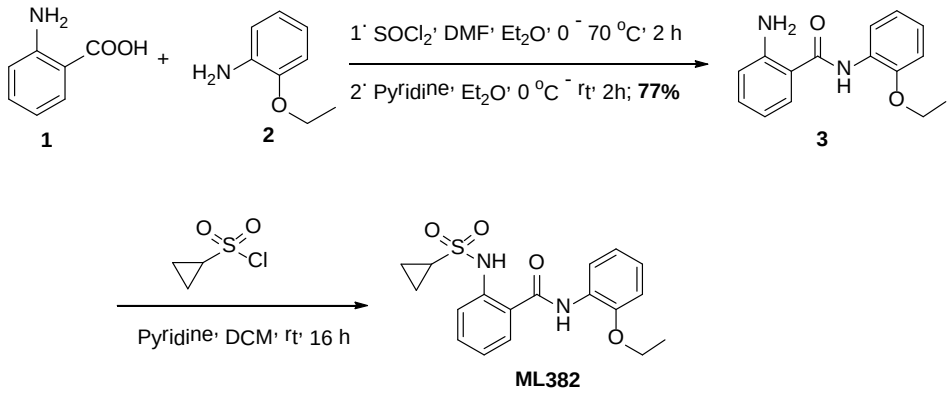
Table of Contents:

General Experimental	S1
Compound Experimentals and Characterization	S2 – S7
In Vitro Pharmacology Methods	S8
DMPK Methods	S9 – S10
Selectivity Panel	S11 – S13
BAM Dose Response Curve	S14
PAMP Dose Response Curve	S15

General Experimental

All reagents and solvents were commercial grade and purified prior to use when necessary. Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies HL 0.25 mm silica gel plates with UV indicator. Visualization was accomplished by irradiation under a 254 nm UV lamp and/or the use of an iodine chamber. Chromatography on silica gel was performed using Silica Gel 60 (230-400 mesh) from Sorbent Technologies. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 (400 and 100 MHz, respectively) NMR instrument. Chemical shifts are reported in ppm from the solvent resonance as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet), coupling constant (Hz), and number of protons. Low resolution mass spectra were obtained on an Agilent 6130 Quadrupole LC/MS with electrospray ionization (RT = retention time). Optical rotations were measured on a JASCO P-2000 digital polarimeter at room temperature. Concentration (c) in g/100 mL and solvent are given in parentheses. Preparative purification was performed on a Gilson chromatograph using a Luna 5u C18(2) 100A AXIA column (30x50 mm) using a water/acetonitrile gradient. Chiral separations were performed on a Thar Investigator II supercritical fluid chromatograph (SFC) using Lux Cellulose 4 (10x250 mm), Chiralpak IA (10x250 mm), and Chiralpak ID (10x250 mm) columns.

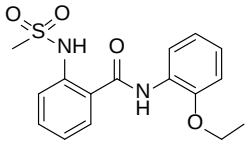


General Procedure:

2-amino-N-(2-ethoxyphenyl)benzamide (3). To a dry argon-filled flask was added anthrinilic acid, 1, (1 eq), and 3 drops of dimethylformamide (DMF) and ether as solvent. To the solution, thionyl chloride (2 eq) was added dropwise at 0 oC. The mixture was refluxed for 2 hours. On completion, ether and remaining thionyl chloride were removed under reduced pressure. The mixture was cooled to 0 oC, followed by addition of 0.5 ml pyridine, 2,4-dimethoxyaniline, 2, (1.2 eq) and ether as solvent. The solution was allowed to warm up to room temperature and stir for 2 h. After removing solvents under reduced pressure, the crude product was purified via reverse phase preparative chromatography (Gilson, Acetonitrile-0.5% NH4OH in water), which yielded 2-amino-N-(2-ethoxyphenyl)benzamide, **3**, as yellow solid, 77% yield. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 8.48-8.46 (m, 1H), 7.52-7.50 (m, 1H), 7.30-7.26 (m, 1H), 7.10-7.00 (m, 2H), 6.94-6.92 (m, 1H), 6.75 (t, 2H, J = 6.87 Hz), 5.62 (s, 2H), 4.18-4.13 (m, 2H), 1.50 (t, 3H, J = 6.94 Hz) ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 167.3, 149.3, 147.7, 132.6, 128.0, 127.2, 123.7, 121.0, 119.9, 117.6, 116.9, 116.7, 111.0, 64.3, 15.0. LCMS: R_T = 1.01 min, >98% @ 215 and 254 nm, m/z = 257.0 $[M + H]^+$.

2-(cyclopropanesulfonamido)-N-(2-ethoxyphenyl)benzamide, 4l (ML382). To dry vial was added 2-amino-N-(2-ethoxyphenyl)benzamide, **3**, (1 eq), cyclopropanesulfonyl chloride (2 eq), pyridine (4 eq) and DCM as solvent. The mixture was stirred for 16 h at room temperature. On completion, the crude product was purified by reverse phase preparative chromatography (Gilson, Acetonitrile-0.5% NH4OH in water). LCMS: RT = 1.13 min, >95% @ 215 and 254 nm, m/z = 360.9 $[M]^+$. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 10.45 (s, 1H), 8.67 (s, 1H), 8.45-8.43 (m, 1H), 7.85 (d, J = 8.38 Hz, 1H), 7.64 (d, J = 8.02 Hz, 1H), 7.56-7.52 (m, 1H), 7.28-7.22 (m, 1H), 7.15-7.11 (m, 1H), 7.06-7.02 (m, 1H), 6.96-6.94 (m, 1H), 4.22-4.16 (m, 2H), 2.56-2.50 (m, 1H), 1.51 (t, J = 6.97 Hz, 3H), 1.27-1.25 (m, 2H), 0.95-0.92 (m, 2H).

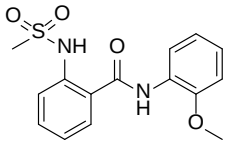
All analogs were made following the General Procedure above:



4a

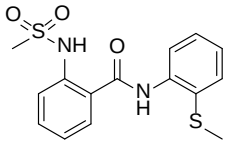
N-(2-ethoxyphenyl)-2-(methylsulfonamido)benzamide (4a)

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 1.49 (t, *J* = 7.0 Hz, 3H), 3.05 (s, 3H), 4.14-4.19 (q, *J* = 7.0 Hz, 2H), 6.92-6.94(m, 1H), 6.99-7.03(m, 1H), 7.09-7.13 (m, 1H), 7.19-7.23 (m, 1H), 7.52-7.56 (m, 1H), 7.64-7.66(m, 1H), 7.78-7.80 (m, 1H), 8.38-8.41 (m, 1H), 8.67 (s, 1H), 10.58 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 14.9, 39.9, 64.4, 111.1, 120.1, 120.2, 121.0, 121.1, 123.6, 124.9, 126.9, 127.0, 133.3, 139.7, 147.8. LC/MS: R_T = 1.068 min, *m/z* = 334.9 $[M]^+$.



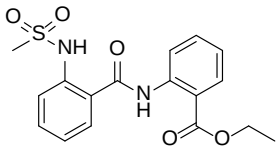
4b

***N*-(2-Methoxyphenyl)-2-(methylsulfonamido)benzamide (4b).** Purchased compound.



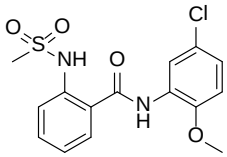
4c

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 2.43 (s, 3H), 3.06 (s, 3H), 7.14-7.18 (m, 1H), 7.21-7.25 (m ,1H), 7.34-7.38 (m, 1H), 7.53-7.58 (m , 1H), 7.74-7.81 (m ,1H), 8.34 (d , *J* = 8.2 Hz, 1H), 9.22 (s, 1H), 10.63 (s, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ (ppm): 166.58, 140.06, 137.46, 133.71, 132.97, 129.07, 127.21, 126.77, 125.57, 123.65, 121.18, 120.54, 120.07, 40.12, 19.19. LC/MS: R_T = 1.008 min, m/z = 336.9 $[M]^+$.



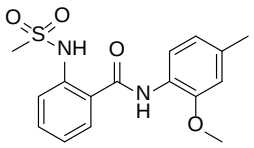
4d

Ethyl 2-(2-(methylsulfonamido)benzamido)benzoate (4d). Purchased compound.



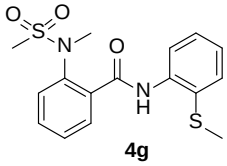
4e

***N*-(5-Chloro-2-methoxyphenyl)-2-(methylsulfonamido)benzamide (4e).** Purchased compound.



4f

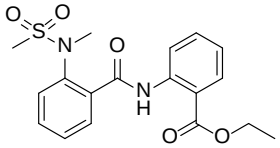
***N*-(2-Methoxy-4-methylphenyl)-2-(methylsulfonamido)benzamide (4f).** Purchased compound.



4g

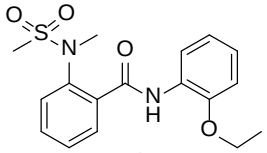
2-(N-methylmethylsulfonamido)-N-(2-(methylthio)phenyl)benzamide (4g).

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 2.40 (s, 3H), 2.98 (s, 3H), 3.39 (s, 3H), 7.11-7.15 (m, 3H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.45-7.50 (m, 3H), 7.52-7.57 (m ,3H), 7.80 (d, *J* = 7.2 Hz, 1H), 8.38 (d, *J* = 8.1 Hz, 1H), 9.13 (s, 1H). ^{13}C NMR (100.6 MHz , $CDCl_3$) δ (ppm): 165.61, 138.87, 138.08, 136.47, 132.65, 131.81, 129.68, 128.97, 128.72, 128.63, 126.85, 125.20, 121.48, 39.27, 37.33, 19.05. LC/MS: R_T = 0.970 min, *m/z* = 350.8 $[M]^+$.



4h

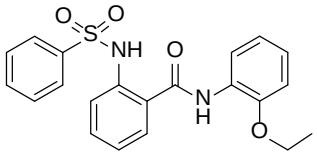
Ethyl 2-(2-(*N*-methylmethylsulfonamido)benzamido)benzoate (4h). Purchased compound.



4i

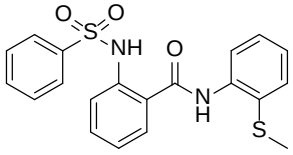
N-(2-ethoxyphenyl)-2-(N-methylmethylsulfonamido)benzamide (4i).

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 1.42 (t, *J* = 7.0 Hz, 3H), 2.99 (s, 3H), 3.33 (s, 3H), 4.11-4.16 (q, *J* = 7.0 Hz, 2H), 6.89-6.91 (m, 1H), 6.96-7.00 (m, 1H), 7.05-7.09 (m ,1H), 7.44-7.48 (m ,2H), 7.51-7.55 (m ,1H), 7.79-7.81 (m ,1H), 8.49-8.52 (dd, *J* = 1.3, 8.0 Hz, 1H), 8.93 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.28, 147.78, 138.64, 136.38, 131.70, 129.89, 129.01, 128.88, 127.69, 124.50, 120.94, 120.43, 111.18, 64.31, 39.19, 37.80, 14.82. LC/MS: R_T = 1.002 min, *m/z* = 348.9 $[M]^+$.



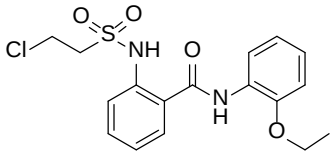
4j

***N*-(2-Ethoxyphenyl)-2-(phenylsulfonamido)benzamide (4j).** Purchased compound.



4k

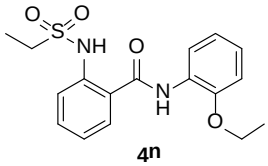
***N*-(2-(methylthio)phenyl)-2-(phenylsulfonamido)benzamide (4k).** Purchased compound.



4m

2-(2-chloroethylsulfonamido)-N-(2-ethoxyphenyl)benzamide (4m).

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 1.49 (t, *J* = 7.0 Hz, 3H), 3.53-3.57 (m, 2H), 3.83-3.87 (m, 2H), 4.14-4.20 (q, *J* = 7.0 Hz, 2H), 6.92-6.94 (dd, *J* = 1.1, 8.1 Hz, 1H), 7.00-7.04 (m, 1H), 7.10-7.14 (m, 1H), 7.21-7.23 (m ,1H), 7.53-7.57 (m ,1H), 7.65-7.67 (dd, *J* = 1.3, 8.0 Hz, 1H), 7.79-7.81 (dd, *J* = 0.7, 8.3 Hz, 1H), 8.37-8.39 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.68 (s, 1H), 10.80 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 15.0, 36.2, 53.9, 64.5, 111.1, 120.4, 120.7, 121.2, 122.1, 124.2, 124.6, 124.9, 126.8, 133.1, 138.6, 147.8, 166.1. LC/MS: R_T = 1.232 min, m/z = 382.8 $[M]^+$.

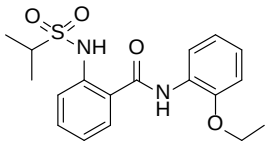


4n

N-(2-ethoxyphenyl)-2-(ethylsulfonamido)benzamide (4n).

^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 1.36 (t, *J* = 7.5 Hz, 3H), 1.47-1.51 (t, *J* = 6.9 Hz, 3H), 3.14-3.19 (q, *J* = 7.5 Hz, 2H), 4.14-4.19 (q, *J* = 6.9 Hz, 2H), 6.91-6.94 (m ,1H), 6.99-7.03 (m ,1H), 7.08-7.13 (m ,1H), 7.16-7.20 (m ,1H), 7.49-7.53 (m, 1H), 7.62-7.64 (dd, *J* = 1.1 Hz, 7.9, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 8.39-8.41 (dd, *J* = 1.4, 7.9 Hz, 1H), 8.66 (s, 1H), 10.60 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 8.2,

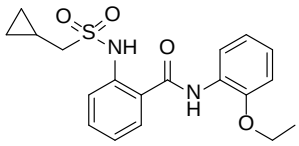
14.9, 46.5, 64.4, 111.1, 119.7, 120.2, 120.8, 121.0, 123.3, 124.8, 126.9, 127.0, 133.3, 139.9, 147.8, 166.2.
LC/MS: R_T = 1.129 min, *m/z* = 348.9 $[M]^+$.



4o

***N*-(2-Ethoxyphenyl)-2-(1-methylethylsulfonamido)benzamide (4o).**

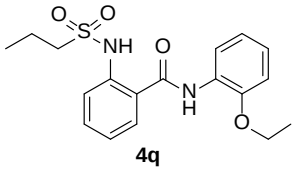
LC/MS: >98% @ 215 and 254 nm, *m/z* = 362.9 $[M]^+$.



4p

2-(Cyclopropylmethylsulfonamido)-*N*-(2-ethoxyphenyl)benzamide (4p).

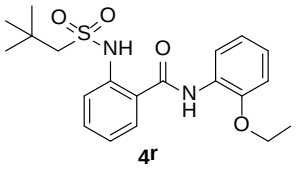
LC/MS: >98% @ 215 and 254 nm, *m/z* = 374.9 $[M]^+$.



4q

***N*-(2-Ethoxyphenyl)-2-(propylsulfonamido)benzamide (4q).**

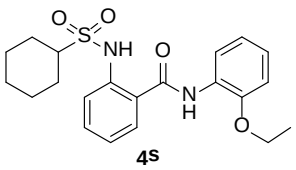
LC/MS: >98% @ 215 and 254 nm, *m/z* = 362.9 $[M]^+$.



4r

2-(2,2-Dimethylpropylsulfonamido)-N-(2-ethoxyphenyl)benzamide (4r).

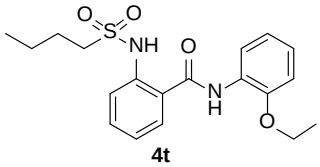
LC/MS: >98% @ 215 and 254 nm, *m/z* = 390.9 $[M]^+$.



4s

2-(Cyclohexanesulfonamido)-N-(2-ethoxyphenyl)benzamide (4s).

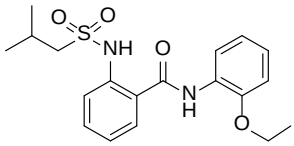
LC/MS: >98% @ 215 and 254 nm, *m/z* = 402.9 $[M]^+$.



4t

2-(Butylsulfonamido)-*N*-(2-ethoxyphenyl)benzamide (4t).

LC/MS: >98% @ 215 and 254 nm, *m/z* = 376.9 $[M]^+$.



4u

***N*-(2-Ethoxyphenyl)-2-(2-methylpropylsulfonamido)benzamide (4u).**

LC/MS: >98% @ 215 and 254 nm, *m/z* = 376.9 $[M]^+$.

In Vitro Pharmacology Methods

Cell based Ca^{2+} imaging assay to detect MrgX1 activation. The purpose of this assay is to identify test compounds that act as an allosteric agonist for MrgX1. This assay employs a HEK293 cell line that stably expresses MrgX1 protein. The cells, which loaded with fluorescent dye-Flou4, are treated with test compounds, followed by measurement of calcium flux. Those HEK293 cells stably expressing MrgX1 were plated into 96-well plates. On the following day, cells were incubated with Fluo4 solution at 37 °C for 30min and at RT for 30 min after removing media. 10 μM compounds were added to the assay buffer with dye for 80 sec followed by adding 10 nM BAM8-22 for 75 sec and recorded the change of fluorescence by Flexstation3 imaging plate reader. Compound effect was evaluated by the calculated fluorescence ratio. If the compound causes more than 3 times the standard deviation of the B-scores of the library compounds, the compound is then considered to be active as an agonist of the MrgX1 protein.

***In Vitro* DMPK Methods:**

The metabolism of ML382 was investigated in human, rat and mouse hepatic microsomes (BD Biosciences, Billerica, MA) using substrate depletion methodology (% test article remaining). A potassium phosphate-buffered reaction mixture (0.1 M, pH 7.4) of test article (1 µM) and microsomes (0.5 mg/mL) was pre-incubated (5 min) at 37°C prior to the addition of NADPH (1 mM). The incubations, performed in 96-well plates, were continued at 37 °C under ambient oxygenation and aliquots (80 µL) were removed at selected time intervals (0, 3, 7, 15, 25 and 45 min). Protein was precipitated by the addition of chilled acetonitrile (160 µL), containing glyburide as an internal standard (50 ng/mL), and centrifuged at 3000 rpm (4°C) for 10 min. Resulting supernatants were transferred to new 96-well plates in preparation for LC/MS/MS analysis. The in vitro half-life ($t_{1/2}$, min, Eq. 1), intrinsic clearance (CL_{int}, mL/min/kg, Eq. 2) and subsequent predicted hepatic clearance (CL_{hep}, mL/min/kg, Eq. 3) were determined employing the following equations:

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

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

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

Plasma Protein Binding. Protein binding of ML382 was determined in human, rat and mouse plasma via equilibrium dialysis employing Single-Use RED Plates with inserts (ThermoFisher Scientific, Rochester, NY). Briefly plasma (220 µL) was added to the 96 well plate containing test article (5 µL) and mixed thoroughly. Subsequently, 200 µL of the plasma-test article mixture was transferred to the *cis* chamber (red) of the RED plate, with an accompanying 350 µL of phosphate buffer (25 mM, pH 7.4) in the *trans* chamber. The RED plate was sealed and incubated 4 h at 37 °C with shaking. At completion, 50 µL aliquots from each chamber were diluted 1:1 (50 µL) with either plasma (*cis*) or buffer (*trans*) and transferred to a new 96 well plate, at which time ice-cold acetonitrile (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rpm, 10 min) and supernatants transferred to a new 96 well plate. The sealed plate was stored at -20 °C until LC/MS/MS analysis.

Liquid Chromatography/Mass Spectrometry Analysis. *In vitro experiments.* ML382 was analyzed via electrospray ionization (ESI) on an AB Sciex API-4000 (Foster City, CA) triple-quadrupole instrument that was coupled with Shimadzu LC-10AD pumps (Columbia, MD) and a Leap Technologies CTC PAL auto-sampler (Carrboro, NC). Analytes were separated by gradient elution using a Fortis C18 2.1 x 50 mm, 3.5 µm column (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 °C. HPLC mobile phase A was 0.1% NH_4OH (pH unadjusted), mobile phase B was acetonitrile. The gradient started at 30% B after a 0.2 min hold and was linearly increased to 90% B over 0.8 min; held at 90% B for 0.5 min and returned to 30% B in 0.1 min followed by a re-equilibration (0.9 min). The total run time was 2.5 min and the HPLC flow rate was 0.5 mL/min. The source temperature was set at 500°C and mass spectral analyses were performed using multiple reaction monitoring (MRM) utilizing a Turbo-Ionspray® source in positive ionization mode (5.0 kV spray voltage). LC/MS/MS analysis was performed employing a

TSQ Quantum^{ULTRA} that was coupled to a ThermoSurveyor LC system (Thermoelectron Corp., San Jose, CA) and a Leap Technologies CTC PAL auto-sampler (Carrboro, NC). Chromatographic separation of analytes was achieved with an Acquity BEH C18 2.1 x 50 mm, 1.7 µm column (Waters, Taunton, MA).

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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:

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_I	n_H
271700	Serotonin (5-Hydroxytryptamine) 5-HT2B	hum	10 µM	63			

* A standard error of the mean is presented where results are based on multiple, independent determinations.
ham=Hamster; hum=Human

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Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_I	n_H	R
Compound: ML382, PT #: 1176873										
200510	Adenosine A_1	344150	hum	2	10 µM	-1				
200610	Adenosine A_{2A}	344151	hum	2	10 µM	-26				1
200720	Adenosine A_3	344148	hum	2	10 µM	10				
203100	Adrenergic α_{1A}	344152	rat	2	10 µM	1				
203200	Adrenergic α_{1B}	344240	rat	2	10 µM	2				
203400	Adrenergic α_{1D}	344154	hum	2	10 µM	6				
203630	Adrenergic α_{2A}	344155	hum	2	10 µM	-13				
204010	Adrenergic β_1	344146	hum	2	10 µM	-3				
204110	Adrenergic β_2	344156	hum	2	10 µM	-11				
206000	Androgen (Testosterone)	344120	hum	2	10 µM	4				
212510	Bradykinin B_1	344119	hum	2	10 µM	28				
212620	Bradykinin B_2	344191	hum	2	10 µM	14				
214510	Calcium Channel L-Type, Benzothiazepine	344415	rat	2	10 µM	-1				
214600	Calcium Channel L-Type, Dihydropyridine	344157	rat	2	10 µM	-6				
216000	Calcium Channel N-Type	344192	rat	2	10 µM	-12				
217030	Cannabinoid CB_1	344158	hum	2	10 µM	-8				
219500	Dopamine D_1	344159	hum	2	10 µM	12				
219700	Dopamine D_{2S}	344160	hum	2	10 µM	-10				
219800	Dopamine D_3	344161	hum	2	10 µM	-18				
219900	Dopamine $D_{4.2}$	344162	hum	2	10 µM	-10				
224010	Endothelin ET_A	344193	hum	2	10 µM	7				
224110	Endothelin ET_B	344122	hum	2	10 µM	-12				
225510	Epidermal Growth Factor (EGF)	344089	hum	2	10 µM	9				
226010	Estrogen ERα	344194	hum	2	10 µM	-3				
226600	$GABA_A$, Flunitrazepam, Central	344164	rat	2	10 µM	11				
226500	$GABA_A$, Muscimol, Central	344163	rat	2	10 µM	0				
228610	$GABA_{B1A}$	344149	hum	2	10 µM	14				
232030	Glucocorticoid	344197	hum	2	10 µM	2				
232700	Glutamate, Kainate	344198	rat	2	10 µM	-1				
232810	Glutamate, NMDA, Agonism	344199	rat	2	10 µM	0				
232910	Glutamate, NMDA, Glycine	344200	rat	2	10 µM	4				
233000	Glutamate, NMDA, Phencyclidine	344165	rat	2	10 µM	5				
239610	Histamine H_1	344166	hum	2	10 µM	-12				
239710	Histamine H_2	344145	hum	2	10 µM	0				

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
R=See Remarks (if any) at end of this section.

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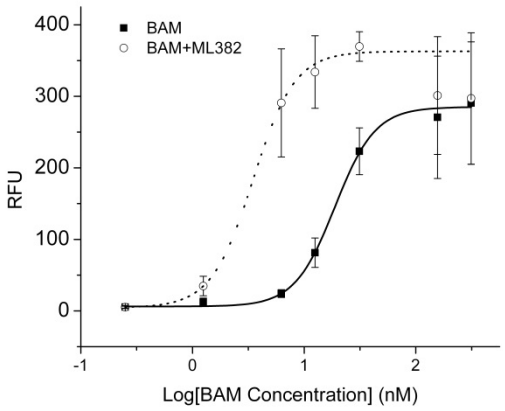
Experimental Results

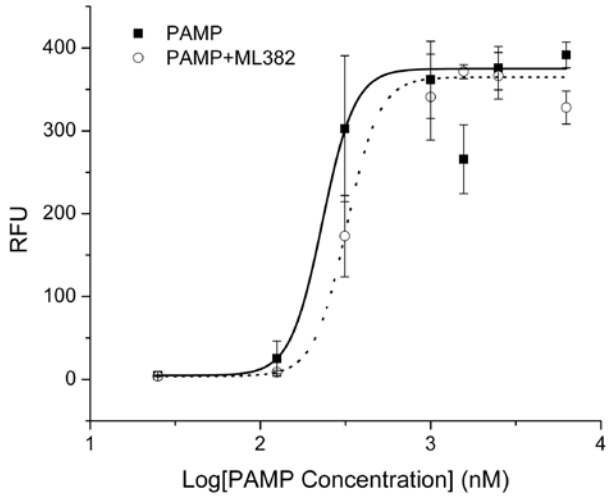
Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_I	n_H	R
239820	Histamine H_3	344201	hum	2	10 µM	2				
241000	Imidazoline I_2, Central	344181	rat	2	10 µM	-7				
243520	Interleukin IL-1	344202	mouse	2	10 µM	5				
250460	Leukotriene, Cysteinyl $CysLT_1$	344203	hum	2	10 µM	0				
251600	Melatonin MT_1	344138	hum	2	10 µM	7				
252610	Muscarinic M_1	344167	hum	2	10 µM	9				
252710	Muscarinic M_2	344168	hum	2	10 µM	-17				
252810	Muscarinic M_3	344169	hum	2	10 µM	-4				
257010	Neuropeptide Y Y_1	344221	hum	2	10 µM	1				
257110	Neuropeptide Y Y_2	344205	hum	2	10 µM	2				
258590	Nicotinic Acetylcholine	344139	hum	2	10 µM	12				
258700	Nicotinic Acetylcholine α1, Bungarotoxin	344140	hum	2	10 µM	0				
260130	Opiate δ_1 (OP1, DOP)	344206	hum	2	10 µM	0				
260210	Opiate κ(OP2, KOP)	344207	hum	2	10 µM	4				
260410	Opiate µ(OP3, MOP)	344171	hum	2	10 µM	2				
264500	Phorbol Ester	344172	mouse	2	10 µM	0				
265010	Platelet Activating Factor (PAF)	344214	hum	2	10 µM	15				
265600	Potassium Channel [K_{ATP}]	344173	ham	2	10 µM	10				
265900	Potassium Channel hERG	344174	hum	2	10 µM	2				
268420	Prostanoid EP_4	344175	hum	2	10 µM	12				
268700	Purinergic P2X	344208	rabbit	2	10 µM	33				
268810	Purinergic P2Y	344209	rat	2	10 µM	20				
270000	Rolipram	344176	rat	2	10 µM	11				
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	344211	hum	2	10 µM	-2				
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	344177	hum	2	10 µM	63				
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	344210	hum	2	10 µM	8				
278110	Sigma σ_1	344178	hum	2	10 µM	-1				
279510	Sodium Channel, Site 2	344180	rat	2	10 µM	7				
255520	Tachykinin NK_1	344170	hum	2	10 µM	8				
285900	Thyroid Hormone	344215	rat	2	10 µM	1				
220320	Transporter, Dopamine (DAT)	344123	hum	2	10 µM	-5				
226400	Transporter, GABA	344196	rat	2	10 µM	10				
204410	Transporter, Norepinephrine (NET)	344141	hum	2	10 µM	0				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	344179	hum	2	10 µM	5				

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



BAM:
EC_{50} = 18.67 ± 1.06 nM
BAM + 5μM ML382:
EC_{50} = 2.87 ± 1.22 nM
[95% confidence interval]

Supplemental Figure 1. ML382 enhances the potency of BAM8-22 on MrgX1-expressing HEK293 cells. Dose response curve of BAM8-22 in presence (red circles) or absence of ML382 (black squares) determined by Ca^{2+} imaging assay. The EC_{50} of BAM8-22 in presence of maximal amount of ML382 (5 μM) is 2.87 ± 1.22 nM (95% confidence interval). In the absence of ML382, the EC_{50} of BAM8-22 is 18.67 ± 1.06 nM (95% confidence interval). RFU: relative fluorescence units. The fitting was dose response fitting. The experiments were repeated three times. Error bars represent as Mean ± SEM.



PAMP:
EC_{50}=211.86 ± 51.98 nM
PAMP + 5 μM ML382:
EC_{50} = 316.12 ± 17.8 nM
[95% confidence interval]

Supplemental Figure 2. ML382 has no effect on MrgX2-expressing HEK293 cells. Dose response curve of PAMP (an agonist of MrgX2)[11] in presence (red circles) or absence of ML382 (black squares) determined by Ca^{2+} imaging assay. The EC_{50} of PAMP in presence of 5 μM ML382 is 316.12 ± 17.8 nM (95% confidence interval). In the absence of ML382, the EC_{50} of BAM8-22 is 211.86 ± 51.98 nM (95% confidence interval). RFU: relative fluorescence units. The fitting was dose response fitting. The experiments were repeated three times. Error bars represent as Mean ± SEM.