Supporting Information

Indolinyl-thiazole based inhibitors of Scavenger Receptor-BI (SR-BI)-mediated lipid transport

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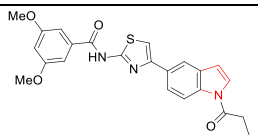
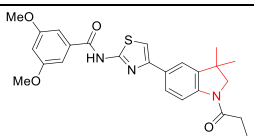
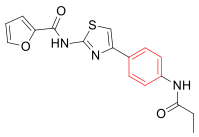
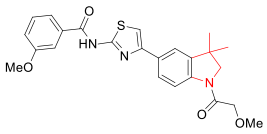
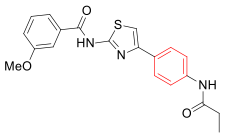
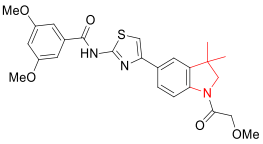
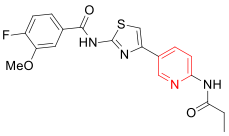
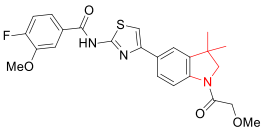
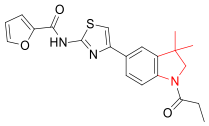
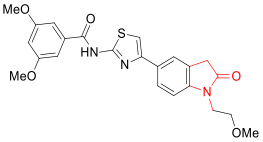
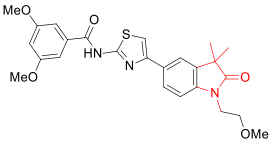
Contents

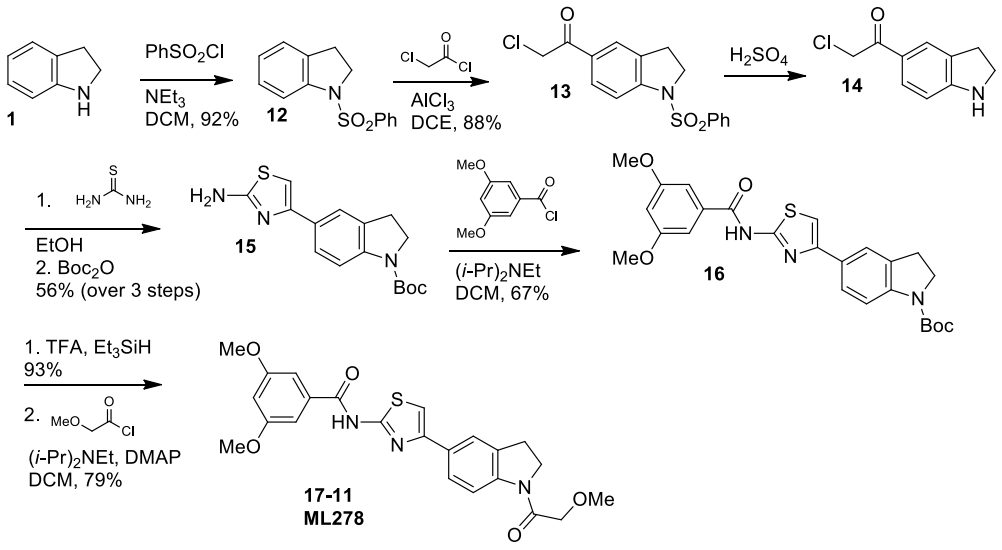
1. Additional SAR data

Table S1. Modifications to indoline ring[a]

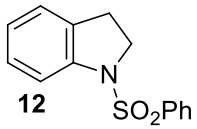
Cmp	Structure	IC_{50} (μM)[a]	Cmp	Structure	IC_{50} (μM)[a]
18		0.089	**23**		0.012
19		2.0	**24**		0.20
20		0.085 ±0.02	**25**		0.09
21		0.2	**26**		0.40
22		0.020	**27**		0.07
			28		0.09

[a] Average of at least two measurements in dil uptake assay, ± standard of mean when n > 2.

2. Synthesis and characterization data for 17-11 (ML278)

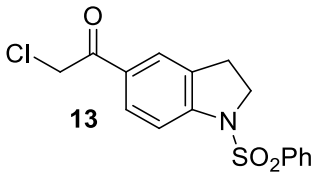


General Details. All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on a Bruker 300 MHz or Varian UNITY INOVA 500 MHz spectrometer as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm; δ) relative to tetramethylsilane, $CDCl_3$ solvent, or d_6-DMSO (^{1}H δ 0, ^{13}C δ 77.16, or ^{13}C δ 39.5, respectively). NMR data are reported as follows: chemical shifts, multiplicity (obs. = obscured, app = apparent, br = broad, s = singlet, d = doublet, t = triplet, m = multiplet, comp = complex overlapping signals); coupling constant(s) in Hz; integration. Unless otherwise indicated, NMR data were collected at 25 °C. Flash chromatography was performed using 40-60 μm Silica Gel on a Teledyne Isco Combiflash R_f system. Tandem liquid chromatography/mass spectrometry (LCMS) was performed on a Waters 2795 separations module and Waters 3100 mass detector. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. High-resolution mass spectra were obtained at the MIT Mass Spectrometry Facility with a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance mass spectrometer. Compound purity and identity were determined by UPLC-MS (Waters, Milford, MA). Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive electrospray ionization. Mobile Phase A consisted of either 0.1% ammonium hydroxide or 0.1% trifluoroacetic acid in water, while mobile Phase B consisted of the same additives in acetonitrile. The gradient ran from 5% to 95% mobile Phase B over 0.8 minutes at 0.45 ml/min. An Acquity BEH C18, 1.7 μm, 1.0 x 50 mm column was used with column temperature maintained at 65 °C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/ml, and 0.25 μl of this solution was injected.



12

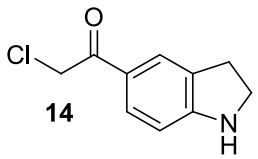
1-(Phenylsulfonyl)indoline (12): Indoline (1.4 ml, 12.6 mmol) was placed in a round-bottom flask equipped with a magnetic stirbar and dissolved in dichloromethane (42.0 ml). After cooling to 0 °C, triethylamine (3.5 ml, 25.2 mmol) was added to the solution, followed by benzenesulfonyl chloride (1.7 ml, 13.3 mmol). The reaction was stirred for 30 minutes while warming to room temperature. Saturated sodium bicarbonate solution (aqueous, 30 ml) was added to quench the reaction. The layers were separated, and the aqueous layer was extracted with dichloromethane (3 x 25 ml). The combined organics were washed with brine (25 ml), then shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give a light tan solid. The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 80/20) to give the title compound as a light pink solid (3.00 g, 92 %). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.82 – 7.77 (m, 2H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.20 (t, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.93 (t, *J* = 8.4 Hz, 2H), 2.89 (t, *J* = 8.4 Hz, 2H); **MS** (ESI^+): 260 (M+H).



13

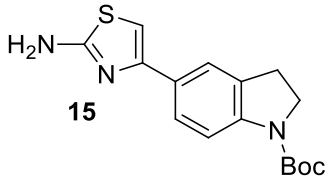
2-Chloro-1-(1-(phenylsulfonyl)indolin-5-yl)ethanone (13): A round-bottom flask was charged with a magnetic stirbar and anhydrous aluminum chloride (1.70 g, 12.7 mmol) then placed under nitrogen atmosphere. Anhydrous 1,2-dichloroethane (28 ml) was added to produce a pale yellow suspension. Chloroacetyl chloride (1.0 ml, 12.7 mmol) was added slowly by syringe. The mixture was stirred at room temperature for 30 minutes. A solution of 1-(phenylsulfonyl)indoline (3.00 g, 11.6 mmol) in anhydrous 1,2-dichloroethane (8.0 ml) was slowly added to the reaction by syringe. The syringe was rinsed twice with anhydrous 1,2-dichloroethane (3.0 ml), and the rinses were added to the reaction. The reaction, now a dark green mixture, was heated to 50 °C and stirred for 4 hours. Additional portions of aluminum chloride (1.30 g, 9.7 mmol) and chloroacetyl chloride (0.75 ml, 9.5 mmol) were dissolved in anhydrous 1,2-dichloroethane (10 ml) and added to the reaction. After stirring 2 more hours at 50 °C, 5 ml of anhydrous 1,2-dichloroethane containing another 0.8 g (6.0 mmol) of aluminum chloride and 0.5 ml (6.4 mmol) of chloroacetyl chloride was added. Stirring at 50 °C was continued for another hour to complete the reaction. The dark red mixture was slowly poured into ice water (approx.250 ml) and further diluted with dichloromethane (200 ml). The resulting cloudy, orange mixture was stirred while warming to room temperature, after which the layers were separated and the aqueous phase was extracted with dichloromethane (3 x 100 ml). The combined organic layers were washed with water (100 ml) and brine (100 ml), then shaken over magnesium sulfate, filtered, and

concentrated under reduced pressure to give a brown solid (3.91 g). The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 0/100) to give the title compound as a tan solid (3.41 g, 88 %). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.87 – 7.81 (m, 3H), 7.75 – 7.69 (m, 2H), 7.65 – 7.57 (m, 1H), 7.54 – 7.47 (m, 2H), 4.63 (s, 2H), 4.01 (t, *J* = 8.6 Hz, 2H), 3.05 (t, *J* = 8.6 Hz, 2H); **MS** (ESI^+): 336 (M+H).



14

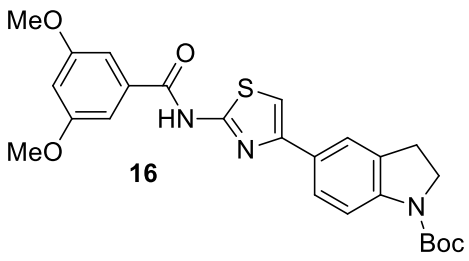
2-Chloro-1-(indolin-5-yl)ethanone (14): A microwave tube was charged with a magnetic stirbar and 2-chloro-1-(1-(phenylsulfonyl)indolin-5-yl)ethanone (3.26 g, 9.54 mmol). Concentrated sulfuric acid (9.0 ml) was added, and the resulting suspension was microwaved for 10 minutes at 100 °C. The reaction was carefully poured into ice water (500 ml). The dark mixture was stirred while warming to room temperature then treated with 10% (w/v) aqueous sodium hydroxide (approximately 200 ml) until the pH >10. This mixture was then extracted with dichloromethane (3 x 250 ml). The combined extracts were washed with brine (200 ml), then shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give a brown solid (1.52 g). This material was used immediately without further purification. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.72 (d, *J* = 1.2 Hz, 1H), 7.69 (dd, *J* = 8.3, 1.6 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 4.60 (s, 2H), 3.70 (t, *J* = 8.6 Hz, 2H), 3.09 (t, *J* = 8.6 Hz, 2H); **MS** (ESI^+): 196 (M+H).



15

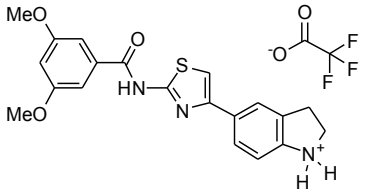
***tert*-Butyl 5-(2-aminothiazol-4-yl)indoline-1-carboxylate (15):** 2-Chloro-1-(indolin-5-yl)ethanone (1.52 g, 7.75 mmol) was placed in a microwave vial and dissolved in anhydrous ethanol (30.0 mL) to give an opaque, black solution. Thiourea (0.66 g, 8.67 mmol) was added and the resulting mixture was microwaved for 30 minutes at 120 °C. 4-(*N*,*N*-Dimethylamino)pyridine (95.0 mg, 0.78 mmol) and *N*,*N*-diisopropylethylamine (1.5 ml, 9.3 mmol) were added to the reaction mixture. Neat di-*tert*-butyl dicarbonate (2.0 mL, 8.52 mmol) was added last, and the reaction was stirred at room temperature for 1 hour. The opaque, red-brown mixture was concentrated under reduced pressure to give a red-brown solid. This material was partitioned between water (50 ml) and ethyl acetate (75 ml) and stirred at room temperature until everything dissolved. The layers were separated and the aqueous phase was extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give an orange-brown solid. The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 40/60) to give the title compound as an orange solid (1.72 g, 56 % over three steps). **^{1}H NMR**

(300 MHz, d_6-DMSO): δ 7.61 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 6.99 (s, 1H), 6.85 (s, 1H), 3.92 (t, *J* = 8.7 Hz, 2H), 3.07 (t, *J* = 8.7 Hz, 2H), 1.51 (s, 9H); **MS** (ESI^+): 318 (M+H).



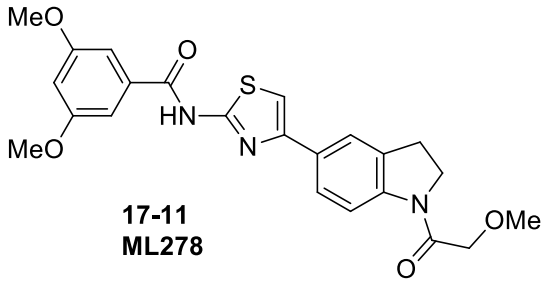
16

***tert*-Butyl 5-(2-(3,5-dimethoxybenzamido)thiazol-4-yl)indoline-1-carboxylate (16):** In a round-bottom flask equipped with a magnetic stirbar, *tert*-butyl 5-(2-aminothiazol-4-yl)indoline-1-carboxylate (0.500 g, 1.58 mmol) was combined with 4-(*N*,*N*-dimethylamino)pyridine (19.0 mg, 0.16 mmol). Dichloromethane (4.0 ml) was added to produce an orange suspension that was cooled to 0 °C. *N*,*N*-Diisopropylethylamine (0.33 ml, 1.89 mmol) was added followed by a solution of 3,5-dimethoxybenzoyl chloride (0.35 g, 1.73 mmol) in dichloromethane (1.00 ml). The bright orange mixture was stirred for 1 hour while warming to room temperature. The resulting clear, red-brown solution was diluted with saturated sodium bicarbonate solution (aqueous, 10 ml) and dichloromethane (10 ml). The layers were separated, and the aqueous phase was extracted with dichloromethane (3 x 10 ml). The combined organics were washed with brine (10 ml) then shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give a thick, orange-brown oil. This material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 70/30) to give the title compounds as a light yellow solid (0.51 g, 67%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 9.84 (s, 1H), 7.60 (d, *J* = 12.2 Hz, 1H), 7.58 (s, 1H), 7.07 (s, 1H), 7.04 (s, 1H), 7.03 (s, 1H), 6.64 (t, *J* = 2.2 Hz, 1H), 4.01 (t, *J* = 8.7 Hz, 2H), 3.84 (s, 6H), 3.12 (t, *J* = 8.7 Hz, 2H), 1.58 (s, 9H); **MS** (ESI^+): 482 (M+H).



***N*-(4-(Indolin-5-yl)thiazol-2-yl)-3,5-dimethoxybenzamide, trifluoroacetic acid salt:** *tert*-Butyl 5-(2-(3,5-dimethoxybenzamido)thiazol-4-yl)indoline-1-carboxylate (0.38 g, 0.79 mmol) was placed in a round-bottom flask with a magnetic stirbar and dissolved in dichloromethane (5.3 mL) to give a clear, yellow solution. Triethylsilane (1.3 mL, 7.93 mmol) was added followed by 2,2,2-trifluoroacetic acid (1.2 mL, 15.9 mmol), and the reaction quickly turned light orange and produced a gas. The reaction was stirred at room temperature for 1 hour. The light pink reaction was concentrated under reduced pressure to give a rose-colored solid. The crude material was suspended in diethyl ether (10 ml) and filtered. The collected solids were washed

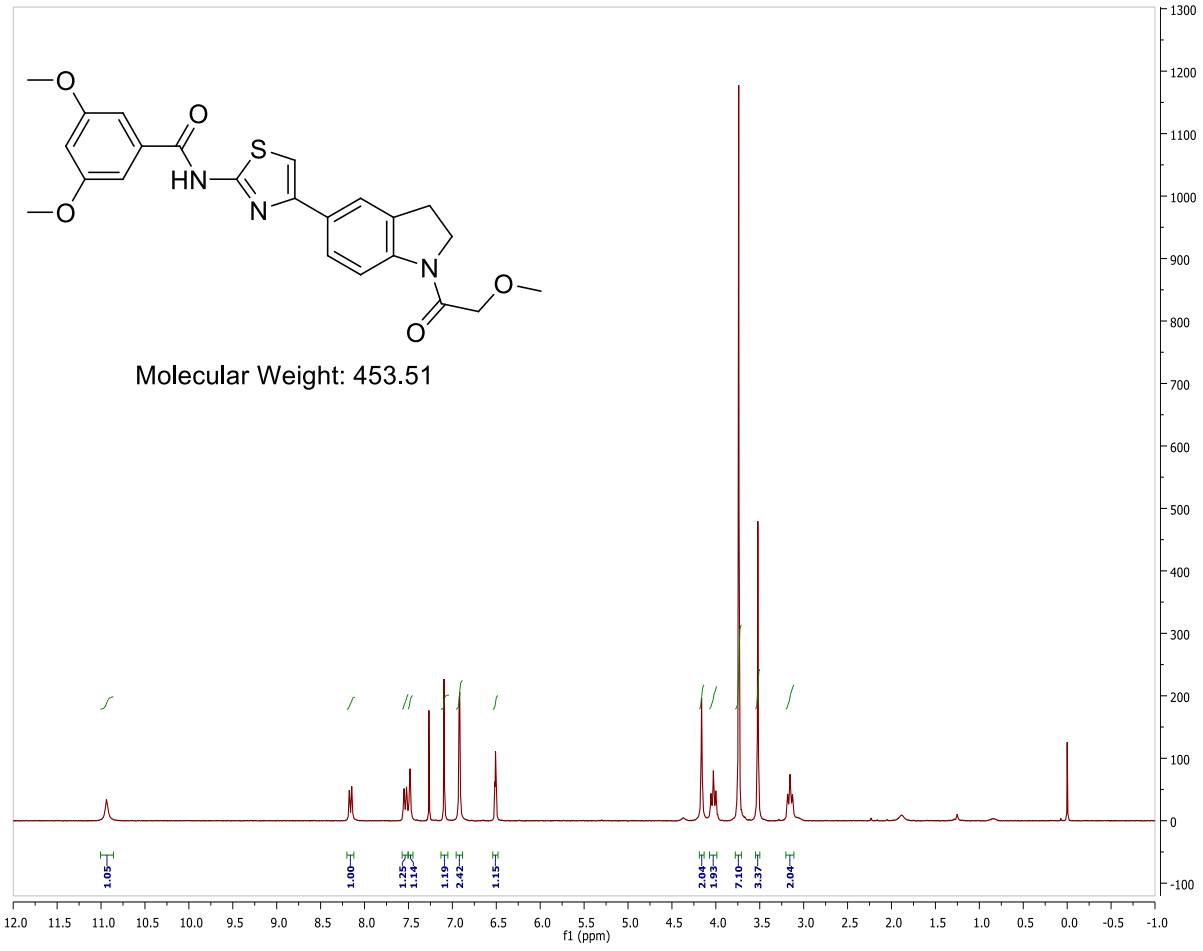
with additional diethyl ether and air dried on the filter to give the title compound as light purple solid (0.36 g, 93 %). **^{1}H NMR (300 MHz, CD_3OD):** δ 8.05 (s, 1H), 7.99 (d, *J* = 10.0 Hz, 1H), 7.51 (s, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.20 (s, 1H), 7.20 (s, 1H), 6.74 (s, 1H), 3.87 (s, 6H), 3.85 (t, *J* = 7.8 Hz, 2H), 3.35 (t, *J* = 7.7 Hz, 2H); **MS** (ESI^+): 382 (M+H).



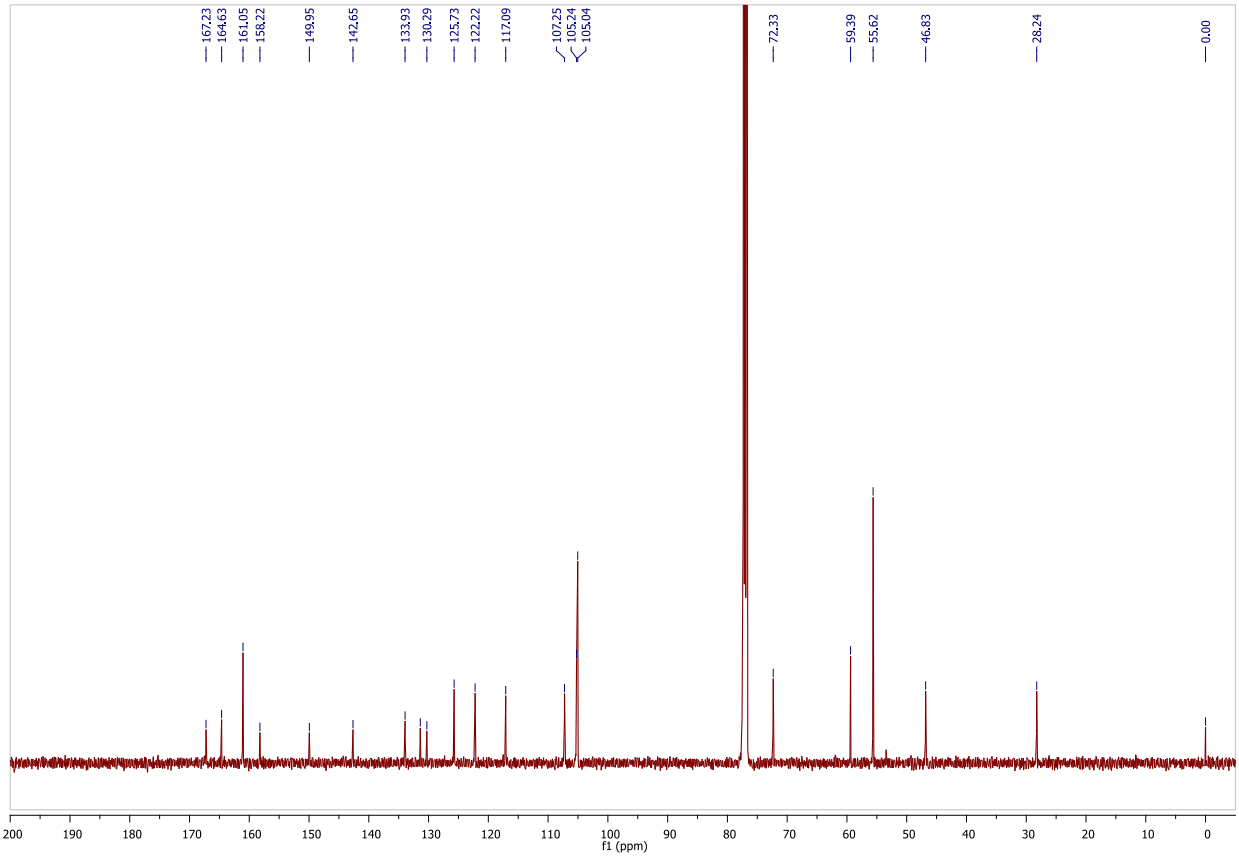
17-11
ML278

3,5-Dimethoxy-*N*-(4-(1-(2-methoxyacetyl)indolin-5-yl)thiazol-2-yl)benzamide (17-11, ML278): A round-bottom flask equipped with a magnetic stir bar was charged with 5-(2-(3,5-dimethoxybenzamido)thiazol-4-yl)indolinium 2,2,2-trifluoroacetate (75.0 mg, 0.15 mmol). Dichloromethane (1.5 ml) was added to produce a purple suspension that was cooled to 0 °C. 4-(*N*,*N*-Dimethylamino)pyridine (1.8 mg, 0.015 mmol) was added, followed by *N*,*N*-diisopropylethylamine (79 µl, 0.45 mmol) to give a clear, dark solution. 2-Methoxyacetyl chloride (15 µl, 0.17 mmol) was added last. The reaction was warmed to room temperature and stirred for 1 hour. The cloudy, tan mixture was diluted with dichloromethane (2 ml) and quenched with saturated sodium bicarbonate (aqueous, 2 ml). The layers were separated, and the aqueous portion was extracted with additional hot ethyl acetate (5 x 3 ml), with both layers heated to ~50 °C. The combined organic layers were shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give a light tan solid. The crude material was purified by column chromatography over silica gel (dichloromethane/methanol: 100/0 to 97/3) to give the title compound as an off-white solid (54.3 mg, 79 %).**^{1}H NMR (300 MHz, $CDCl_3$):** δ 10.14 (s, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.60 (s, 1H), 7.11 (s, 1H), 7.01 (d, *J* = 1.9 Hz, 3H), 6.61 (t, *J* = 2.0 Hz, 1H), 4.18 (s, 2H), 4.07 (t, *J* = 8.3 Hz, 2H), 3.81 (s, 8H), 3.53 (s, 4H), 3.22 (t, *J* = 8.2 Hz, 2H). **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 167.2, 164.6, 161.1, 158.2, 150.0, 142.7, 133.9, 131.4, 130.3, 125.7, 122.2, 117.1, 107.3, 105.2, 105.0, 72.3, 59.4, 55.6, 46.8, 28.2. **HRMS** (ESI^+): calculated for $C_{23}H_{24}N_3O_5S$ [M+H] 454.1437, found 454.1420.

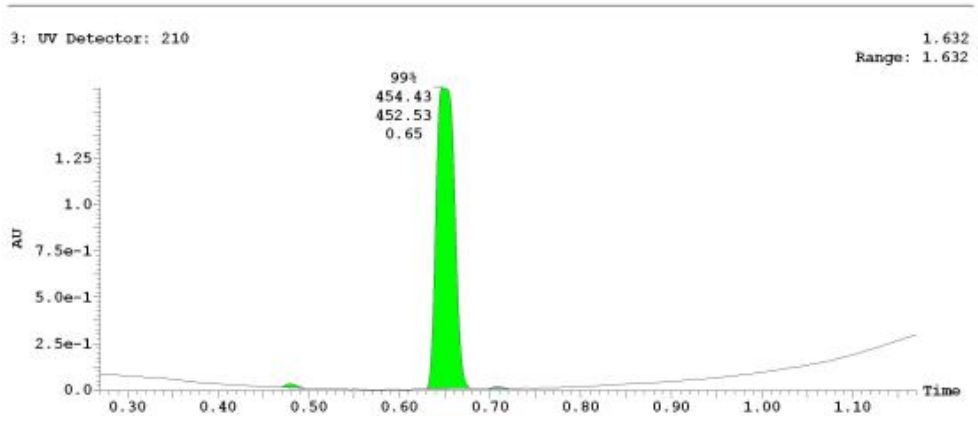
^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Probe 1 (ML278)



^{13}C NMR Spectrum (125 MHz, $CDCl_3$) of Probe 1 (ML278)

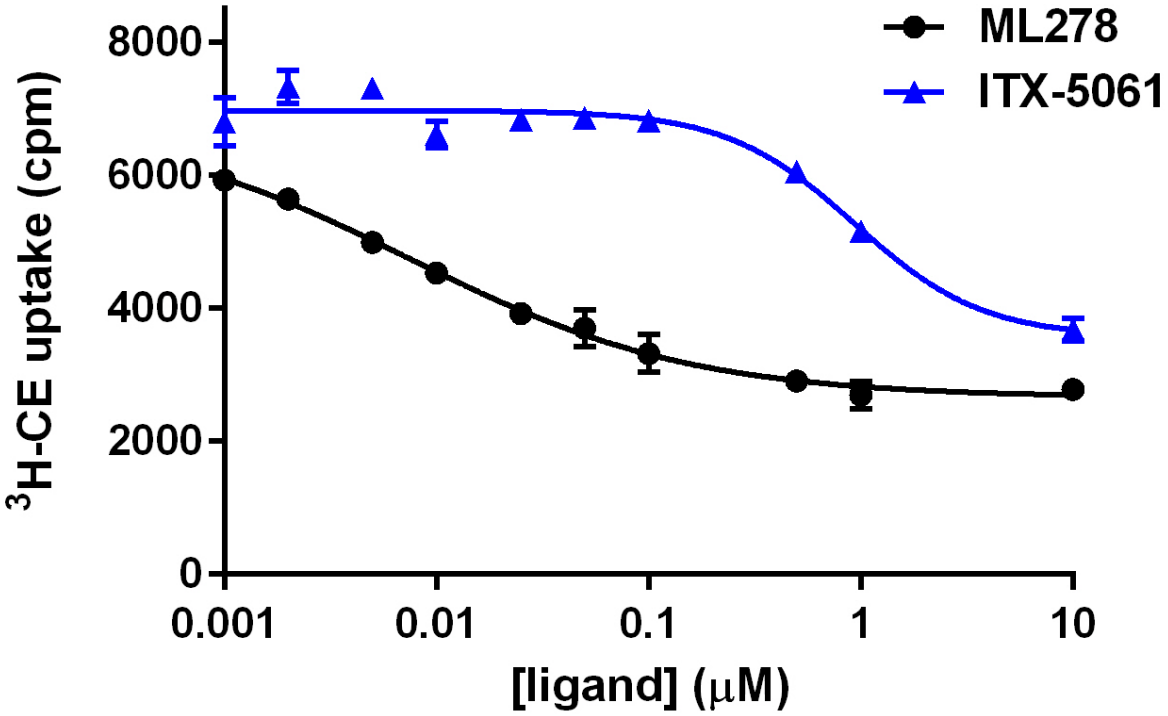


UPLC-MS Chromatogram of Probe 1 FW = 453.51 (ML278)



3. Dose-response for [^{3}H]cholesteryl ester uptake assay

Figure S1. Dose-response for [^{3}H]cholesteryl oleate ester uptake from HDL particles to ldlA[m-SR-BI] cells. Non-selective uptake (~5% of maximal uptake, see assay protocol) was quantified and subtracted from each uptake measurement prior to analysis. Points indicate the mean of n = 3, error bars indicate S.E.M. Uptake with no added inhibitor (ligand concentration = 0) was 7762 ± 302 cpm. IC_{50}s were determined (Table S2) and curves were plotted using non-linear regression analysis (4 parameters), using GraphPad Prism v. 6.



4. Probe comparison

Table S2. Comparison of SR-BI probes to prior art compounds[a]

Property	BLT-1	ITX-5061	ML278 (17-11)
Inhibition of DiI-HDL uptake in ldlA[mSR-BI] cells: IC_{50}	0.011±0.002 μM	0.254 μM	0.006±0.0003 μM
Inhibition of [^{3}H]CE-HDL uptake in ldlA[mSR-BI] cells: IC_{50}	0.05 μM	0.94 μM	0.007 μM
Increase in Alexa488-HDL binding with ldlA[mSR-BI] cells: IC_{50}	0.09 μM	NT	0.035 μM
Selectivity; receptor mediated endocytosis	No effect at up to 35 μM	No effect at up to 35 μM	No effect at up to 35 μM
Reversible	No[1]	NT	Yes
Cellular toxicity in ldlA[mSR-BI]	CC_{50} = 2.1 μM at 24 h	Non-toxic with CC_{50} >35 μM at 24 h	Non-toxic with CC_{50} >35 μM at 24 h
PBS Solubility (1% DMSO, pH 7.4, 23 °C)	1.3 μM	<1 μM	0.57 μM
Plasma protein binding, % bound (mouse, human)	NT	NT	>99%, 94%
Plasma stability, % remaining after 5 h (mouse, human)	NT	NT	>99%, >99%
Microsomal stability, % remaining after 1 h (mouse, human)	NT	NT	75%, 48%

[a] Average of at least two measurements in DiI uptake assay, ± standard error of mean when n > 2. NT = not tested.

5. Compound profiling protocols

Solubility. Solubility was determined in phosphate buffered saline (PBS) pH 7.4 with 1% DMSO. Each compound was prepared in duplicate at 100 μM in both 100% DMSO and PBS with 1% DMSO. Compounds were allowed to equilibrate at room temperature with a 250 rpm orbital shake for 24 hours. After equilibration, samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer. The DMSO samples were used to create a two-point calibration curve to which the response in PBS was fit.

Plasma Protein Binding. Plasma protein binding was determined by equilibrium dialysis using the Rapid Equilibrium Dialysis (RED) device (Pierce Biotechnology, Rockford, IL) for both human and mouse plasma. Each compound was prepared in duplicate at 5 μM in plasma (0.95% acetonitrile, 0.05% DMSO) and added to one side of the membrane (200 μl) with PBS pH 7.4 added to the other side (350 μl). Compounds were incubated at 37 ºC for 5 hours with a 250-rpm orbital shake. After incubation, samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer.

Plasma Stability. Plasma stability was determined at 37 ºC at 5 hours in both human and mouse plasma. Each compound was prepared in duplicate at 5 μM in plasma diluted 50/50 (v/v) with PBS pH 7.4 (0.95% acetonitrile, 0.05% DMSO). Compounds were incubated at 37 ºC for 5 hours with a 250-rpm orbital shake with time points taken at 0 hours and 5 hours. Samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer.

Microsomal Stability. Microsomal stability was determined at 37 ºC at 60 minutes in both human and mouse microsomes. Each compound was prepared in duplicate at 1 uM with 0.3 mg/mL microsomes in PBS pH 7.4 (1% DMSO). Compounds were incubated at 37 ºC for 60 minutes with a 350-rpm orbital shake with time points taken at 0 minutes and 60 minutes. Samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by selected ion recording (SIR) on a single quadrupole mass spectrometer.

6. Assay protocols

Materials and Reagents

- DiI-HDL, custom purified HDL particles derived from human blood were prepared and labeled with 1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate (DiI; Catalog No. D-282; Invitrogen, Carlsbad, CA). See section 5e) for details.
- Alexa 488 HDL, human HDL particles labeled with the Alexa Fluor® 488 Protein Labeling Kit (Catalog No. A-10235, Invitrogen; Carlsbad, CA) were purified and labeled by the Assay Provider.
- CellTiter-Glo® Luminescent Cell Viability Assay was purchased from Promega (Catalog No. G7573; Madison, WI).
- Radiolabeled cholesterol [1,2-^{3}H(N)]-, 1 mCi (37 MBq) was obtained from PerkinElmer – NEN (Catalog No. NET139001MC; Waltham, MA).
- Alexa Fluor-594 conjugated human transferrin (Catalog No. T-13343) was obtained from Invitrogen.

Cell Lines

The following cell lines were used in this study:

- ldlA[mSR-BI] is a Chinese hamster ovary (CHO) cell line that overexpresses murine SR-BI, isoform 1 (NP_058021) and lacks the LDL receptor was obtained from the Assay Provider (Krieger Laboratory). This cell line was used for the primary assay and several secondary assays. A variant of this cell line expressing mutant SR-BI, where a cysteine required for interaction with BLT-1 is mutated to serine (C384S SR-BI), was used in several secondary assays.
- [ldlA7] is the parental cell line to ldlA[mSR-BI] cells, and does not overexpresses SR-BI, and can be used to rule out compound activity independent of SR-BI.

a) DiI-HDL Uptake Assay

ldlA[mSR-BI] cells were plated into 384-well plates at 30 µl per well and incubated overnight. As a measurable surrogate for cholesterol uptake, human HDL particles were treated with the lipophilic fluorescent dye DiI and exposed to ldlA[mSR-BI] cells in lipoprotein-free media (Ham's F12/0.5% fatty acid-free Bovine Serum Albumin [BSA]/25 mM HEPES pH 7.4 plus 10 µg protein/ml DiI-HDL). Cells took up the Dil via SR-BI over 3 hours in the presence of compound. After significant uptake of the DiI, the cells became fluorescent. The level of fluorescence correlates with the amount of Dil uptake and can be measured with a standard plate reader. The uptake of lipid (represented by DiI) was inhibited by the compound BLT-1 or with an excess of HDL untreated with DiI.

The ldlA[mSR-BI] cells used in the assay were a CHO cell line lacking expression of the LDL receptors and overexpressing the Scavenger receptor (SR-BI). Inhibitors of SR-BI and HDL-mediated uptake lead to a reduction in fluorescence in this assay. Fluorescence was measured using a PerkinElmer EnVision plate reader. Primary HTS data were analyzed in Genedata Screener Assay Analyzer, and were normalized against DMSO and 1 µM BLT-1 (positive control). For the HTS, the average of two replicates was used to rank order activity and to choose compounds for retests. For dose studies, the curves were generated with Genedata Condeseo and showed percent (%) activity for the individual doses.

Detailed protocol

DiI-HDL Labeling Mix
2200 ml of Ham's/0.5% BSA/25 mM HEPES pH 7.4
DiI-HDL to 10 ug protein/ml final concentration (Stocks vary from 2.81-4 mg/mL)

Ham's/0.5% BSA/25 mM HEPES Assay Media (1 liter)
5 g BSA powder (Sigma fatty acid-free)

1 Liter Ham's F12 base media
25 ml 1 M HEPES pH 7.4

ldlA[mSR-BI] Chinese Hamster Ovary (CHO) cells were maintained in Ham's F12K (Cellgro Catalog No. 10-080-CV)/10% Fetal Calf Serum (Hyclone Catalog No. SH30071.03, Lot No. ATG32533)/ 1x Penicillinstreptomycin-L-Glutamine (Gibco Catalog No.10378-016)/200 ug/mL Geniticin (Gibco Catalog No.10131-027, Lot No.802967). Cells were fluid changed every 2 days and/or split upon reaching 80% confluency. For the primary HTS, cells were thawed at 6 million cells per Falcon T225 flask. After 2 days, the cells were passed to a Corning Hyperflask and plated after 3 days in the Hyperflask with a fluid change the day prior to plating.

On Day 0:

1. Plate 10,000 ldlA[mSR-BI] cells in 30 uL per well in Ham's F12K (Cellgro Catalog No. 10-080-CV)/5% Fetal Calf Serum (Hyclone Catalog No. SH30071.03, Lot No. ATG32533)/ 1x Penicillinstreptomycin-L-Glutamine (Gibco Catalog No. 10378-016) with a Thermo Combi Multi-drop fluid handler.

2. Use Aurora black 384-well, square, clear-bottomed, image-quality plates (Aurora Catalog No. 1022-11330) for the assay.

On Day 1:

1. Remove media with aspirator (Bio-Tek ELX405 plate washer).
2. Add 30 uL Ham's F12/0.5% Bovine Serum Albumin (fatty acid-free) /25 mM HEPES pH 7.4 (Invitrogen) + 10 ug protein/ml DiI-HDL with Thermo Combi fluid handler (slow speed setting).
3. Pin transfer 100 nL compounds and positive control (1 uM BLT-1). **It is recommended that low solubility compounds (such as ML278) be sonicated in DMSO to ensure complete dissolution prior to preparing stock solutions.**
4. Incubate 3 hours @ 37°C in humidified cell culture incubator.
5. Remove media with aspirator.
6. Wash twice with 30 uL PBS (+Ca and Mg) using the Thermo Combi on slow speed setting.
7. Analyze DiI-HDL uptake with Perkin-Elmer EnVision plate reader (Bodipy TMR mirror #405, excitation filter is Photometric 550 (#312) and emission filter is Cy3 595 (#229) with bottom read.

b) HDL Binding Assay

HDL binding was assessed using Alexa Fluor 488-labeled HDL particles. For this assay, the Alexa 488 dye was covalently bound to apolipoprotein components of the HDL particle via primary amines; thus, no transfer of the fluorophore to cell membranes occurs. In this manner, direct binding of the HDL particles to SR-BI can be measured. As a positive control, BLT-1 was used at 1 μM, which is known to increase binding of HDL to SR-BI. It is possible that a compound can reduce binding of HDL to the receptor, and this would lead to a decrease in signal. This assay is used to characterize the mechanism of action of a particular compound; therefore, any outcome in the assay is acceptable. Data were normalized against DMSO and BLT-1 (positive control) wells in Genedata Assay Analyzer. Curves were generated with Genedata Condeseo and showed percent (%) activity for the individual doses.

Detailed protocol

ldlA[mSR-BI] Chinese Hamster Ovary (CHO) cells were maintained in Ham's F12K (Cellgro Catalog No. 10-080-CV)/10% Fetal Calf Serum (Hyclone Catalog No. SH30071.03, Lot No. ATG32533)/ 1x Penicillin-streptomycin-L-Glutamine (Gibco 10378-016)/200 ug/mL Geniticin (Gibco Catalog No.10131-027, Lot No.802967). Cells were fluid changed every 2 days and/or split upon reaching 80% confluency.

On Day 0:

1. Plate 10,000 ldlA[mSR-BI] cells in 30 uL per well in Ham's F12K (Cellgro Catalog No. 10-080-CV)/5% Fetal Calf Serum (Hyclone Catalog No. SH30071.03, Lot No. ATG32533)/ 1x Penicillin-streptomycin-L-Glutamine (Gibco 10378-016).
2. Use Aurora black, 384-well, square, clear-bottomed image-quality plates (Aurora Catalog No.1022-11330) for the assay.

On Day 1:

1. Remove media with aspirator (ELX405 plate washer).
2. Add 30 uL Ham's F12/0.5% Bovine Serum Albumin (fatty acid-free) (Sigma Catalog No. A8806-5G) /25 mM HEPES pH 7.4 (Invitrogen) plus 10 ug/mL Alexa 488-HDL with Thermo Combi fluid handler (slow speed setting).
3. Pin transfer 100 nL compounds and positive control (1 uM BLT-1).
4. Incubate 3 hours @ 37°C in a humidified cell culture incubator.
5. Remove media with aspirator.
6. Wash twice with 30 uL PBS (+Ca and Mg) using the Thermo Combi on slow speed setting.
7. Analyze Alexa-488-HDL binding with Perkin-Elmer EnVision plate reader (FITC mirror #403) with bottom read

c) Cholesterol Ester Uptake Assay

The goal of this assay is to verify compounds that disrupt the binding of HDL particles to SR-BI scavenger receptor using an alternative means of labeling cholesteryl esters and avoiding any type of fluorescence measurement. To measure this binding event, HDL particles are loaded with [^{3}H]cholesteryl oleate ester and added to mSR-BI cells. Cells take up HDL and the [^{3}H]cholesteryl ester via the SR-BI scavenger receptor in 2 to 3 hours. After significant uptake of the HDL, the radiolabel can be detected by liquid scintillation counting. The level of radioactivity correlates with the amount of [^{3}H]cholesteryl ester uptake. The uptake can be inhibited by the compound BLT-1 or when co-treated with an excess of unlabeled HDL. The ldlA[mSR-BI] cells utilized in the assay are a CHO cell line lacking expression of the LDL receptors and overexpressing the scavenger receptor, SR-BI. Inhibitors of SR-BI and HDL uptake will have a reduction in liquid scintillation counts.

Detailed protocol

On Day 0:

1. Plate 50,000 ldlA[mSR-BI] cells in 1 mL per well in Ham's F12K/5% Fetal Calf Serum/ 1x Penicillin-streptomycin-L-Glutamine/G418.

On Day 2:

2. Wash cells with prewarmed (37°C) Ham's F12K medium plus 0.5% (wt/vol) fatty acid free BSA prior to adding radioactive lipoprotein.
3. Preincubate cells with 200 uL of inhibitors at indicated concentration or non-labeled HDL (at a 40 fold excess for measurement of non-specific activity) in Ham's F12/0.5% BSA/0.5%DMSO (vol/vol) for 1 hr at 37°C in a humidified cell culture incubator. **It is recommended that low solubility compounds (such as ML278) be sonicated in DMSO to ensure complete dissolution prior to preparing stock solutions.**
4. Add 50 uL Ham's F12/0.5% BSA/0.5%DMSO plus 50 ug/mL [^{3}H]CE-HDL to reach a final concentration of 10 ug/mL and incubate 2 hours at 37°C in a humidified cell culture incubator
5. Remove the medium at the end of incubation at 4C
6. Wash the plates 2X with Tris-BSA buffer and 1X Tris-HCl buffer.
7. Add 1 ml isopropanol, and incubate for 20 min at 4C.
8. Collect 1 ml isopropanol/lysate into counting tube, add 4 ml liquid scintillation buffer, and prepare for counting.
9. Specific uptake is the difference between total and nonspecific activities.
10. Data were analyzed using Prism 6 (GraphPad Software).
11. All calculated errors represent standard errors of the mean.

e) HDL isolation and labeling

Human HDL was isolated from donors by density gradient ultracentrifugation and labeled with [^{3}H]cholesteryl ester ([^{3}H]CE, [^{3}H]CE-HDL) as described previously.[2,3,4,5,6]

7. References

[1] Yu, M.; Romer, K. A.; Nieland, T. J. F.; Xu, S.; Saenz-Vash, V.; Penman, M.; Yesilaltay, A.; Carr, S. A.; Krieger, M. Exoplasmic cysteine Cys384 of the HDL receptor SR-BI is critical for its sensitivity to a small-molecule inhibitor and normal lipid transport activity. *Proc. Nat. Acad. Sci. USA* **2011**, *108*, 12243–12248.
[2] Nieland, T. J. F.; Xu, S.; Penman, M.; Krieger, M. Negatively Cooperative Binding of High-Density Lipoprotein to the HDL Receptor SR-BI, *Biochemistry* **2011**, *50*, 1818–1830.
[3] Chung, B. H.; Wilkinson, T.; Geer, J. C.; Segrest, J. P. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor, *J Lipid Res*. **1980**, *21*, 284–291.
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[5] Goldstein, J. L.; Basu, S. K.; Brown, M. S. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol*. **1983**, *98*, 241–260.
[6] Gwynne, J. T.; Mahaffee, D. D. Rat adrenal uptake and metabolism of high density lipoprotein cholesteryl ester. *J. Biol. Chem*. **1989**, *264*, 8141–8150.

8. Off-target screening data for 17-11 (ML278)

Services Performed

LeadProfilingScreen (Total # of Assays: 67)

Study Objectives

To evaluate, in LeadProfilingScreen, the activity of test compound BRD-K83634925-001-01-1 (Ricerca # 1162315).

Study Signatures

Hua-Chou Hsing
Study Director for Binding Assays

Gonzalo Castillo, Ph.D.
Technical Director

"This study was conducted according to the procedures described in this report. All data presented are authentic, accurate and correct to the best of our knowledge."

Table of Contents

Summary

STUDY OBJECTIVE

To evaluate, in Radioligand Binding assays, the activity of compound BRD-K83634925-001-01-1 (BRD-9, PT# 1162315).

METHODS

Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Assays were performed under conditions described in the accompanying "Methods" section of this report.

Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). Where inhibition constants (K_i) are presented, the K_i values were calculated using the equation of Cheng and Prusoff (Cheng, Y., Prusoff, W.H., Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the K_D of the ligand (obtained experimentally at **Ricerca Biosciences, LLC**). Where presented, the Hill coefficient (n_H), defining the slope of the competitive binding curve, was calculated using MathIQ™. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Where IC_{50}, K_i, and/or n_H data are presented without Standard Error of the Mean (SEM), data are insufficient to be quantitative, and the values presented (K_i, IC_{50}, n_H) should be interpreted with caution.

RESULTS

A summary of results meeting the significance criteria is presented in the following sections. Complete results are presented under the section labeled "Experimental Results". Individual responses, if requested, are presented in the section labeled "Individual Responses".

SUMMARY/CONCLUSION

Significant results are displayed in the following table(s) in rank order of potency for estimated IC_{50} and/or K_i values.

Summary of Significant Results

Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report. All other results are expressed in terms of that assay's quantitation method.

- For primary assays, only the lowest concentration with a significant response judged by the assays' criteria, is shown in this summary.
- Where applicable, either the secondary assay results with the lowest dose/concentration meeting the significance criteria or, if inactive, the highest dose/concentration that did not meet the significance criteria is shown.
- Unless otherwise requested, primary screening in duplicate with quantitative data (e.g., IC_{50} ± SEM, K_i ± SEM and n_H) are shown where applicable for individual requested assays. In screening packages, primary screening in duplicate with semi-quantitative data (e.g., estimated IC_{50}, K_i and n_H) are shown where applicable (concentration range of 4 log units); available secondary functional assays are carried out (30 mM) and MEC or MIC determined only if active in primary assays >50% at 1 log unit below initial test concentration.

Significant responses (≥ 50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

No significant results noted.

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
Compound: BRD-K83634925-001-01-1, PT #: 1162315										
200510	Adenosine A_1	317535	hum	2	10 µM	20				
200610	Adenosine A_{2A}	317540	hum	2	10 µM	24				
200720	Adenosine A_3	317577	hum	2	10 µM	43				
203100	Adrenergic α_{1A}	317536	rat	2	10 µM	3				
203200	Adrenergic α_{1B}	317547	rat	2	10 µM	13				
203400	Adrenergic α_{1D}	317537	hum	2	10 µM	5				
203620	Adrenergic α_{2A}	317534	hum	2	10 µM	2				
204010	Adrenergic β_1	317532	hum	2	10 µM	2				
204110	Adrenergic β_2	317533	hum	2	10 µM	4				
285010	Androgen (Testosterone) AR	317485	rat	2	10 µM	21				
212510	Bradykinin B_1	317578	hum	2	10 µM	-7				
212620	Bradykinin B_2	317579	hum	2	10 µM	3				
214510	Calcium Channel L-Type, Benzothiazepine	317482	rat	2	10 µM	16				
214600	Calcium Channel L-Type, Dihydropyridine	317531	rat	2	10 µM	20				
216000	Calcium Channel N-Type	317580	rat	2	10 µM	-17				
217030	Cannabinoid CB_1	317529	hum	2	10 µM	8				
219500	Dopamine D_1	317541	hum	2	10 µM	6				
219700	Dopamine D_{2S}	317543	hum	2	10 µM	-3				
219800	Dopamine D_3	317544	hum	2	10 µM	7				
219900	Dopamine $D_{4.2}$	317552	hum	2	10 µM	-6				
224010	Endothelin ET_A	317641	hum	2	10 µM	12				
224110	Endothelin ET_B	317642	hum	2	10 µM	5				
225510	Epidermal Growth Factor (EGF)	317582	hum	2	10 µM	14				
226010	Estrogen ERα	317583	hum	2	10 µM	6				
226600	$GABA_A$, Flunitrazepam, Central	317545	rat	2	10 µM	-5				
226500	$GABA_A$, Muscimol, Central	317556	rat	2	10 µM	8				
228610	$GABA_{B1A}$	317584	hum	2	10 µM	-12				
232030	Glucocorticoid	317476	hum	2	10 µM	-24				
232700	Glutamate, Kainate	317586	rat	2	10 µM	4				
232810	Glutamate, NMDA, Agonism	317587	rat	2	10 µM	-16				
232910	Glutamate, NMDA, Glycine	317588	rat	2	10 µM	-1				
233000	Glutamate, NMDA, Phencyclidine	317557	rat	2	10 µM	1				
239610	Histamine H_1	317546	hum	2	10 µM	9				
239710	Histamine H_2	317514	hum	2	10 µM	8				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
ham=Hamster; hum=Human

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
239820	Histamine H_3	317553	hum	2	10 µM	0				
241000	Imidazoline I_2, Central	317539	rat	2	10 µM	15				
243520	Interleukin IL-1	317484	mouse	2	10 µM	-10				
250460	Leukotriene, Cysteinyl $CysLT_1$	317589	hum	2	10 µM	-6				
251600	Melatonin MT_1	317513	hum	2	10 µM	-3				
252610	Muscarinic M_1	317554	hum	2	10 µM	11				
252710	Muscarinic M_2	317527	hum	2	10 µM	-9				
252810	Muscarinic M_3	317528	hum	2	10 µM	0				
257010	Neuropeptide Y Y_1	317468	hum	2	10 µM	-8				
257110	Neuropeptide Y Y_2	317592	hum	2	10 µM	-1				
258590	Nicotinic Acetylcholine	317521	hum	2	10 µM	0				
258700	Nicotinic Acetylcholine α1, Bungarotoxin	317522	hum	2	10 µM	-3				
260130	Opiate δ_1 (OP1, DOP)	317525	hum	2	10 µM	-8				
260210	Opiate κ(OP2, KOP)	317524	hum	2	10 µM	2				
260410	Opiate µ(OP3, MOP)	317526	hum	2	10 µM	-15				
264500	Phorbol Ester	317558	mouse	2	10 µM	-4				
265010	Platelet Activating Factor (PAF)	317496	hum	2	10 µM	23				
265600	Potassium Channel [K_{ATP}]	317559	ham	2	10 µM	-1				
265900	Potassium Channel hERG	317560	hum	2	10 µM	-4				
268420	Prostanoid EP_4	317561	hum	2	10 µM	2				
268700	Purinergic P_{2X}	317590	rabbit	2	10 µM	7				
268810	Purinergic P_{2Y}	317591	rat	2	10 µM	9				
270000	Rolipram	317562	rat	2	10 µM	8				
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	317510	hum	2	10 µM	0				
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	317551	hum	2	10 µM	24				
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	317593	hum	2	10 µM	22				
278110	Sigma σ_1	317523	hum	2	10 µM	-20				
255520	Tachykinin NK_1	317474	hum	2	10 µM	7				
285900	Thyroid Hormone	317488	rat	2	10 µM	10				
220320	Transporter, Dopamine (DAT)	317469	hum	2	10 µM	-1				
226400	Transporter, GABA	317538	rat	2	10 µM	32				
204410	Transporter, Norepinephrine (NET)	317520	hum	2	10 µM	4				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	317549	hum	2	10 µM	9				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
ham=Hamster; hum=Human

Experimental Results

MDS has an exclusive, worldwide limited use license from Synaptic Pharmaceutical Corporation to perform these assays: Adrenergic Alpha 1D, Adrenergic Alpha 2B, and Dopamine D5 for safety and selectivity profiling. MDS' license excludes performing those assays in connection with drug discovery or development activities where the principal therapeutic mechanism of action of the test compound involves selective binding to a licensed receptor. Customers may contact Synaptic directly if they believe they need a broader license.

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
ham=Hamster; hum=Human

Methods

200510 Adenosine A_1

Source:	Human recombinant CHO cells	**Ligand:**	1.0 nM [^{3}H] DPCPX
Vehicle:	1% DMSO	**Non-Specific Ligand:**	100 µM R(-)-PIA
Incubation Time/Temp:	90 minutes @ 25°C	**Specific Binding:**	85% *
Incubation Buffer:	20 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 100 mM NaCl	**Quantitation Method:**	Radioligand Binding
Kd:	1.40 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	2.70 pmole/mg Protein *

200610 Adenosine A_{2A}

Source:	Human recombinant HEK-293 cells	**Ligand:**	0.050 µM [^{3}H] CGS-21680
Vehicle:	1% DMSO	**Non-Specific Ligand:**	50.0 µM NECA
Incubation Time/Temp:	90 minutes @ 25°C	**Specific Binding:**	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 2 U/mL Adenosine Deaminase	**Quantitation Method:**	Radioligand Binding
Kd:	0.064 µM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	7.0 pmole/mg Protein *

200720 Adenosine A_3

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.50 nM [^{125}I] AB-MECA
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM IB-MECA
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	83% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	5.90 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.80 pmole/mg Protein *

203100 Adrenergic α_{1A}

Source:	Wistar Rat submaxillary gland	**Ligand:**	0.25 nM [^{3}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:	0.17 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.18 pmole/mg Protein *

* Historical Values

Methods

203200 Adrenergic α_{1B}

Source:	Wistar Rat liver	**Ligand:**	0.25 nM [^{3}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:**	≥50% of max stimulation or inhibition
		Bmax:	0.18 pmole/mg Protein *

203400 Adrenergic α_{1D}

Source:	Human recombinant HEK-293 cells	**Ligand:**	0.60 nM [^{3}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:**	≥50% of max stimulation or inhibition
		Bmax:	0.17 pmole/mg Protein *

203620 Adrenergic α_{2A}

Source:	Human recombinant insect Sf9 cells	**Ligand:**	1.0 nM [^{3}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:**	≥50% of max stimulation or inhibition
		Bmax:	4.60 pmole/mg Protein *

204010 Adrenergic β_1

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.030 nM [^{125}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:**	≥50% of max stimulation or inhibition
		Bmax:	0.072 pmole/mg Protein *

* Historical Values

Methods

204110 Adrenergic β_2

Source:	Human recombinant CHO cells	**Ligand:**	0.20 nM [^{3}H] CGP-12177
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM ICI-118551
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 5.0 mM $MgCl_2$, 120 mM NaCl	**Quantitation Method:**	Radioligand Binding
Kd:	0.44 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.44 pmole/mg Protein *

285010 Androgen (Testosterone) AR

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

212510 Bradykinin B_1

Source:	Human IMR-90 cells	**Ligand:**	0.50 nM [^{3}H] (Des-Arg^{10})-Kallidin
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM (Des-Arg^9, Leu^8)-Bradykinin
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	80% *
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	**Quantitation Method:**	Radioligand Binding
Kd:	0.17 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.55 pmole/mg Protein *

212620 Bradykinin B_2

Source:	Human recombinant Chem-1 cells	**Ligand:**	0.50 nM [^{3}H] Bradykinin
Vehicle:	1% DMSO	**Non-Specific Ligand:**	5.0 µM Bradykinin
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 0.2% BSA, 1 mM $CaCl_2$, 5 mM $MgCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	0.85 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	9.40 pmole/mg Protein *

* Historical Values

Methods

214510 Calcium Channel L-Type, Benzothiazepine

Source:	Wistar Rat brain	**Ligand:**	2.0 nM [^{3}H] Diltiazem
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 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:**	0.10 nM [^{3}H] Nitrendipine
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 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}I] ω-Conotoxin GVIA
Vehicle:	1% DMSO	**Non-Specific Ligand:**	0.10 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 CB_1

Source:	Human recombinant Chem-1 cells	**Ligand:**	2.0 nM [^{3}H] SR141716A
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	5.90 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	15.0 pmole/mg Protein *

* Historical Values

Methods

219500 Dopamine D_1

Source:	Human recombinant CHO cells	**Ligand:**	1.40 nM [^{3}H] SCH-23390
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM (+)-Butaclamol
Incubation Time/Temp:	2 hours @ 37°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	**Quantitation Method:**	Radioligand Binding
Kd:	1.40 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.63 pmole/mg Protein *

219700 Dopamine D_{2S}

Source:	Human recombinant CHO cells	**Ligand:**	0.16 nM [^{3}H] Spiperone
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Haloperidol
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	**Quantitation Method:**	Radioligand Binding
Kd:	0.090 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.60 pmole/mg Protein *

219800 Dopamine D_3

Source:	Human recombinant CHO cells	**Ligand:**	0.70 nM [^{3}H] Spiperone
Vehicle:	1% DMSO	**Non-Specific Ligand:**	25.0 µM S(-)-Sulpiride
Incubation Time/Temp:	2 hours @ 37°C	**Specific Binding:**	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	**Quantitation Method:**	Radioligand Binding
Kd:	0.36 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

219900 Dopamine $D_{4.2}$

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.50 nM [^{3}H] Spiperone
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Haloperidol
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	**Quantitation Method:**	Radioligand Binding
Kd:	0.32 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.55 pmole/mg Protein *

* Historical Values

Methods

224010 Endothelin ET_A

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.030 nM [^{125}I] Endothelin-1
Vehicle:	1% DMSO	**Non-Specific Ligand:**	0.10 µM Endothelin-1
Incubation Time/Temp:	2 hours @ 37°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.1% BSA, 0.5 mM $CaCl_2$, 0.05% Tween-20	**Quantitation Method:**	Radioligand Binding
Kd:	0.048 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.35 pmole/mg Protein *

224110 Endothelin ET_B

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.10 nM [^{125}I] Endothelin-1
Vehicle:	1% DMSO	**Non-Specific Ligand:**	0.10 µM Endothelin-1
Incubation Time/Temp:	2 hours @ 25°C	**Specific Binding:**	75% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	0.085 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	4.30 pmole/mg Protein *

225510 Epidermal Growth Factor (EGF)

Source:	Human A431 cells	**Ligand:**	0.080 nM [^{125}I] EGF (human)
Vehicle:	1% DMSO	**Non-Specific Ligand:**	0.10 µM EGF (human)
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM HEPES, pH 7.7, 0.1% BSA, 1.2 mM $CaCl_2$, 5 mM KCl, 1.2 mM $MgSO_4$, 138 mM NaCl	**Quantitation Method:**	Radioligand Binding
Kd:	0.17 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	5.50 pmole/mg Protein *

226010 Estrogen ERα

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

* Historical Values

Methods

226600 $GABA_A$, Flunitrazepam, Central

Source:	Wistar Rat brain (minus cerebellum)	**Ligand:**	1.0 nM [^{3}H] Flunitrazepam
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Diazepam
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	91% *
Incubation Buffer:	50 mM Phosphate Buffer, pH 7.4	**Quantitation Method:**	Radioligand Binding
Kd:	4.40 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.20 pmole/mg Protein *

226500 $GABA_A$, Muscimol, Central

Source:	Wistar Rat brain (minus cerebellum)	**Ligand:**	1.0 nM [^{3}H] Muscimol
Vehicle:	1% DMSO	**Non-Specific Ligand:**	0.10 µM Muscimol
Incubation Time/Temp:	10 minutes @ 4°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	**Quantitation Method:**	Radioligand Binding
Kd:	3.80 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.80 pmole/mg Protein *

228610 $GABA_{B1A}$

Source:	Human recombinant CHO cells	**Ligand:**	4.0 nM [^{3}H] CGP-54626
Vehicle:	1% DMSO	**Non-Specific Ligand:**	3.0 mM GABA
Incubation Time/Temp:	3 hours @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2.5 mM $CaCl_2$, 0.1% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	3.30 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	48.0 pmole/mg Protein *

232030 Glucocorticoid

Source:	Human recombinant Insect cells	**Ligand:**	5.0 nM [^{3}H] Dexamethasone
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Dexamethasone
Incubation Time/Temp:	1 day @ 4°C	**Specific Binding:**	97% *
Incubation Buffer:	5 mM KH_2PO_4, 8 mM $Na_2HPO_4 \cdot 12H_2O$, pH7.4, 137 mM NaCl, 2.7 mM KCl, 0.2% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	4.60 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg *

* Historical Values

Methods

232700 Glutamate, Kainate

Source:	Wistar Rat brain (minus cerebellum)	**Ligand:**	5.0 nM [^{3}H] Kainic acid
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 mM L-Glutamic acid
Incubation Time/Temp:	60 minutes @ 4°C	**Specific Binding:**	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	**Quantitation 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:	Wistar Rat cerebral cortex	**Ligand:**	2.0 nM [^{3}H] CGP-39653
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 mM L-Glutamic acid
Incubation Time/Temp:	20 minutes @ 4°C	**Specific Binding:**	70% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	**Quantitation Method:**	Radioligand Binding
Kd:	0.019 µM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	2.30 pmole/mg Protein *

232910 Glutamate, NMDA, Glycine

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

233000 Glutamate, NMDA, Phencyclidine

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

* Historical Values

Methods

239610 Histamine H_1

Source:	Human recombinant CHO-K1 cells	**Ligand:**	1.20 nM [^{3}H] Pyrilamine
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM Pyrilamine
Incubation Time/Temp:	3 hours @ 25°C	**Specific Binding:**	94% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$, 100 mM NaCl, 250 mM Sucrose	**Quantitation Method:**	Radioligand Binding
Kd:	1.10 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	6.70 pmole/mg Protein *

239710 Histamine H_2

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

239820 Histamine H_3

Source:	Human recombinant CHO-K1 cells	**Ligand:**	0.40 nM [^{3}H] N-α-Methylhistamine (NAMH)
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM R(-)-α-Methylhistamine (RAMH)
Incubation Time/Temp:	2 hours @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.1% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	0.38 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	2.0 pmole/mg Protein *

241000 Imidazoline I_2, Central

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

* Historical Values

Methods

243520 Interleukin IL-1

Source:	Mouse 3T3-SWISS cells	**Ligand:**	0.10 nM [^{125}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	**Quantitation Method:**	Radioligand Binding
Kd:	0.25 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	820 R/cell Protein *

250460 Leukotriene, Cysteinyl 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 Benzamidine, 0.1 mM PMSF	**Quantitation Method:**	Radioligand Binding
Kd:	0.21 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	3.0 pmole/mg Protein *

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	**Quantitation Method:**	Radioligand Binding
Kd:	0.054 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	3.50 pmole/mg Protein *

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	**Quantitation Method:**	Radioligand Binding
Kd:	0.26 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	2.0 pmole/mg Protein *

* Historical Values

Methods

252710 Muscarinic M_2

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	**Quantitation Method:**	Radioligand Binding
Kd:	0.58 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	5.10 pmole/mg Protein *

252810 Muscarinic M_3

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	**Quantitation Method:**	Radioligand Binding
Kd:	0.75 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	5.40 pmole/mg Protein *

257010 Neuropeptide Y Y_1

Source:	Human SK-N-MC cells	**Ligand:**	0.015 nM [^{125}I] Peptide YY
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM Neuropeptide Y (human, rat)
Incubation Time/Temp:	60 minutes @ 37°C	**Specific Binding:**	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1% BSA, 0.01% Bacitracin	**Quantitation Method:**	Radioligand Binding
Kd:	0.24 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.58 pmole/mg protein *

257110 Neuropeptide Y Y_2

Source:	Human KAN-TS cells	**Ligand:**	10 pM [^{125}I] Peptide YY
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM Neuropeptide Y (13-36) (porcine)
Incubation Time/Temp:	2 hours @ 37°C	**Specific Binding:**	90% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% Bacitracin	**Quantitation Method:**	Radioligand Binding
Kd:	0.012 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.5 pmole/mg Protein *

* Historical Values

Methods

258590 Nicotinic Acetylcholine

Source:	Human IMR-32 cells	**Ligand:**	0.10 nM [^{125}I] Epibatidine
Vehicle:	1% DMSO	**Non-Specific Ligand:**	300 µM (-)-Nicotine
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	97% *
Incubation Buffer:	20 mM HEPES, pH 7.5, 150 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$.	**Quantitation Method:**	Radioligand Binding
Kd:	0.22 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.46 pmole/mg Protein *

258700 Nicotinic Acetylcholine α1, Bungarotoxin

Source:	Human RD cells	**Ligand:**	0.60 nM [^{125}I] α-Bungarotoxin
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM α-Bungarotoxin
Incubation Time/Temp:	2 hours @ 25°C	**Specific Binding:**	85% *
Incubation Buffer:	150 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	1.10 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg Protein *

260130 Opiate δ_1 (OP1, DOP)

Source:	Human recombinant HEK-293 cells	**Ligand:**	1.30 nM [^{3}H] Naltrindole
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM Naltrindole
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	0.27 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	7.60 pmole/mg Protein *

260210 Opiate κ(OP2, KOP)

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

* Historical Values

Methods

260410 Opiate μ(OP3, MOP)

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

264500 Phorbol Ester

Source:	ICR Mouse brain	**Ligand:**	3.0 nM [^{3}H] PDBu
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 μM PDBu
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	80% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.4, 5 mM $CaCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	8.70 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	26.0 pmole/mg Protein *

265010 Platelet Activating Factor (PAF)

Source:	Human platelets	**Ligand:**	0.12 nM [^{3}H] PAF
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 μM PAF
Incubation Time/Temp:	3 hours @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 100 mM KCl, 5 mM EDTA, 5 mM $MgCl_2$, 0.25% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	0.13 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	120 R/cell *

265600 Potassium Channel [K_{ATP}]

Source:	Hamster pancreatic HIT-T15 beta cells	**Ligand:**	5.0 nM [^{3}H] Glyburide
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 μM Glyburide
Incubation Time/Temp:	2 hours @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	50 mM MOPS, pH 7.4, 0.1 mM $CaCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	0.64 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg Protein *

* Historical Values

Methods

265900 Potassium Channel hERG

Source:	Human recombinant HEK-293 cells	**Ligand:**	1.50 nM [^{3}H] Astemizole
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Astemizole
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	10 mM HEPES, pH 7.4, 0.1% BSA, 5 mM KCl, 0.8 mM $MgCl_2$, 130 mM NaCl, 1 mM EGTA, 10 mM Glucose	**Quantitation Method:**	Radioligand Binding
Kd:	6.80 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	6.30 pmole/mg Protein *

268420 Prostanoid EP_4

Source:	Human recombinant Chem-1 cells	**Ligand:**	1.0 nM [^{3}H] Prostaglandin E_2 (PGE_2)
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Prostaglandin E_2 (PGE_2)
Incubation Time/Temp:	2 hours @ 25°C	**Specific Binding:**	90% *
Incubation Buffer:	10 mM MES, pH 6.0, 1 mM EDTA, 10 mM $MgCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	0.69 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	4.30 pmole/mg Protein *

268700 Purinergic P_{2X}

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

268810 Purinergic P_{2Y}

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

* Historical Values

Methods

270000 Rolipram

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

271110 Serotonin (5-Hydroxytryptamine) 5-HT$_{1A}$

Source:	Human recombinant CHO-K1 cells	**Ligand:**	1.50 nM [^{3}H] 8-OH-DPAT
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 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 $MgSO_4$	**Quantitation Method:**	Radioligand Binding
Kd:	2.0 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.30 pmole/mg Protein *

271700 Serotonin (5-Hydroxytryptamine) 5-HT$_{2B}$

Source:	Human recombinant CHO-K1 cells	**Ligand:**	1.20 nM [^{3}H] Lysergic acid diethylamide (LSD)
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 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	**Quantitation Method:**	Radioligand Binding
Kd:	2.10 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.10 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:**	10.0 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 $MgCl_2$	**Quantitation Method:**	Radioligand Binding
Kd:	0.20 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	11.0 pmole/mg Protein *

* Historical Values

Methods

278110 Sigma σ_1

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

255520 Tachykinin NK_1

Source:	Human recombinant CHO cells	**Ligand:**	0.80 nM [^{3}H] Substance P
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM L-703,606
Incubation Time/Temp:	90 minutes @ 4°C	**Specific Binding:**	90% *
Incubation Buffer:	20 mM HEPES, pH 7.4, 1 mM $MnCl_2$, 0.1% BSA	**Quantitation Method:**	Radioligand Binding
Kd:	2.10 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	1.70 pmole/mg Protein *

285900 Thyroid Hormone

Source:	Wistar Rat liver	**Ligand:**	0.030 nM [^{125}I] Triiodothyronine
Vehicle:	1% DMSO	**Non-Specific Ligand:**	1.0 µM Triiodothyronine
Incubation Time/Temp:	18 hours @ 4°C	**Specific Binding:**	77% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10% Glycerol, 2 mM EDTA, 5 mM DTT	**Quantitation Method:**	Radioligand Binding
Kd:	0.034 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	0.16 pmole/mg Protein *

220320 Transporter, Dopamine (DAT)

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

* Historical Values

Methods

226400 Transporter, GABA

Source:	Wistar Rat cerebral cortex	**Ligand:**	6.0 nM [^{3}H] GABA
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM NO-711
Incubation Time/Temp:	20 minutes @ 25°C	**Specific Binding:**	80% *
Incubation Buffer:	10 mM HEPES, pH 7.5, 120 mM NaCl, 4 mM $Ca(CH_3COO)_2$, 10 µM Isoguvacine, 10 µM S(-)-Baclofen	**Quantitation Method:**	Radioligand Binding
Kd:	0.30 µM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	60.0 pmole/mg Protein *

204410 Transporter, Norepinephrine (NET)

Source:	Human recombinant MDCK cells	**Ligand:**	0.20 nM [^{125}I] RTI-55
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Desipramine
Incubation Time/Temp:	3 hours @ 4°C	**Specific Binding:**	75% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 µM Leupeptin, 10 µM PMSF	**Quantitation Method:**	Radioligand Binding
Kd:	0.024 µM *	**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	**Ligand:**	0.40 nM [^{3}H] Paroxetine
Vehicle:	1% DMSO	**Non-Specific Ligand:**	10.0 µM Imipramine
Incubation Time/Temp:	60 minutes @ 25°C	**Specific Binding:**	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl	**Quantitation Method:**	Radioligand Binding
Kd:	0.078 nM *	**Significance Criteria:**	≥50% of max stimulation or inhibition
		Bmax:	4.40 pmole/mg Protein *

* Historical Values

Reference Compounds

Cat #	Assay Name	Reference Compound	Historical IC_{50}*	Historical K_i	Historical n_H	Concurrent Batch *	Concurrent IC_{50}*
200510	Adenosine A_1	R(-)-PIA	0.83 µM	0.49 µM	0.90	317535	0.36 µM
200610	Adenosine A_{2A}	CGS-21680	0.13 µM	0.079 µM	1.0	317540	0.11 µM
200720	Adenosine A_3	IB-MECA	0.78 nM	0.72 nM	0.80	317577	0.91 nM
203100	Adrenergic α_{1A}	Prazosin	0.69 nM	0.28 nM	0.90	317536	0.25 nM
203200	Adrenergic α_{1B}	Prazosin	0.27 nM	0.15 nM	1.0	317547	0.16 nM
203400	Adrenergic α_{1D}	Prazosin	0.88 nM	0.43 nM	0.70	317537	0.68 nM
203620	Adrenergic α_{2A}	Yohimbine	8.40 nM	3.10 nM	0.90	317534	4.48 nM
204010	Adrenergic β_1	S(-)-Propranolol	2.50 nM	1.40 nM	0.80	317532	0.86 nM
204110	Adrenergic β_2	S(-)-Propranolol	0.78 nM	0.54 nM	1.20	317533	0.28 nM
285010	Androgen (Testosterone) AR	Testosterone	6.50 nM	4.30 nM	1.0	317485	3.27 nM
212510	Bradykinin B_1	(Des-Arg^{10})-Kallidin	0.87 nM	0.22 nM	1.10	317578	0.42 nM
212620	Bradykinin B_2	Bradykinin	1.80 nM	1.10 nM	1.0	317579	1.17 nM
214510	Calcium Channel L-Type, Benzothiazepine	Diltiazem	0.036 µM	0.032 µM	0.90	317482	0.021 µM
214600	Calcium Channel L-Type, Dihydropyridine	Nitrendipine	0.72 nM	0.46 nM	0.90	317531	0.26 nM
216000	Calcium Channel N-Type	ω-Conotoxin GVIA	0.034 nM	0.028 nM	1.60	317580	0.026 nM
217030	Cannabinoid CB_1	R(+)-WIN-55,212-2	0.20 µM	0.15 µM	0.70	317529	0.26 µM
219500	Dopamine D_1	R(+)-SCH-23390	1.40 nM	0.70 nM	0.90	317541	1.56 nM
219700	Dopamine D_{2S}	Spiperone	0.25 nM	0.089 nM	1.0	317543	0.086 nM
219800	Dopamine D_3	Spiperone	0.36 nM	0.12 nM	0.90	317544	0.21 nM
219900	Dopamine $D_{4.2}$	Spiperone	0.50 nM	0.20 nM	0.90	317552	0.30 nM
224010	Endothelin ET_A	Endothelin-1	0.23 nM	0.14 nM	1.10	317641	0.17 nM
224110	Endothelin ET_B	Endothelin-1	0.13 nM	0.060 nM	0.90	317642	0.054 nM
225510	Epidermal Growth Factor (EGF)	EGF (human)	1.60 nM	1.10 nM	1.10	317582	0.97 nM
226010	Estrogen ERα	Diethylstilbestrol	0.77 nM	0.22 nM	1.0	317583	0.81 nM
226600	$GABA_A$, Flunitrazepam, Central	Diazepam	0.016 µM	0.013 µM	0.80	317545	0.027 µM
226500	$GABA_A$, Muscimol, Central	GABA	0.032 µM	0.026 µM	0.90	317556	0.025 µM
228610	$GABA_{B1A}$	CGP-54626	6.40 nM	2.90 nM	1.0	317584	4.56 nM
232030	Glucocorticoid	Dexamethasone	3.80 nM	1.80 nM	0.90	317476	2.98 nM
232700	Glutamate, Kainate	L-Glutamic acid	0.24 µM	0.17 µM	0.80	317586	0.18 µM
232810	Glutamate, NMDA, Agonism	L-Glutamic acid	0.41 µM	0.37 µM	0.90	317587	0.18 µM
232910	Glutamate, NMDA, Glycine	MDL 105,519	0.022 µM	0.021 µM	0.60	317588	9.38 nM
233000	Glutamate, NMDA, Phencyclidine	Dizocilpine ((+)-MK-801)	5.10 nM	3.40 nM	0.70	317557	4.71 nM
239610	Histamine H_1	Pyrilamine	3.30 nM	1.60 nM	1.0	317546	2.50 nM
239710	Histamine H_2	Tiotidine	0.022 µM	0.018 µM	1.10	317514	0.020 µM
239820	Histamine H_3	R(-)-α-Methylhistamine (RAMH)	2.30 nM	1.10 nM	1.10	317553	1.74 nM
241000	Imidazoline I_2, Central	Idazoxan	0.012 µM	8.0 nM	1.0	317539	7.50 nM
243520	Interleukin IL-1	IL-1β	0.19 nM	0.14 nM	1.30	317484	0.22 nM

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

Reference Compounds

Cat #	Assay Name	Reference Compound	Historical			Concurrent	
			IC_{50}*	K_i	n_H	Batch *	IC_{50}*
250460	Leukotriene, Cysteinyl CysLT$_1$	LTD_4	0.70 nM	0.29 nM	1.0	317589	1.29 nM
251600	Melatonin MT$_1$	Melatonin	0.21 nM	0.11 nM	0.70	317513	0.45 nM
252610	Muscarinic M$_1$	4-DAMP	4.50 nM	1.10 nM	1.0	317554	3.64 nM
252710	Muscarinic M$_2$	4-DAMP	0.055 µM	0.023 µM	1.0	317527	0.024 µM
252810	Muscarinic M$_3$	4-DAMP	5.10 nM	2.50 nM	1.10	317528	4.70 nM
257010	Neuropeptide Y Y$_1$	Neuropeptide Y (human, rat)	0.22 nM	0.21 nM	1.10	317468	0.44 nM
257110	Neuropeptide Y Y$_2$	Neuropeptide Y (13-36) (porcine)	0.21 nM	0.12 nM	0.90	317592	0.35 nM
258590	Nicotinic Acetylcholine	Epibatidine	0.076 nM	0.052 nM	0.90	317521	0.12 nM
258700	Nicotinic Acetylcholine α1, Bungarotoxin	α-Bungarotoxin	1.10 nM	0.72 nM	1.10	317522	1.30 nM
260130	Opiate δ$_1$ (OP1, DOP)	Naltrindole	0.91 nM	0.16 nM	1.0	317525	1.23 nM
260210	Opiate κ(OP2, KOP)	U-69593	0.016 µM	6.40 nM	0.5	317524	0.013 µM
260410	Opiate µ(OP3, MOP)	DAMGO	0.020 µM	8.10 nM	0.60	317526	0.019 µM
264500	Phorbol Ester	PMA	0.79 nM	0.59 nM	1.0	317558	0.69 nM
265010	Platelet Activating Factor (PAF)	PAF	0.28 nM	0.15 nM	0.90	317496	0.27 nM
265600	Potassium Channel [K_{ATP}]	Glyburide	5.70 nM	0.65 nM	0.80	317559	2.80 nM
265900	Potassium Channel hERG	Astemizole	2.60 nM	2.10 nM	1.10	317560	6.16 nM
268420	Prostanoid EP$_4$	Prostaglandin E_2 (PGE_2)	1.10 nM	0.45 nM	0.90	317561	0.61 nM
268700	Purinergic P_{2X}	α, β-Methylene ATP	0.082 µM	0.018 µM	1.10	317590	0.029 µM
268810	Purinergic P_{2Y}	ATP	0.018 µM	0.018 µM	0.90	317591	0.038 µM
270000	Rolipram	Rolipram	5.70 nM	2.10 nM	1.0	317562	2.66 nM
271110	Serotonin (5-Hydroxytryptamine) 5-HT$_{1A}$	Metergoline	4.10 nM	2.30 nM	0.90	317510	2.45 nM
271700	Serotonin (5-Hydroxytryptamine) 5-HT$_{2B}$	Ketanserin	0.29 µM	0.18 µM	0.60	317551	0.53 µM
271910	Serotonin (5-Hydroxytryptamine) 5-HT$_3$	MDL 72222	0.011 µM	2.50 nM	0.80	317593	0.018 µM
278110	Sigma σ$_1$	Haloperidol	0.021 µM	8.80 nM	0.90	317523	8.84 nM
255520	Tachykinin NK$_1$	L-703,606	3.60 nM	2.60 nM	1.0	317474	1.56 nM
285900	Thyroid Hormone	Triiodothyronine	0.034 nM	0.018 nM	1.0	317488	0.040 nM
220320	Transporter, Dopamine (DAT)	GBR-12909	1.70 nM	1.30 nM	0.90	317469	0.59 nM
226400	Transporter, GABA	NO-711	0.20 µM	0.20 µM	1.10	317538	0.21 µM
204410	Transporter, Norepinephrine (NET)	Desipramine	0.93 nM	0.92 nM	0.60	317520	0.44 nM
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	Fluoxetine	8.60 nM	1.40 nM	0.90	317549	0.013 µM

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

Appendix Notes

The assay # 232020 Glucocorticoid (Ricerca Taiwan Ltd.) was replaced with # 232030 Glucocorticoid (Invitrogen #PV4690). The used material for membrane source was changed from in house to commercial supplier to simplify work process.

Assay #279510 sodium channel site 2 was removed due to backorder of radioligand from the supplier.