x Pen/Strep, was also assessed using the same method.

**Chemical Stability Assessment Protocol**

Compound 45 was evaluated for susceptibility to nucleophilic addition and formation of conjugates by treatment with dithiothreitol (DTT). Supplemental Figure 1 represents the time course experiment with compound 45 under various conditions. The compound was dissolved at 10 μM in PBS at pH 7.4 (1% DMSO) and independently incubated at room temperature with no nucleophile present or 50 μM dithiothreitol (DTT). The test reactions were sampled every hour for eight hours and analyzed by LCMS. The analytical LCMS system utilized for the analysis was a Waters Acquity system with UV-detection and mass-detection (Waters LCT Premier). The analytical method conditions included a Waters Acquity HSS T3 C18 column (2.1 x 50mm, 1.8 μm) and elution with a linear gradient of 1% water to 100% CH₃CN at 0.6 mL/min flow rate. Peaks on the 214 nm chromatographs were integrated using the Waters OpenLynx software. Absolute areas under the curve were compared at each time point to determine relative percent parent remaining. The masses of potential adducts and dimers of compound 45 were searched for in the final samples to determine if any detectable adduct formed or dimerization had occurred. In the case of compound 45, no adducts were detected at any time point using LCMS detection. All samples were prepared in duplicate. Ethacrynic acid, a known Michael acceptor, was used as a positive control.
Supplemental Figure 1. Chemical stability of 45 over 8 h in the presence of a 5-fold concentration of dithiothreitol. Supplemental Table 1 summarizes the percent remaining of 45 at the endpoints of each run in each experiment. The average amount of 45 remaining after 8h when no nucleophile was added was 93.9%, and when 5X DTT was added, nearly 99% of parent 45 remained after the same time period, indicating that 45 was not generally susceptible to degradation or adduct formation in the presence of a thiol-based nucleophile.

Supplemental Table 1. Percent of compound 45 remaining at the conclusion of the experiment (8h)

| Test Condition                                      | Run | Percent Compound 45 Remaining after 8 h | Averaged Percent Compound 45 Remaining after 8 h |
|-----------------------------------------------------|-----|----------------------------------------|-----------------------------------------------|
| ML336 without nucleophile (control)                 | 1   | 96.1                                   | 93.9                                          |
| ML336 without nucleophile (control)                 | 2   | 91.7                                   |                                               |
| ML336 with 5X DTT                                   | 1   | 100.4                                  |                                               |
| ML336 with 5X DTT                                   | 2   | 97.6                                   | 99.0                                          |

Aqueous Stability: Supplemental Figure 2 represents the time course experiment with 45 under various aqueous stability conditions. Compound 45 was dissolved at 10 μM in PBS at pH 7.4 (1% DMSO) or dissolved at 10 μM in 50% PBS/50% acetonitrile at pH 7.4 (1% DMSO). The samples were evaluated over 14 timepoints (0, 1, 2, 3, 4, 5, 6, 7, 8, 16, 24, 32, 40, and 48 h) and analyzed
by LCMS. The analytical LCMS system utilized for the analysis was a Waters Acquity system with UV-detection and mass-detection (Waters LCT Premier). The analytical method conditions included a Waters Acquity HSS T3 C18 column (2.1 x 50mm, 1.8 µm) and elution with a linear gradient of 1% water to 100% CH$_3$CN at 0.6 mL/min flow rate. Peaks on the 214 nm chromatographs were integrated using the Waters OpenLynx software. Absolute areas under the curve were compared at each time point to determine relative percent parent remaining.

**Supplemental Figure 2.** Aqueous stability of Compound 45 (ML336) over 48 h in PBS or PBS/acetonitrile.

In PBS, compound 45 showed an average 82.9% remaining after 48 h; however, no other mass peaks were observed. In order to account for precipitation/solubility effects, compound 45 was also evaluated in 50/50 PBS:acetonitrile and was shown to have an average of 96.0% remaining after 48 h (Supplemental Table 2).

**Supplemental Table 2. Percent of compound 45 remaining after 48 h**

| Test Condition                  | Run | Percent Compound 45 Remaining after 48 h | Averaged Percent Compound 45 Remaining after 48 h |
|--------------------------------|-----|-----------------------------------------|-----------------------------------------------|
| ML336 in PBS                   | 1   | 81.5                                    |                                               |
| ML336 in PBS                   | 2   | 84.3                                    |                                               |
| ML336 in 50/50 PBS: Acetonitrile | 1   | 96.0                                    |                                               |
| ML336 in 50/50 PBS: Acetonitrile | 2   | 96.0                                    |                                               |
**In vitro pharmacology assay protocols**

**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:

\[
\% \text{ of bound parent} = \left( \frac{\text{amount of compound in donor} - 1}{\text{amount of compound in donor}} \right) \times 100
\]

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.

**BBB-PAMPA** – Permeability was assessed using an *in vitro* model for the passive transport through the blood-brain barrier, BBB-PAMPA. For this, the Parallel Artificial Membrane Permeability Assay (PAMPA) in a 96-well format was used. All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). Measurements were performed in an aqueous buffer solution (Pion Inc.) at pH 7.4, in quadruplicate. A “sandwich” plate (Pion Inc.) consisting of a donor bottom plate and an acceptor filter plate was used. The donor wells contained the compounds in 180 µl system solution, and magnetic stir bars. The filter on the bottom of each acceptor well was coated with BBB-1 lipid solution (Pion Inc.) and filled with 200 µl of Brain Sink Buffer, pH 7.4 (Pion Inc.) also containing a surfactant. The permeation time was 60 min. Moderate stirring (equivalent to 40 µm Aqueous Boundary Layer thickness) was applied using the Gut-Box™ (Pion Inc.). The assay DMSO final concentration was 0.5%. After the permeation time, the sandwich was disassembled and the amount of compound present in both the donor and acceptor wells was measured by UV absorbance (250-498 nm) using the Infinite M200 (Tecan US) and compared to spectra obtained from reference standards. Mass balance was used to determine the amount of material embedded in the membrane filter. The effective permeability, Pe, was calculated using the software PAMPA Evolution Plus, version 3.2 (Pion Inc.).
**Inhibition of drug-metabolizing CYP3A4, CYP2C9, and CYP1A2** – Evaluation of the compound as inhibitor of the activity of CYP3A4, CYP2C9, and CYP1A2 was assessed using the P450-Glo™ Assay kits (Promega) in the presence of human liver microsomes (XenoTech). NADPH, a required cofactor for CYP450 metabolism, was provided by the NADPH Regenerating System, Solutions A (BD Biosciences) and B (BD Biosciences). Compound stock solution was initially prepared in 100% DMSO and subsequently diluted in acetonitrile for the assay. The pH of the reactions was maintained at ~ 7.4 with potassium phosphate buffer (BD Biosciences). The reaction was started after adding NADPH and the corresponding luciferin substrate (luciferin-IPA for 3A4, luciferin-H for 2C9, and luciferin 1A2) to the reaction plate containing microsomes and compound (10µM). The reaction was incubated for 20 minutes at 37°C with shaking. Luciferin Detection Reagent was added after the incubation to stop the reaction and initiate a stable glow-type luminescent signal. The luminescence of the metabolite formed, Luciferin, was recorded with the Infinite M200 (Tecan US). All reactions were run in triplicate, except negative controls (no NADPH) which were performed as single reactions. The background luminescence obtained from the negative control reactions was subtracted from the luminescence values obtained for the positive reaction triplicates. The percent of inhibition was calculated by the formula: 100-[RLU at 10µM/RLU at 0µM*100].

**Inhibition of drug-metabolizing CYP2C19** – Evaluation of the compound as inhibitor of the activity of CYP2C19 was assessed using the P450-Glo™ Assay kit (Promega) in the presence of recombinant CYP2C19 (Cypex/XenoTech or Promega). NADPH, a required cofactor for CYP450 metabolism, was provided by the NADPH Regenerating System, Solutions A (BD Biosciences) and B (BD Biosciences). Compound stock solution was initially prepared in 100% DMSO and subsequently diluted in acetonitrile for the assay. The pH of the reactions was maintained at ~ 7.4 with potassium phosphate buffer (BD Biosciences). The reaction was started after adding NADPH to the reaction plate containing the enzyme, the substrate (luciferin-HEGE) and the compound (10µM). The reaction was incubated for 30 minutes at 37°C with shaking. Luciferin Detection Reagent was added after the incubation to stop the reaction and initiate a stable glow-type luminescent signal. The luminescence of the metabolite formed, Luciferin, was recorded with the Infinite M200.
Inhibition of drug-metabolizing CYP2D6 – Evaluation of the compound as inhibitor of the activity of CYP2D6 was assessed using the P450-Glo™ Assay kit (Promega) in the presence of recombinant CYP2D6 (Cypex/XenoTech). NADPH, a required cofactor for CYP450 metabolism, was provided by the NADPH Regenerating System, Solutions A (BD Biosciences) and B (BD Biosciences). Compound stock solution was initially prepared in 100% DMSO and subsequently diluted in acetonitrile for the assay. The pH of the reactions was maintained at ~ 7.4 with potassium phosphate buffer (BD Biosciences). The reaction was started after adding NADPH to the reaction plate containing the enzyme, the substrate (luciferin-ME EGE), and the compound (10µM). The reaction was incubated for 30 minutes at 37°C with shaking. Luciferin Detection Reagent was added after the incubation to stop the reaction and initiate a stable glow-type luminescent signal. The luminescence of the metabolite formed, Luciferin, was recorded with the Infinite M200 (Tecan US). All reactions were run in duplicate, except negative controls (no enzyme) which were performed as single reactions. The background luminescence obtained from the negative control reactions was substracted from the luminescence values obtained for the positive reaction duplicates. The percent of inhibition was calculated by the formula: 100-[RLU at 10µM/RLU at 0µM*100].

(Tecan US). All reactions were run in duplicate, except negative controls (no enzyme) which were performed as single reactions. The background luminescence obtained from the negative control reactions was substracted from the luminescence values obtained for the positive reaction duplicates. The percent of inhibition was calculated by the formula: 100-[RLU at 10µM/RLU at 0µM*100].
**In vivo** brain/plasma exposure assay parameters

Supplemental Table 3. Parameters for evaluating compound 45 exposure in mice brain and plasma

| Parameter          | Result                                                                                                                                 |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Species            | Mouse (C57Bl/6)                                                                                                                       |
| Mice per group     | 3                                                                                                                                      |
| Groups             | 4 = vehicle control, 1 mg/kg dose, 5 mg/kg dose, 10 mg/kg dose                                                                       |
| Administration     | IP                                                                                                                                     |
| Blood collection   | 20 min and 120 min post IP administration                                                                                              |
| Brain collection   | 120 min post IP administration                                                                                                          |
| Formulation        | 0.1 mg/mL of compound 45 in (DMSO:Tw80:H₂O) (10:10:80) = 1 mg/kg dose                                                                |
|                    | 0.5 mg/mL of compound 45 in (DMSO:Tw80:H₂O) (10:10:80) = 5 mg/kg dose                                                                    |
|                    | 1.0 mg/mL of compound 45 in (DMSO:Tw80:H₂O) (10:10:80) = 10 mg/kg dose                                                                   |
| Analytical technique: | LC/MS/MS (368.200→112.100 Da)                                                                                                             |

*In vivo* dose-exposure profiling of compound 45 was conducted using the Rapid Assessment of Compound Exposure (RACE) assay as described. Briefly, three independent cohorts of adult male mice (n=3) were subjected to a single IP dose of compound at 1, 5, or 10 mg/kg. Blood was drawn at 20 and 120 min post-administration, and subsequent LC/MS analysis of pooled samples was performed to determine the overall plasma levels of compound. In this abbreviated study, compound 45 was rapidly absorbed with Cmax =20min, followed by decreasing plasma levels observed over time. The compound exhibited a linear relationship between dose and exposure, with estimated AUC (eAUC) that increased in direct proportion to increased dose. At t=120min, compound 45 exhibited significant distribution to the brain suggesting that the compound will reach sufficiently high levels in the target organ tissue necessary to elicit the desired effect *in vivo*. Compound 45 was well tolerated. Throughout the study the mice exhibited normal behavior and no signs of adverse effects were observed at any dose.

**Materials and methods:**

The Rapid Assessment of Compound Exposure (or RACE) assay, is an experiment used in the early phases of discovery to quickly and efficiently determine if the compound of interest is bioavailable. Male C57Bl6j mice, 6-8 weeks of age, ~20g body weight were purchased from the Jackson Laboratory (Bar Harbor, ME) and allowed to acclimate for 3 days. On the day of the
study, the compound was formulated a vehicle containing DMSO, Tween80, and sterile water (10:10:80) at 0.1 mg/ml, 0.5mg/ml and 1.0mg/mL) and administered IP to 3 mice at 1 mg/kg, 5 mg/kg or 10 mg/kg. An additional cohort of 3 mice received vehicle as control animals. Blood was collected from the mandibular vein at t= 20min and 120 min post-administration in collection tubes containing sodium EDTA 0.5mM. Whole blood samples were placed on wet ice, immediately after collection, and prior to centrifugation to separate the cellular constituents from plasma. Brain tissues were collected from mice under deep anesthesia at t=120min. LC/MS/MS was used to determine final concentration of compound in plasma and brain homogenate. The protocol described herein was approved by the Sanford Burnham Medical Research Institute at Lake Nona IACUC.

**In vivo efficacy study parameters and protocol**

See Supplemental Figure 3. C3H/HeN mice (5-6 week old) were obtained from Charles River Laboratories (Wilmington, MA) and randomly assigned to one of 6 treatment groups (7 mice/group): Group 1-Vehicle control; Group 2-VEEV only; Group 3- VEEV and 5 mg/kg/day ML336. Mice were dosed IP twice per day with 200 µL volume comprised of vehicle only (1% carboxymethylcellulose) or compound formulated in vehicle. Treatments were conducted for four days, beginning 4 hours prior to virus challenge (D0-D3). On D0, dosing started at 4 hours prior to virus challenge and 4 hours post-challenge. At 4 hours after the first administration of compound, mice were infected intranasally with 10 LD$_{50}$ of TC-83. Mice were infected intranasally with 10 times the LD$_{50}$ of TC-83 (Day-0) diluted in 50 µL of PBS. For the vehicle control, PBS was used in place of virus. Mice were weighed from D0-D21 and checked twice a day for mortality and morbidity. The median time-to-death for the group challenged with VEEV was 8 days. P values were generated from comparisons of survival data using the Log-Rank (Mantel-Cox) test using Prism 6 (Graph Pad Software, Inc).
Supplemental Figure 3. Study design for 21 day efficacy study in mice with compound 45
(IP = intraperitoneal; IN = intranasal; BID = twice daily)
## PanLabs Profiling Results for Compound 45

### Supplemental Table 4. Compound 45 Results from Profiling at PanLabs

| Cat #   | Assay Name                        | Batch* | Spec. | Rep. | Conc. | % Inh. |
|---------|----------------------------------|--------|-------|------|-------|--------|
| 200510  | Adenosine A₁                      | 305599 | hum   | 2    | 10 µM | 20.43  |
| 200610  | Adenosine A₂A                     | 305600 | hum   | 2    | 10 µM | 16.97  |
| 200720  | Adenosine A₃                      | 305602 | hum   | 2    | 10 µM | 26.67  |
| 203100  | Adrenergic α₁A                    | 305613 | rat   | 2    | 10 µM | 4.41   |
| 203200  | Adrenergic α₁B                    | 305614 | rat   | 2    | 10 µM | -1.17  |
| 203400  | Adrenergic α₁D                    | 305615 | hum   | 2    | 10 µM | -7.82  |
| 203620  | Adrenergic α₂A                    | 305616 | hum   | 2    | 10 µM | -0.5   |
| 204010  | Adrenergic β₁                      | 305603 | hum   | 2    | 10 µM | 3.56   |
| 204110  | Adrenergic β₂                      | 305604 | hum   | 2    | 10 µM | 6.39   |
| 285010  | Androgen (Testosterone) AR        | 305773 | rat   | 2    | 10 µM | 1.1    |
| 212510  | Bradykinin B₁                      | 305625 | hum   | 2    | 10 µM | 9.23   |
| 212620  | Bradykinin B₂                      | 305626 | hum   | 2    | 10 µM | 3.4    |
| 214510  | Calcium Channel L-Type, Benzothiazepine | 305776 | rat   | 2    | 10 µM | 7.06   |
| 214600  | Calcium Channel L-Type, Dihydropyridine | 305794 | rat   | 2    | 10 µM | 4.51   |
| 216000  | Calcium Channel N-Type            | 305795 | rat   | 2    | 10 µM | 22.74  |
| 217030  | Cannabinoid CB₁                    | 305621 | hum   | 2    | 10 µM | -2.06  |
| 219500  | Dopamine D₁                       | 305797 | hum   | 2    | 10 µM | 2.65   |
| 219700  | Dopamine D₂B                      | 305798 | hum   | 2    | 10 µM | 9.08   |
| 219800  | Dopamine D₃                       | 305799 | hum   | 2    | 10 µM | -1.53  |
| 219900  | Dopamine D₁₂                      | 305800 | hum   | 2    | 10 µM | 11.2   |
| 224010  | Endothelin ETₐ                    | 305622 | hum   | 2    | 10 µM | 7.78   |
| 224110  | Endothelin ET₈                    | 305623 | hum   | 2    | 10 µM | 8.32   |
| 225510  | Epidermal Growth Factor (EGF)     | 305638 | hum   | 2    | 10 µM | -2.63  |
| 226010  | Estrogen ERα                      | 305716 | hum   | 2    | 10 µM | 10.01  |
| 226600  | GABA₁₂, Flunitrazepam, Central    | 305802 | rat   | 2    | 10 µM | 15.23  |
| 226500  | GABA₁₂, Muscimol, Central         | 305801 | rat   | 2    | 10 µM | 0.52   |
| 228610  | GABA₁₂A                          | 305888 | hum   | 2    | 10 µM | 35.94  |
| 232030  | Glucocorticoid                    | 305774 | hum   | 2    | 10 µM | 10.19  |
| 232700  | Glutamate, Kainate                | 305634 | rat   | 2    | 10 µM | 22.27  |
| 232810  | Glutamate, NMDA, Agonism          | 305632 | rat   | 2    | 10 µM | -2.6   |
| 232910  | Glutamate, NMDA, Glycine          | 305635 | rat   | 2    | 10 µM | 12.86  |
| 233000  | Glutamate, NMDA, Phencyclidine    | 305803 | rat   | 2    | 10 µM | 9.28   |
| 239610  | Histamine H₁                      | 305804 | hum   | 2    | 10 µM | 12.31  |
| 239710  | Histamine H₂                      | 305805 | hum   | 2    | 10 µM | 20.43  |

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
## Supplemental Table 4. Compound 45 Results from Profiling at PanLabs - continued

| Catalog # | Assay Name                                      | Species | Replicates | Concentration | % Inh. |
|-----------|------------------------------------------------|---------|------------|---------------|--------|
| 239820    | Histamine H3                                    | hum     | 2          | 10 µM         | 15.75  |
| 241000    | Imidazoline I2, Central                         | rat     | 2          | 10 µM         | 14.42  |
| 243520    | Interleukin IL-1                                 | mouse   | 2          | 10 µM         | 3.11   |
| 250460    | Leukotriene, Cysteinyl CysLT1                   | hum     | 2          | 10 µM         | 10.91  |
| 251600    | Melatonin MT1                                   | hum     | 2          | 10 µM         | 18.02  |
| 252610    | Muscarinic M1                                   | hum     | 2          | 10 µM         | 0.73   |
| 252710    | Muscarinic M2                                   | hum     | 2          | 10 µM         | 2.1    |
| 252810    | Muscarinic M3                                   | hum     | 2          | 10 µM         | 3      |
| 257010    | Neuropeptide Y Y1                               | hum     | 2          | 10 µM         | 25.92  |
| 257110    | Neuropeptide Y Y2                               | hum     | 2          | 10 µM         | -6.1   |
| 258590    | Nicotinic Acetylcholine                          | hum     | 2          | 10 µM         | 10.78  |
| 258700    | Nicotinic Acetylcholine Alpha1, Bungarotoxin     | hum     | 2          | 10 µM         | 0.62   |
| 260130    | Opiate delta1 (OP1, DOP)                        | hum     | 2          | 10 µM         | -2.18  |
| 260210    | Opiate kappa (OP2, KOP)                         | hum     | 2          | 10 µM         | 9.71   |
| 260410    | Opiate mu (OP3, MOP)                            | hum     | 2          | 10 µM         | 25.45  |
| 264500    | Phorbol Ester                                   | mouse   | 2          | 10 µM         | 0      |
| 265010    | Platelet Activating Factor (PAF)                | hum     | 2          | 10 µM         | 7.04   |
| 265600    | Potassium Channel [KATP]                        | ham     | 2          | 10 µM         | 4.77   |
| 265900    | Potassium Channel hERG                          | hum     | 2          | 10 µM         | 29.98  |
| 268420    | Prostanoid EP4                                  | hum     | 2          | 10 µM         | 4.82   |
| 268700    | Purinergic P2X                                  | rabbit  | 2          | 10 µM         | 14.25  |
| 268810    | Purinergic P2Y                                  | rat     | 2          | 10 µM         | 6.41   |
| 270000    | Rolipram                                        | rat     | 2          | 10 µM         | 34.89  |
| 271110    | Serotonin (5-Hydroxytryptamine) 5-HT1A          | hum     | 2          | 10 µM         | 2.12   |
| 271700    | Serotonin (5-Hydroxytryptamine) 5-HT2B          | hum     | 2          | 10 µM         | 10     |
| 271910    | Serotonin (5-Hydroxytryptamine) 5-HT3           | hum     | 2          | 10 µM         | 1.64   |
| 278110    | Sigma1                                          | hum     | 2          | 10 µM         | 6.54   |
| 279510    | Sodium Channel, Site 2                          | rat     | 2          | 10 µM         | 9.27   |
| 255520    | Tachykinin NK1                                  | hum     | 2          | 10 µM         | 8.19   |
| 285900    | Thyroid Hormone                                 | rat     | 2          | 10 µM         | 1.61   |
| 220320    | Transporter, Dopamine (DAT)                     | hum     | 2          | 10 µM         | 10.8   |
| 226400    | Transporter, GABA                               | rat     | 2          | 10 µM         | 0.42   |
| 204410    | Transporter, Norepinephrine (NET)               | hum     | 2          | 10 µM         | 91.1   |
| 274030    | Transporter, Serotonin (5-Hydroxytryptamine) (SERT) | hum     | 2          | 10 µM         | -5.97  |

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
R=See Remarks (if any) at end of this section.
ham=Hamster; hum=Human
### Profiling 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:** 1.4 nM * |
| **Ligand:** 1 nM [³H] DPCPX |
| **Non-Specific Ligand:** 100 µM R(-)-PIA |
| **Specific Binding:** 85% * |
| **Quantitation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 2.7 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:** 0.05 µM [³H] CGS-21680 |
| **Non-Specific Ligand:** 50 µM NECA |
| **Specific Binding:** 85% * |
| **Quantitation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 7 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:** 5.9 nM * |
| **Ligand:** 0.5 nM [³²⁵I] AB-MECA |
| **Non-Specific Ligand:** 1 µM IB-MECA |
| **Specific Binding:** 83% * |
| **Quantitation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 1.8 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 µM Phentolamine |
| **Specific Binding:** 90% * |
| **Quantitation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 0.18 pmole/mg Protein * |

* *Historical Values*
### Profiling Methods:

| Source:  | Adrenergic $\alpha_{1B}$ | Source:  | Adrenergic $\alpha_{1D}$ | Source:  | Adrenergic $\alpha_{2A}$ | Source:  | Adrenergic $\beta_{1}$ |
|----------|--------------------------|----------|--------------------------|----------|--------------------------|----------|--------------------------|
| **Ligand:** | 0.25 nM $[^{3}H]$ Prazosin | **Ligand:** | 0.6 nM $[^{3}H]$ Prazosin | **Ligand:** | 1 nM $[^{3}H]$ MK-912 | **Ligand:** | 0.03 nM $[^{125}I]$ Cyanopindolol |
| **Non-Specific Ligand:** | 10 µM Phentolamine | **Non-Specific Ligand:** | 10 µM Phentolamine | **Non-Specific Ligand:** | 10 µM WB-4101 | **Non-Specific Ligand:** | 100 µM S(-)-Propranolol |
| **Specific Binding:** | 90% * | **Specific Binding:** | 80% * | **Specific Binding:** | 95% * | **Specific Binding:** | 95% * |
| **Quantiation Method:** | Radioligand Binding | **Quantiation Method:** | Radioligand Binding | **Quantiation Method:** | Radioligand Binding | **Quantiation Method:** | Radioligand Binding |
| **Significance Criteria:** | ≥50% of max stimulation or inhibition | **Significance Criteria:** | ≥50% of max stimulation or inhibition | **Significance Criteria:** | ≥50% of max stimulation or inhibition | **Significance Criteria:** | ≥50% of max stimulation or inhibition |
| **Bmax:** | 0.18 pmole/mg Protein * | **Bmax:** | 0.17 pmole/mg Protein * | **Bmax:** | 4.6 pmole/mg Protein * | **Bmax:** | 0.072 pmole/mg Protein * |

| **203200** | **203400** | **203620** | **204010** |
|------------|------------|------------|------------|
| **Source:** | Wistar Rat liver | Human recombinant HEK-293 cells | Human recombinant insect Sf9 cells | Human recombinant CHO-K1 cells |
| **Vehicle:** | 1% DMSO | 1% DMSO | 1% DMSO | 1% DMSO |
| **Incubation Time/Temp:** | 60 minutes @ 25°C | 60 minutes @ 25°C | 60 minutes @ 25°C | 2 hours @ 25°C |
| **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA | 50 mM Tris-HCl, pH 7.4 | 50 mM Tris-HCl, pH 7.4, 12.5 mM MgCl$_2$, 2 mM EDTA | 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 |
| **Kd:** | 0.31 nM * | 0.58 nM * | 0.6 nM * | 0.041 nM * |

* Historical Values
### 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                  | 0.2 nM [³H] CGP-12177      |
| Non-Specific Ligand     | 10 µM ICI-118551           |
| Specific Binding        | 95% *                      |
| Quantitation 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 nM *                    |
| Ligand                  | 1.5 nM [³H] Mibolerone    |
| Non-Specific Ligand     | 10 µM Mibolerone          |
| Specific Binding        | 90% *                     |
| Quantitation 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                  | 0.5 nM [³H] (Des-Arg₁₀)-Kallidin |
| Non-Specific Ligand     | 10 µM (Des-Arg⁹, Leu²)-Bradykinin |
| Specific Binding        | 80% *                     |
| Quantitation 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                  | 0.5 nM [³H] Bradykinin       |
| Non-Specific Ligand     | 5 µM Bradykinin              |
| Specific Binding        | 90% *                       |
| Quantitation Method     | Radioligand Binding          |
| Significance Criteria   | ≥50% of max stimulation or inhibition |
| Bmax                    | 9.4 pmole/mg Protein *       |

* Historical Values
| **214510** Calcium Channel L-Type, Benzothiazepine |
|-----------------------------------------------|
| Source: Wistar Rat brain | Ligand: 2 nM $[^3]$H Diltiazem |
| Vehicle: 1% DMSO | Non-Specific Ligand: 10 µM Diltiazem |
| Incubation Time/Temp: 3 hours @ 4°C | Specific Binding: 73% * |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4, 0.1% BSA | Quantitation Method: Radioligand Binding |
| Kd: 0.016 µM * | Significance Criteria: ≥50% of max stimulation or inhibition |
| Bmax: 0.21 pmole/mg Protein * |

| **214600** Calcium Channel L-Type, Dihydropyridine |
|-----------------------------------------------|
| Source: Wistar Rat cerebral cortex | Ligand: 0.1 nM $[^3]$H Nitrendipine |
| Vehicle: 1% DMSO | Non-Specific Ligand: 1 µM Nifedipine |
| Incubation Time/Temp: 90 minutes @ 25°C | Specific Binding: 91% * |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | Quantitation Method: Radioligand Binding |
| Kd: 0.18 nM * | Significance Criteria: ≥50% of max stimulation or inhibition |
| Bmax: 0.23 pmole/mg Protein * |

| **216000** Calcium Channel N-Type |
|-----------------------------------------------|
| Source: Wistar Rat frontal brain | Ligand: 10 pM $[^{125}]$ω-Conotoxin GVIA |
| Vehicle: 1% DMSO | Non-Specific Ligand: 0.1 µM ω-Conotoxin GVIA |
| Incubation Time/Temp: 30 minutes @ 4°C | Specific Binding: 96% * |
| Incubation Buffer: 20 mM Tris-HCl, pH 7.4, 0.5% BSA | Quantitation Method: Radioligand Binding |
| Kd: 0.051 nM * | Significance Criteria: ≥50% of max stimulation or inhibition |
| Bmax: 0.88 pmole/mg Protein * |

| **217030** Cannabinoid CB1 |
|-----------------------------------------------|
| Source: Human recombinant Chem-1 cells | Ligand: 2 nM $[^3]$H SR141716A |
| Vehicle: 1% DMSO | Non-Specific Ligand: 10 µM R(+)-WIN-55,212-2 |
| Incubation Time/Temp: 90 minutes @ 37°C | Specific Binding: 70% * |
| Incubation Buffer: 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA | Quantitation Method: Radioligand Binding |
| Kd: 5.9 nM * | Significance Criteria: ≥50% of max stimulation or inhibition |
| Bmax: 15 pmole/mg Protein * |

* Historical Values
| **219500** Dopamine D<sub>1</sub> |  | **219700** Dopamine D<sub>2S</sub> |  | **219800** Dopamine D<sub>3</sub> |  | **219900** Dopamine D<sub>4.2</sub> |
| --- | --- | --- | --- | --- | --- | --- |
| **Source:** Human recombinant CHO cells | **Ligand:** 1.4 nM [³H] SCH-23390 | **Source:** Human recombinant CHO cells | **Ligand:** 0.16 nM [³H] Spiperone | **Source:** Human recombinant CHO cells | **Ligand:** 0.7 nM [³H] Spiperone | **Source:** Human recombinant CHO-K1 cells | **Ligand:** 0.5 nM [³H] Spiperone |
| **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 10 µM (+)-Butaclamol | **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 10 µM Haloperidol | **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 25 µM S(-)-Sulpiride | **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 10 µM Haloperidol |
| **Incubation Time/Temp:** 2 hours @ 37°C | **Specific Binding:** 90% * | **Incubation Time/Temp:** 2 hours @ 25°C | **Specific Binding:** 90% * | **Incubation Time/Temp:** 2 hours @ 37°C | **Specific Binding:** 85% * | **Incubation Time/Temp:** 2 hours @ 25°C | **Specific Binding:** 90% * |
| **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl | **Quantiation Method:** Radioligand Binding | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl | **Quantiation Method:** Radioligand Binding | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl | **Quantiation Method:** Radioligand Binding | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl | **Quantiation Method:** Radioligand Binding |
| **Kd:** 1.4 nM * | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Kd:** 0.09 nM * | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Kd:** 0.36 nM * | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Kd:** 0.32 nM * | **Significance Criteria:** ≥50% of max stimulation or inhibition |
|  | **Bmax:** 0.63 pmole/mg Protein * |  | **Bmax:** 1.6 pmole/mg Protein * |  | **Bmax:** 1.1 pmole/mg Protein * |  | **Bmax:** 0.55 pmole/mg Protein * |

* Historical Values
### 224010 Endothelin ET<sub>A</sub>

**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 CaCl<sub>2</sub>, 0.05% Tween-20  
**K<sub>d</sub>:** 0.048 nM *  
**Ligand:** 0.03 nM [¹²⁵I] Endothelin-1  
**Non-Specific Ligand:** 0.1 µM Endothelin-1  
**Specific Binding:** 90% *  
**Quantiation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**B<sub>max</sub>:** 0.35 pmole/mg Protein *

### 224110 Endothelin ET<sub>B</sub>

**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 CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 0.5% BSA  
**K<sub>d</sub>:** 0.085 nM *  
**Ligand:** 0.1 nM [¹²⁵I] Endothelin-1  
**Non-Specific Ligand:** 0.1 µM Endothelin-1  
**Specific Binding:** 75% *  
**Quantiation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**B<sub>max</sub>:** 4.3 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 CaCl<sub>2</sub>, 5 mM KCl, 1.2 mM MgSO<sub>4</sub>, 138 mM NaCl  
**K<sub>d</sub>:** 0.17 nM *  
**Ligand:** 0.08 nM [¹²⁵I] EGF (human)  
**Non-Specific Ligand:** 0.1 µM EGF (human)  
**Specific Binding:** 90% *  
**Quantiation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**B<sub>max</sub>:** 5.5 pmole/mg Protein *

### 226010 Estrogen ER<sub>α</sub>

**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  
**K<sub>d</sub>:** 0.2 nM *  
**Ligand:** 0.5 nM [³H] Estradiol  
**Non-Specific Ligand:** 1 µM Diethylstilbestrol  
**Specific Binding:** 85% *  
**Quantiation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**B<sub>max</sub>:** 1400 pmole/mg Protein *

* Historical Values
### 226600  GABA<sub>A</sub>, Flunitrazepam, Central

| Source                      | Wistar Rat brain (minus cerebellum) |
|-----------------------------|-------------------------------------|
| Vehicle                     | 1% DMSO                             |
| Incubation Time/Temp        | 60 minutes @ 25°C                   |
| Incubation Buffer           | 50 mM Phosphate Buffer, pH 7.4      |
| K<sub>d</sub>               | 4.4 nM *                            |
| Ligand                      | 1 nM [³H] Flunitrazepam             |
| Non-Specific Ligand         | 10 µM Diazepam                      |
| Specific Binding            | 91% *                               |
| Quantitation Method         | Radioligand Binding                 |
| Significance Criteria       | ≥50% of max stimulation or inhibition |
| B<sub>max</sub>             | 1.2 pmole/mg Protein *              |

### 226500  GABA<sub>A</sub>, Muscimol, Central

| Source                      | Wistar Rat brain (minus cerebellum) |
|-----------------------------|-------------------------------------|
| Vehicle                     | 1% DMSO                             |
| Incubation Time/Temp        | 10 minutes @ 4°C                    |
| Incubation Buffer           | 50 mM Tris-HCl, pH 7.4              |
| K<sub>d</sub>               | 3.8 nM *                            |
| Ligand                      | 1 nM [³H] Muscimol                  |
| Non-Specific Ligand         | 0.1 µM Muscimol                     |
| Specific Binding            | 90% *                               |
| Quantitation Method         | Radioligand Binding                 |
| Significance Criteria       | ≥50% of max stimulation or inhibition |
| B<sub>max</sub>             | 1.8 pmole/mg Protein *              |

### 228610  GABA<sub>B1A</sub>

| Source                      | Human recombinant CHO cells         |
|-----------------------------|-------------------------------------|
| Vehicle                     | 1% DMSO                             |
| Incubation Time/Temp        | 3 hours @ 25°C                      |
| Incubation Buffer           | 50 mM Tris-HCl, pH 7.4, 2.5 mM CaCl₂, 0.1% BSA |
| K<sub>d</sub>               | 3.3 nM *                            |
| Ligand                      | 4 nM [³H] CGP-54626                 |
| Non-Specific Ligand         | 3 mM GABA                           |
| Specific Binding            | 90% *                               |
| Quantitation Method         | Radioligand Binding                 |
| Significance Criteria       | ≥50% of max stimulation or inhibition |
| B<sub>max</sub>             | 48 pmole/mg Protein *               |

### 232030  Glucocorticoid

| Source                      | Human recombinant Insect cells      |
|-----------------------------|-------------------------------------|
| Vehicle                     | 1% DMSO                             |
| Incubation Time/Temp        | 1 day @ 4°C                         |
| Incubation Buffer           | 5 mM KH₂PO₄, 8 mM Na₂HPO₄·12H₂O, pH7.4, 137 mM NaCl, 2.7 mM KCl, 0.2% BSA |
| K<sub>d</sub>               | 4.6 nM *                            |
| Ligand                      | 5 nM [³H] Dexamethasone             |
| Non-Specific Ligand         | 10 µM Dexamethasone                 |
| Specific Binding            | 97% *                               |
| Quantitation Method         | Radioligand Binding                 |
| Significance Criteria       | ≥50% of max stimulation or inhibition |
| B<sub>max</sub>             | 1 pmole/mg Protein *                |

* Historical Values
### 232700 Glutamate, Kainate

| Source                              | Ligand: 5 nM $[^3]H$ Kainic acid |
|------------------------------------|----------------------------------|
| Vehicle: 1% DMSO                    | Non-Specific Ligand: 1 mM L-Glutamic acid |
| Incubation Time/Temp: 60 minutes @ 4°C | Specific Binding: 80% * |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | Quantiation Method: Radioligand Binding |
| Kd: 0.012 µM *                      | Significance Criteria: ≥50% of max stimulation or inhibition |
|                                     | Bmax: 0.35 pmole/mg Protein * |

### 232810 Glutamate, NMDA, Agonism

| Source                              | Ligand: 2 nM $[^3]H$ CGP-39653 |
|------------------------------------|----------------------------------|
| Vehicle: 1% DMSO                    | Non-Specific Ligand: 1 mM L-Glutamic acid |
| Incubation Time/Temp: 20 minutes @ 4°C | Specific Binding: 70% * |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | Quantiation Method: Radioligand Binding |
| Kd: 0.019 µM *                      | Significance Criteria: ≥50% of max stimulation or inhibition |
|                                     | Bmax: 2.3 pmole/mg Protein * |

### 232910 Glutamate, NMDA, Glycine

| Source                              | Ligand: 0.33 nM $[^3]H$ MDL 105,519 |
|------------------------------------|----------------------------------|
| Vehicle: 1% DMSO                    | Non-Specific Ligand: 10 µM MDL 105,519 |
| Incubation Time/Temp: 30 minutes @ 4°C | Specific Binding: 85% * |
| Incubation Buffer: 50 mM HEPES, pH 7.7 | Quantiation Method: Radioligand Binding |
| Kd: 6 nM *                          | Significance Criteria: ≥50% of max stimulation or inhibition |
|                                     | Bmax: 3.7 pmole/mg Protein * |

### 233000 Glutamate, NMDA, Phencyclidine

| Source                              | Ligand: 4 nM $[^3]H$ TCP |
|------------------------------------|----------------------------------|
| Vehicle: 1% DMSO                    | Non-Specific Ligand: 1 µM Dizocilpine ((+)-MK-801) |
| Incubation Time/Temp: 45 minutes @ 25°C | Specific Binding: 94% * |
| Incubation Buffer: 10 mM Tris-HCl, pH 7.4 | Quantiation Method: Radioligand Binding |
| Kd: 8.4 nM *                        | Significance Criteria: ≥50% of max stimulation or inhibition |
|                                     | Bmax: 0.78 pmole/mg Protein * |

* Historical Values
| Source: Histamine H₁ | Source: Histamine H₂ | Source: Histamine H₃ | Source: Imidazoline I₂, Central |
|---------------------|---------------------|---------------------|-------------------------------|
| **Ligand:** 1.2 nM [³H] Pyrilamine | **Ligand:** 0.1 nM [¹²⁵I] Aminopotentidine | **Ligand:** 0.4 nM [³H] N-α-Methylhistamine (NAMH) | **Ligand:** 2 nM [³H] Idazoxan |
| **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO |
| **Incubation Time/Temp:** 3 hours @ 25°C | **Incubation Time/Temp:** 2 hours @ 25°C | **Incubation Time/Temp:** 2 hours @ 25°C | **Incubation Time/Temp:** 30 minutes @ 25°C |
| **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 2 mM MgCl₂, 100 mM NaCl, 250 mM Sucrose | **Incubation Buffer:** 50 mM Phosphate, pH 7.4 | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.1% BSA | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA |
| **Kd:** 1.1 nM | **Kd:** 0.45 nM | **Kd:** 0.38 nM | **Kd:** 4 nM |
| **Non-Specific Ligand:** 1 µM Pyrilamine | **Non-Specific Ligand:** 3 µM Tiotidine | **Non-Specific Ligand:** 1 µM R(-)-α-Methylhistamine (RAMH) | **Non-Specific Ligand:** 1 µM Idazoxan |
| **Specific Binding:** 94% * | **Specific Binding:** 90% * | **Specific Binding:** 90% * | **Specific Binding:** 85% * |
| **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 6.7 pmole/mg Protein * | **Bmax:** 6.9 pmole/mg Protein * | **Bmax:** 2 pmole/mg Protein * | **Bmax:** 0.14 pmole/mg Protein * |

* Historical Values
| **243520** Interleukin IL-1 | **250460** Leukotriene, Cysteiny CysLT₁ | **251600** Melatonin MT₁ | **252610** Muscarinic M₁ |
|----------------------------|--------------------------------|-----------------------|-----------------------|
| **Source:** Mouse 3T3-SWISS cells | **Source:** Human recombinant CHO-K1 cells | **Source:** Human recombinant CHO-K1 cells | **Source:** Human recombinant CHO-K1 cells |
| **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO | **Vehicle:** 1% DMSO |
| **Incubation Time/Temp:** 2 hours @ 37°C | **Incubation Time/Temp:** 30 minutes @ 25°C | **Incubation Time/Temp:** 3 hours @ 25°C | **Incubation Time/Temp:** 2 hours @ 25°C |
| **Incubation Buffer:** RPMI 1640, 20 mM HEPES, pH 7.4, 0.1% Sodium Azide, 1% BSA | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 5 mM CaCl₂, 5 mM MgCl₂, 100 µg/ml Bacitracin, 1 mM Benzamidine, 0.1 mM PMSF | **Incubation Buffer:** 25 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.5% BSA | **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM EDTA |
| **Kd:** 0.25 nM | **Kd:** 0.21 nM | **Kd:** 0.054 nM | **Kd:** 0.26 nM |
| **Ligand:** 0.1 nM [¹²⁵I] Interleukin-1β | **Ligand:** 0.3 nM [³H] LTD₄ | **Ligand:** 0.05 nM [¹²⁵I] 2-Iodomelatonin | **Ligand:** 0.8 nM [³H] N-Methylscopolamine |
| **Non-Specific Ligand:** 10 µM Interleukin-1β | **Non-Specific Ligand:** 0.3 µM LTD₄ | **Non-Specific Ligand:** 1 µM 6-Chloromelatonin | **Non-Specific Ligand:** 1 µM Atropine |
| **Specific Binding:** 80% | **Specific Binding:** 93% | **Specific Binding:** 97% | **Specific Binding:** 95% |
| **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding |
| **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 820 R/cell Protein | **Bmax:** 3 pmole/mg Protein | **Bmax:** 3.5 pmole/mg Protein | **Bmax:** 2 pmole/mg Protein |

* Historical Values
### 252710 Muscarinic M₂

| 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 MgCl₂, 1 mM EDTA |
| Kd                      | 0.58 nM *                      |

| Ligand                  | 0.8 nM [³H] N-Methylscopolamine |
|-------------------------|---------------------------------|
| Non-Specific Ligand     | 1 µM Atropine                   |
| Specific Binding        | 95% *                           |
| Quantitation Method     | Radioligand Binding             |
| Significance Criteria   | ≥50% of max stimulation or inhibition |
| Bmax                    | 5.1 pmole/mg Protein *           |

* Historical Values

### 252810 Muscarinic M₃

| 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 MgCl₂, 1 mM EDTA |
| Kd                      | 0.75 nM *                      |

| Ligand                  | 0.8 nM [³H] N-Methylscopolamine |
|-------------------------|---------------------------------|
| Non-Specific Ligand     | 1 µM Atropine                   |
| Specific Binding        | 95% *                           |
| Quantitation Method     | Radioligand Binding             |
| Significance Criteria   | ≥50% of max stimulation or inhibition |
| Bmax                    | 5.4 pmole/mg Protein *           |

* Historical Values

### 257010 Neuropeptide Y Y₁

| 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 MgCl₂, 2.5 mM CaCl₂, 0.1% BSA, 0.01% Bacitracin |
| Kd                      | 0.24 nM *                      |

| Ligand                  | 0.015 nM [¹²⁵I] Peptide YY     |
|-------------------------|--------------------------------|
| Non-Specific Ligand     | 1 µM Neuropeptide Y (human, rat) |
| Specific Binding        | 80% *                          |
| Quantitation Method     | Radioligand Binding            |
| Significance Criteria   | ≥50% of max stimulation or inhibition |
| Bmax                    | 0.58 pmole/mg Protein *         |

### 257110 Neuropeptide Y Y₂

| 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 CaCl₂, 1 mM MgCl₂, 0.1% Bacitracin |
| Kd                      | 0.012 nM *                     |

| Ligand                  | 10 pM [¹²⁵I] Peptide YY        |
|-------------------------|--------------------------------|
| Non-Specific Ligand     | 1 µM Neuropeptide Y (13-36) (porcine) |
| Specific Binding        | 90% *                          |
| Quantitation Method     | Radioligand Binding            |
| Significance Criteria   | ≥50% of max stimulation or inhibition |
| Bmax                    | 0.5 pmole/mg Protein *          |

* Historical Values
| Source | Ligand | Vehicle | Non-Specific Ligand | Specific Binding | Quantitation Method | Significance Criteria | Bmax |
|--------|--------|---------|--------------------|-----------------|---------------------|----------------------|------|
| Nicotinic Acetylcholine Human IMR-32 cells | 0.1 nM $[^{125}]$ Epibatidine | 1% DMSO | 300 µM (-)-Nicotine | 97% * | Radioligand Binding | ≥50% of max stimulation or inhibition | 0.46 pmole/mg Protein * |
| Nicotinic Acetylcholine α, Bungarotoxin Human RD cells | 0.6 nM $[^{125}]$ α-Bungarotoxin | 1% DMSO | 1 µM α-Bungarotoxin | 85% * | Radioligand Binding | ≥50% of max stimulation or inhibition | 1 pmole/mg Protein * |
| Opiate δ$_1$ (OP1, DOP) Human recombinant HEK-293 cells | 1.3 nM $[^{3}H]$ Naltrindole | 1% DMSO | 1 µM Naltrindole | 95% * | Radioligand Binding | ≥50% of max stimulation or inhibition | 7.6 pmole/mg Protein * |
| Opiate κ(OP2, KOP) Human recombinant HEK-293 cells | 0.6 nM $[^{3}H]$ Diprenorphine | 1% DMSO | 10 µM Naloxone | 90% * | Radioligand Binding | ≥50% of max stimulation or inhibition | 1.1 pmole/mg Protein * |

* Historical Values
| **260410** Opiate µ (OP3, MOP) | Ligand: 0.6 nM[^3]H Diprenorphine | Non-Specific Ligand: 10 µM Naloxone |
| Source: Human recombinant CHO-K1 cells | Specific Binding: 90% * | Specific Binding: 90% * |
| Vehicle: 1% DMSO | Quantitation Method: Radioligand Binding | Quantitation Method: Radioligand Binding |
| Incubation Time/Temp: 60 minutes @ 25°C | Significance Criteria: ≥50% of max stimulation or inhibition | Significance Criteria: ≥50% of max stimulation or inhibition |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4 | Bmax: 3.8 pmole/mg Protein * | Bmax: 3.8 pmole/mg Protein * |
| Kd: 0.41 nM * |  |

| **264500** Phorbol Ester | Ligand: 3 nM[^3]H PDBu | Non-Specific Ligand: 1 µM PDBu |
| Source: ICR Mouse brain | Specific Binding: 80% * | Specific Binding: 80% * |
| Vehicle: 1% DMSO | Quantitation Method: Radioligand Binding | Quantitation Method: Radioligand Binding |
| Incubation Time/Temp: 60 minutes @ 25°C | Significance Criteria: ≥50% of max stimulation or inhibition | Significance Criteria: ≥50% of max stimulation or inhibition |
| Incubation Buffer: 20 mM Tris-HCl, pH 7.4, 5 mM CaCl2 | Bmax: 26 pmole/mg Protein * | Bmax: 26 pmole/mg Protein * |
| Kd: 8.7 nM * |  |

| **265010** Platelet Activating Factor (PAF) | Ligand: 0.12 nM[^3]H PAF | Non-Specific Ligand: 1 µM PAF |
| Source: Human platelets | Specific Binding: 90% * | Specific Binding: 90% * |
| Vehicle: 1% DMSO | Quantitation Method: Radioligand Binding | Quantitation Method: Radioligand Binding |
| Incubation Time/Temp: 3 hours @ 25°C | Significance Criteria: ≥50% of max stimulation or inhibition | Significance Criteria: ≥50% of max stimulation or inhibition |
| Incubation Buffer: 50 mM Tris-HCl, pH 7.4, 100 mM KCl, 5 mM EDTA, 5 mM MgCl2, 0.25% BSA | Bmax: 120 R/cell * | Bmax: 120 R/cell * |
| Kd: 0.13 nM * |  |

| **265600** Potassium Channel [KATP] | Ligand: 5 nM[^3]H Glyburide | Non-Specific Ligand: 1 µM Glyburide |
| Source: Hamster pancreatic HIT-T15 beta cells | Specific Binding: 90% * | Specific Binding: 90% * |
| Vehicle: 1% DMSO | Quantitation Method: Radioligand Binding | Quantitation Method: Radioligand Binding |
| Incubation Time/Temp: 2 hours @ 25°C | Significance Criteria: ≥50% of max stimulation or inhibition | Significance Criteria: ≥50% of max stimulation or inhibition |
| Incubation Buffer: 50 mM MOPS, pH 7.4, 0.1 mM CaCl2 | Bmax: 1 pmole/mg Protein * | Bmax: 1 pmole/mg Protein * |
| Kd: 0.64 nM * |  |

* Historical Values
| **265900** Potassium Channel hERG | **268420** Prostanoid EP<sub>4</sub> | **268700** Purinergic P<sub>2X</sub> | **268810** Purinergic P<sub>2Y</sub> |
|----------------------------------|-------------------------------------|----------------------------------|----------------------------------|
| **Source:** Human recombinant HEK-293 cells | **Ligand:** 1.5 nM [³H] Astemizole | **Ligand:** 8 nM [³H] α, β-Methylene-ATP | **Ligand:** 0.1 nM [³S] ATP-αS |
| **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 10 µM Astemizole | **Non-Specific Ligand:** 100 µM β, γ-Methylene ATP | **Non-Specific Ligand:** 10 µM ADP-βS |
| **Incubation Time/Temp:** 60 minutes @ 25°C | **Specific Binding:** 90% | **Specific Binding:** 80% | **Specific Binding:** 87% |
| **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 | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding |
| **Kd:** 6.8 nM | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 6.3 pmole/mg Protein | **Bmax:** 4.3 pmole/mg Protein | **Bmax1:** 2 pmole/mg Protein | **Bmax1:** 16 pmole/mg Protein |

| **Source:** Human recombinant Chem-1 cells | **Ligand:** 1 nM [³H] Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) | **Ligand:** 2 pmole/mg Protein | **Ligand:** 790 pmole/mg Protein |
| **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 10 µM Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) | **Non-Specific Ligand:** 2 pmole/mg Protein | **Non-Specific Ligand:** 10 µM ADP-βS |
| **Incubation Time/Temp:** 2 hours @ 25°C | **Specific Binding:** 90% | **Specific Binding:** 80% | **Specific Binding:** 87% |
| **Incubation Buffer:** 10 mM MES, pH 6.0, 1 mM EDTA, 10 mM MgCl₂ | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding |
| **Kd:** 0.69 nM | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Bmax:** 6.3 pmole/mg Protein | **Bmax:** 4.3 pmole/mg Protein | **Bmax1:** 2 pmole/mg Protein | **Bmax1:** 790 pmole/mg Protein |

| **Source:** New Zealand derived albino Rabbit urinary bladder | **Ligand:** 8 nM [³H] α, β-Methylene-ATP | **Ligand:** 2 pmole/mg Protein | **Ligand:** 16 pmole/mg Protein |
| **Vehicle:** 1% DMSO | **Non-Specific Ligand:** 100 µM β, γ-Methylene ATP | **Non-Specific Ligand:** 10 µM ADP-βS | **Non-Specific Ligand:** 10 µM ADP-βS |
| **Incubation Time/Temp:** 30 minutes @ 25°C | **Specific Binding:** 80% | **Specific Binding:** 87% | **Specific Binding:** 87% |
| **Incubation Buffer:** 50 mM Tris-HCl, pH 7.4 | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding | **Quantiation Method:** Radioligand Binding |
| **Kd1:** 2.2 nM | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition | **Significance Criteria:** ≥50% of max stimulation or inhibition |
| **Kd2:** 2.2 µM | **Bmax1:** 2 pmole/mg Protein | **Bmax2:** 790 pmole/mg Protein | **Bmax:** 16 pmole/mg Protein |

* Historical Values
| **270000** Rolipram | **Source:** | Wistar Rat brain | **Ligand:** | 1.8 nM $[^3]$H Rolipram |
| | **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10 µM Rolipram |
| | **Incubation Time/Temp:** | 60 minutes @ 4°C | **Specific Binding:** | 90% * |
| | **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4 | **Quantiation Method:** | Radioligand Binding |
| | **Kd:** | 1 nM * | **Significance Criteria:** | ≥50% of max stimulation or inhibition |
| | **Bmax:** | | **Bmax:** | 0.31 pmole/mg Protein * |

| **271110** Serotonin (5-Hydroxytryptamine) 5-HT$_{1A}$ | **Source:** | Human recombinant CHO-K1 cells | **Ligand:** | 1.5 nM $[^3]$H 8-OH-DPAT |
| | **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10 µM Metergoline |
| | **Incubation Time/Temp:** | 60 minutes @ 25°C | **Specific Binding:** | 75% * |
| | **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 0.1% Ascorbic Acid, 0.5 mM EDTA, 10 mM MgSO$_4$ | **Quantiation Method:** | Radioligand Binding |
| | **Kd:** | 2 nM * | **Significance Criteria:** | ≥50% of max stimulation or inhibition |
| | **Bmax:** | | **Bmax:** | 1.3 pmole/mg Protein * |

| **271700** Serotonin (5-Hydroxytryptamine) 5-HT$_{2B}$ | **Source:** | Human recombinant CHO-K1 cells | **Ligand:** | 1.2 nM $[^3]$H Lysergic acid diethylamide (LSD) |
| | **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10 µM Serotonin (5-HT) |
| | **Incubation Time/Temp:** | 60 minutes @ 37°C | **Specific Binding:** | 80% * |
| | **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 4 mM CaCl$_2$, 0.1% Ascorbic Acid | **Quantiation Method:** | Radioligand Binding |
| | **Kd:** | 2.1 nM * | **Significance Criteria:** | ≥50% of max stimulation or inhibition |
| | **Bmax:** | | **Bmax:** | 1.1 pmole/mg Protein * |

| **271910** Serotonin (5-Hydroxytryptamine) 5-HT$_{3}$ | **Source:** | Human recombinant HEK-293 cells | **Ligand:** | 0.69 nM $[^3]$H GR-65630 |
| | **Vehicle:** | 1% DMSO | **Non-Specific Ligand:** | 10 µM MDL 72222 |
| | **Incubation Time/Temp:** | 60 minutes @ 25°C | **Specific Binding:** | 90% * |
| | **Incubation Buffer:** | 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM MgCl$_2$ | **Quantiation Method:** | Radioligand Binding |
| | **Kd:** | 0.2 nM * | **Significance Criteria:** | ≥50% of max stimulation or inhibition |
| | **Bmax:** | | **Bmax:** | 11 pmole/mg Protein * |

* Historical Values
### Sigma σ1

**Source:** Human Jurkat cells  
**Vehicle:** 1% DMSO  
**Incubation Time/Temp:** 4 hours @ 25°C  
**Incubation Buffer:** 5 mM Potassium Phosphate, pH 7.5  
**Kd:** 5.8 nM *  
**Ligand:** 8 nM [³H] Haloperidol  
**Non-Specific Ligand:** 10 µM Haloperidol  
**Specific Binding:** 80% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 0.71 pmole/mg Protein *

### Tachykinin NK1

**Source:** Human recombinant CHO cells  
**Vehicle:** 1% DMSO  
**Incubation Time/Temp:** 90 minutes @ 4°C  
**Incubation Buffer:** 20 mM HEPES, pH 7.4, 1 mM MnCl₂, 0.1% BSA  
**Kd:** 2.1 nM *  
**Ligand:** 0.8 nM [³H] Substance P  
**Non-Specific Ligand:** 10 µM L-703,606  
**Specific Binding:** 90% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 1.7 pmole/mg Protein *

### Thyroid Hormone

**Source:** Wistar Rat liver  
**Vehicle:** 1% DMSO  
**Incubation Time/Temp:** 18 hours @ 4°C  
**Incubation Buffer:** 20 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10% Glycerol, 2 mM EDTA, 5 mM DTT  
**Kd:** 0.034 nM *  
**Ligand:** 0.03 nM [¹²⁵I] Triiodothyronine  
**Non-Specific Ligand:** 1 µM Triiodothyronine  
**Specific Binding:** 77% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 0.16 pmole/mg Protein *

### Transporter, Dopamine (DAT)

**Source:** Human recombinant CHO-K1 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:** 0.58 nM *  
**Ligand:** 0.15 nM [¹²⁵I] RTI-55  
**Non-Specific Ligand:** 10 µM Nomifensine  
**Specific Binding:** 90% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 0.047 pmole/mg Protein *

* Historical Values
### 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:** 0.3 µM *  
**Ligand:** 6 nM [3H] GABA  
**Non-Specific Ligand:** 10 µM NO-711  
**Specific Binding:** 80% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 60 pmole/mg Protein *

### 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:** 0.2 nM [125I] RTI-55  
**Non-Specific Ligand:** 10 µM Desipramine  
**Specific Binding:** 75% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 2.5 pmole/mg Protein *

### 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:** 0.4 nM [3H] Paroxetine  
**Non-Specific Ligand:** 10 µM Imipramine  
**Specific Binding:** 95% *  
**Quantitation Method:** Radioligand Binding  
**Significance Criteria:** ≥50% of max stimulation or inhibition  
**Bmax:** 4.4 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 | 0.9 | 305599  | 0.42 µM  |
| 200610  | Adenosine A_{2A}      | CGS-21680                    | 0.13 µM  | 0.079 µM | 1   | 305600  | 0.11 µM  |
| 200720  | Adenosine A_{3}       | IB-MECA                      | 0.78 nM  | 0.72 nM | 0.8  | 305602  | 1.08 nM  |
| 203100  | Adrenergic α_{1A}     | Prazosin                     | 0.69 nM  | 0.28 nM | 0.9  | 305613  | 0.25 nM  |
| 203200  | Adrenergic α_{1B}     | Prazosin                     | 0.27 nM  | 0.15 nM | 1    | 305614  | 0.23 nM  |
| 203400  | Adrenergic α_{1D}     | Prazosin                     | 0.88 nM  | 0.43 nM | 0.7  | 305615  | 0.64 nM  |
| 203620  | Adrenergic α_{2A}     | Yohimbine                    | 8.4 nM   | 3.1 nM  | 0.9  | 305616  | 4.61 nM  |
| 204010  | Adrenergic β_{1}      | (S-) Propranolol             | 2.5 nM   | 1.4 nM  | 0.8  | 305603  | 0.88 nM  |
| 204110  | Adrenergic β_{2}      | (S-) Propranolol             | 0.78 nM  | 0.54 nM | 1.2  | 305604  | 0.4 nM   |
| 285010  | Androgen (Testosterone) AR | Testosterone               | 6.5 nM   | 4.3 nM  | 1    | 305773  | 4.88 nM  |
| 212510  | Bradykinin B_{1}     | (Des-Arg^{10})-Kallidin      | 0.87 nM  | 0.22 nM | 1.1  | 305625  | 0.31 nM  |
| 212620  | Bradykinin B_{2}     | Diltiazem                    | 0.036 µM | 0.032 µM | 0.9  | 305776  | 0.02 µM  |
| 214510  | Calcium Channel L-Type, Benzothiazepine | Diltiazem           | 0.072 µM | 0.046 µM | 0.9  | 305794  | 0.24 nM  |
| 214600  | Calcium Channel L-Type, Dihydropyridine | Nitrendipine       | 0.034 nM | 0.028 nM | 1.6  | 305795  | 0.031 nM |
| 216000  | Calcium Channel N-Type | w-Conotoxin GVIA           | 0.072 nM | 0.046 nM | 0.9  | 305794  | 0.24 nM  |
| 217030  | Cannabinoid CB_{1}    | R(+)-WIN-55,212-2            | 0.072 µM | 0.046 µM | 0.9  | 305794  | 0.24 nM  |
| 219500  | Dopamine D_{1}        | R(+)-SCH-23390              | 1.4 nM   | 0.7 nM  | 0.9  | 305797  | 0.95 nM  |
| 219700  | Dopamine D_{2b}       | Spiperone                    | 0.25 nM  | 0.089 nM | 1    | 305798  | 0.22 nM  |
| 219800  | Dopamine D_{3}        | Spiperone                    | 0.36 nM  | 0.12 nM | 0.9  | 305799  | 0.41 nM  |
| 219900  | Dopamine D_{2}        | Spiperone                    | 0.5 nM   | 0.2 nM  | 0.9  | 305800  | 0.3 nM   |
| 224010  | Endothelin ET_{A}     | Endothelin-1                 | 0.23 nM  | 0.14 nM | 1.1  | 305622  | 0.094 nM |
| 224110  | Endothelin ET_{B}     | Endothelin-1                 | 0.13 nM  | 0.06 nM | 0.9  | 305623  | 0.059 nM |
| 225510  | Epidermal Growth Factor (EGF) | EGF (human)             | 1.6 nM   | 1.1 nM  | 1.1  | 305638  | 3.69 nM  |
| 226010  | Estrogen ER_{A}       | Diethylstilbestrol           | 0.77 nM  | 0.22 nM | 1    | 305716  | 0.98 nM  |
| 226600  | GABA_{A}, Flunitrazepam, Central | Diazepam              | 0.016 µM | 0.013 µM | 0.8  | 305802  | 0.019 µM |
| 226500  | GABA_{A}, Muscimol, Central | GABA               | 0.032 µM | 0.026 µM | 0.9  | 305801  | 0.024 µM |
| 228610  | GABA_{B1A}            | CGP-54626                   | 6.4 nM   | 2.9 nM  | 1    | 305888  | 3.56 nM  |
| 232030  | Glucocorticoid        | Dexamethasone               | 3.8 nM   | 1.8 nM  | 0.9  | 305774  | 5.27 nM  |
| 232700  | Glutamate, Kainate    | L-Glutamic acid             | 0.24 µM  | 0.17 µM | 0.8  | 305634  | 0.16 µM  |
| 232810  | Glutamate, NMDA, Agonism | L-Glutamic acid         | 0.41 µM  | 0.37 µM | 0.9  | 305632  | 0.17 µM  |
| 232910  | Glutamate, NMDA, Glycine | MDL 105,519             | 0.022 µM | 0.021 µM | 0.6  | 305635  | 9.34 nM  |
| 233000  | Glutamate, NMDA, Phencyclidine | Dizocilpine ((+)-MK-801) | 5.1 nM   | 3.4 nM  | 0.7  | 305803  | 3.86 nM  |
| 239610  | Histamine H_{1}       | Pyrilamine                  | 3.3 nM   | 1.6 nM  | 1    | 305804  | 2.59 nM  |
| 239710  | Histamine H_{2}       | Tiotidine                   | 0.022 µM | 0.018 µM | 1.1  | 305805  | 0.027 µM |
| 239820  | Histamine H_{3}       | R(-)-α-Methylhistamine (RAMH) | 2.3 nM   | 1.1 nM  | 1.1  | 305806  | 1.05 nM  |
| 241000  | Imidazoline I_{2}, Central | Idazoxan              | 0.012 µM | 8 nM    | 1    | 305807  | 7 nM     |
| 243520  | Interleukin IL-1      | IL-1β                       | 0.19 nM  | 0.14 nM | 1.3  | 305639  | 0.36 nM  |

* Batch: Represents compounds tested concurrently in the same assay(s).
| Batch | Compounds                                                                 | EC50 | IC50 | Ratio | Batch | EC50 | IC50 | Ratio |
|-------|---------------------------------------------------------------------------|------|------|-------|-------|------|------|-------|
| 250460 | Leukotriene, Cysteinyl CysLT1 | LTD4 | 0.7 nM | 0.29 nM | 1 | 305750 | 1.24 nM |
| 251600 | Melatonin MT1 | Melatonin | 0.21 nM | 0.11 nM | 0.7 | 305752 | 0.31 nM |
| 252610 | Muscarinic M1 | 4-DAMP | 4.5 nM | 1.1 nM | 1 | 305608 | 4.67 nM |
| 252710 | Muscarinic M2 | 4-DAMP | 0.055 µM | 0.023 µM | 1 | 305609 | 0.035 µM |
| 252810 | Muscarinic M3 | 4-DAMP | 5.1 nM | 2.5 nM | 1.1 | 305610 | 2.29 nM |
| 257010 | Neuropeptide Y Y1 | Neuropeptide Y (human, rat) | 0.22 nM | 0.21 nM | 1.1 | 305636 | 0.51 nM |
| 257110 | Neuropeptide Y Y2 | Neuropeptide Y (13-36) (porcine) | 0.21 nM | 0.12 nM | 0.9 | 305637 | 0.49 nM |
| 258590 | Nicotinic Acetylcholine | Epibatidine | 0.076 nM | 0.052 nM | 0.9 | 305811 | 0.078 nM |
| 258700 | Nicotinic Acetylcholine α, Bungarotoxin | α-Bungarotoxin | 1.1 nM | 0.72 nM | 1.1 | 305812 | 1.93 nM |
| 260130 | Opiate δ (OP1, DOP) | Naltirindole | 0.91 nM | 0.16 nM | 1 | 305631 | 1.12 nM |
| 260210 | Opiate κ(OP2, KOP) | U-69593 | 0.016 µM | 6.4 nM | 0.5 | 305988 | 0.012 µM |
| 260410 | Opiate μ(OP3, MOP) | DAMGO | 0.02 µM | 8.1 nM | 0.6 | 305815 | 0.038 µM |
| 264500 | Phorbol Ester | PMA | 0.79 nM | 0.59 nM | 1 | 305816 | 1.08 nM |
| 265010 | Platelet Activating Factor (PAF) | PAF | 0.28 nM | 0.15 nM | 0.9 | 305687 | 0.25 nM |
| 265600 | Potassium Channel [KATP] | Glyburide | 5.7 nM | 0.65 nM | 0.8 | 305817 | 4.13 nM |
| 265900 | Potassium Channel hERG | Astemizole | 2.6 nM | 2.1 nM | 1.1 | 305818 | 3.69 nM |
| 268420 | Prostanoid EP4 | Prostaglandin E2 (PGE2) | 1.1 nM | 0.45 nM | 0.9 | 305621 | 1.25 nM |
| 268700 | Purinergic P2X | α, β-Methylene ATP | 0.082 µM | 0.018 µM | 1.1 | 305713 | 0.029 µM |
| 268810 | Purinergic P2Y | ATP | 0.018 µM | 0.018 µM | 0.9 | 305714 | 0.019 µM |
| 270000 | Rolipram | Rolipram | 5.7 nM | 2.1 nM | 1 | 305820 | 3.24 nM |
| 271110 | Serotonin (5-Hydroxytryptamine) 5-HT1A | Metergoline | 4.1 nM | 2.3 nM | 0.9 | 305821 | 1.93 nM |
| 271700 | Serotonin (5-Hydroxytryptamine) 5-HT2A | Ketanserin | 0.29 µM | 0.18 µM | 0.6 | 305822 | 0.41 µM |
| 271910 | Serotonin (5-Hydroxytryptamine) 5-HT3 | MDL 72222 | 0.011 µM | 2.5 nM | 0.8 | 305720 | 0.017 µM |
| 278110 | Sigma σ1 | Haloperidol | 0.021 µM | 8.8 nM | 0.9 | 305823 | 6.99 nM |
| 255520 | Tachykinin NK1 | L-703,606 | 3.6 nM | 2.6 nM | 1 | 305810 | 7.25 nM |
| 285900 | Thyroid Hormone | Triiodothyronine | 0.034 nM | 0.018 nM | 1 | 305624 | 0.04 nM |
| 220320 | Transporter, Dopamine (DAT) | GBR-12909 | 1.7 nM | 1.3 nM | 0.9 | 305619 | 0.8 nM |
| 226400 | Transporter, GABA | NO-711 | 0.2 µM | 0.2 µM | 1.1 | 305882 | 0.21 µM |
| 204410 | Transporter, Norepinephrine (NET) | Desipramine | 0.93 nM | 0.92 nM | 0.6 | 305618 | 0.67 nM |
| 274030 | Transporter, Serotonin (5-Hydroxytryptamine) (SERT) | Fluoxetine | 8.6 nM | 1.4 nM | 0.9 | 305620 | 6.95 nM |

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

**REFERENCES**

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