Design and Synthesis of γ- and δ-Lactam M_1 Positive Allosteric Modulators (PAMs): Convulsion and Cholinergic Toxicity of an M_1-Selective PAM with Weak Agonist Activity

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Supporting Information

Contents

Animal Welfare Statement

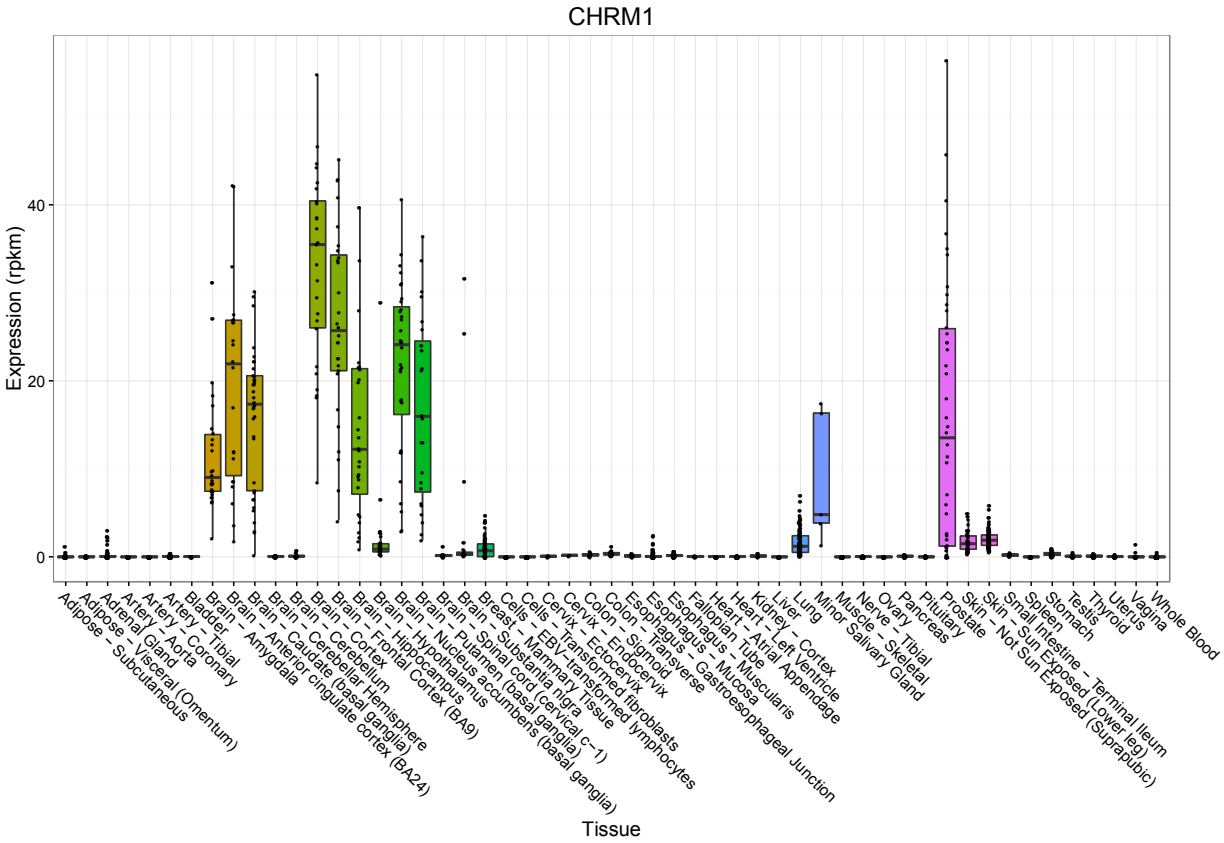
All procedures performed on animals in these experiments were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health (NIH: 8th edition) and approved by an Institutional Animal Care and Use Committee (IACUC).

Abbreviations List

bis(pinacolato)diboron (B_2pin_2), 1,1′-bis(diphenylphosphino)ferrocene (dppf) and tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$, hours post dose (HPD), acetylcholine (ACh), positive allosteric modulator (PAM), adverse event (AE), gastrointestinal (GI), cardiovascular (CV), powder X-ray diffraction (PXRD); guinea pig ileum (GPI), longitudinal muscle myenteric plexus (LMMP), muscarinic acetylcholine receptor (mAChR), central nervous system (CNS), non-human primate (NHP), area under the curve (AUC), Alzheimer's disease (AD), multi-drug resistance protein (MDR), human liver microsome (HLM), lipophilic efficiency (LipE), pharmacokinetics (PK), Morris water maze (MWM), and amphetamine stimulated locomotor activity (aLMA).

M_1- M_5 Human mRNA Expression Levels from The Genotype-Tissue Expression (GTEx) Project

M1



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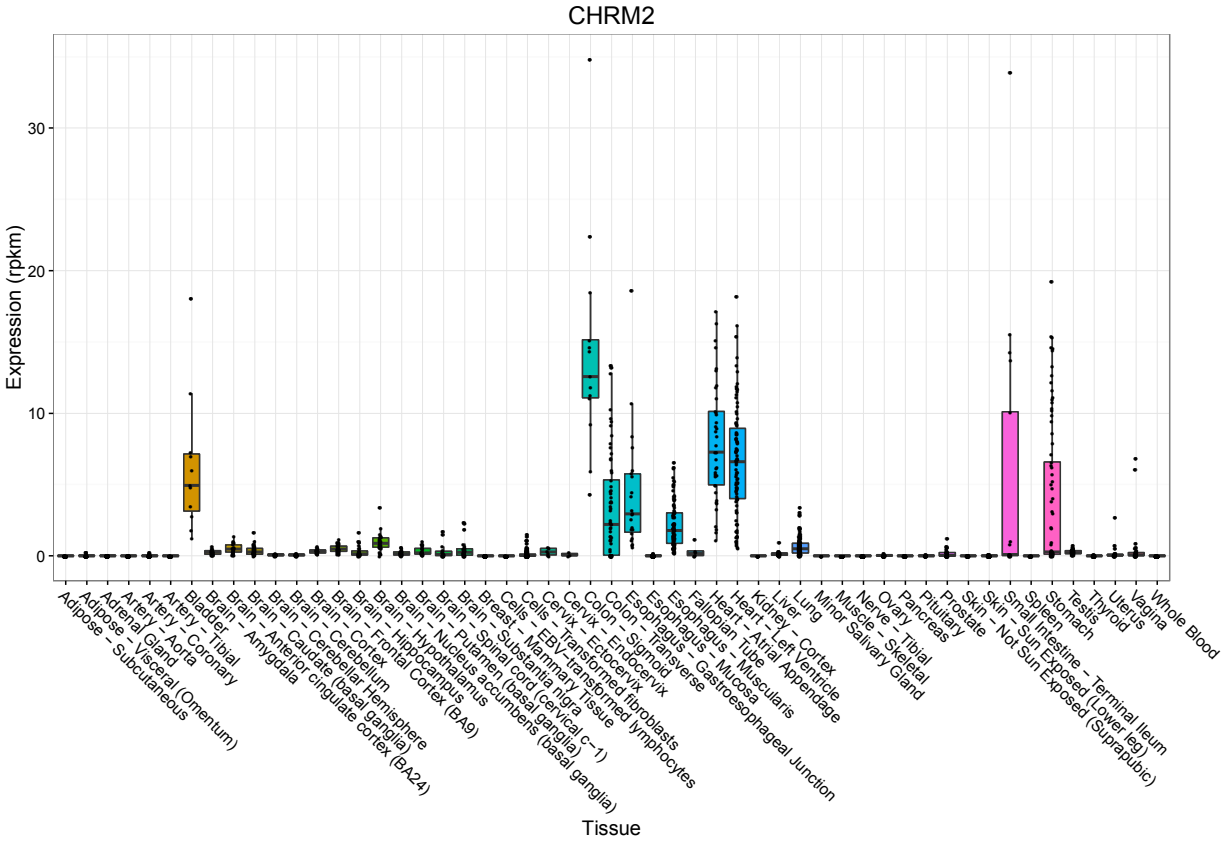
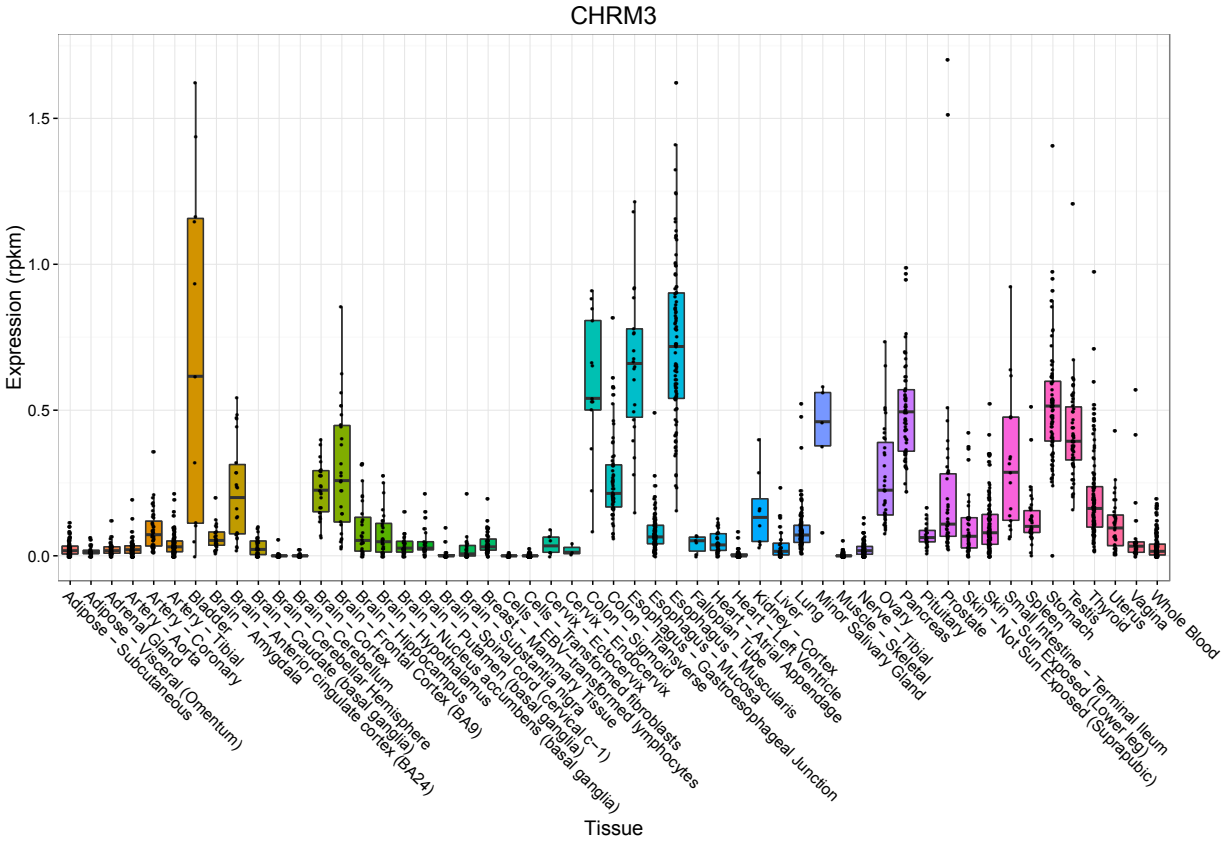

CHRM2
Expression (rpkm)
30
20
10
0
Adipose - Subcutaneous
Adipose - Visceral (Omentum)
Adrenal Gland
Artery - Aorta
Artery - Coronary
Artery - Tibial
Bladder
Brain - Amygdala
Brain - Anterior cingulate cortex (BA24)
Brain - Caudate (basal ganglia)
Brain - Cerebellar Hemisphere
Brain - Cerebellum
Brain - Cortex
Brain - Frontal Cortex (BA9)
Brain - Hippocampus
Brain - Hypothalamus
Brain - Nucleus accumbens (basal ganglia)
Brain - Putamen (basal ganglia)
Brain - Spinal cord (cervical c-1)
Brain - Substantia nigra
Breast - Mammary Tissue
Cells - EBV-transformed lymphocytes
Cells - Transformed fibroblasts
Cervix - Ectocervix
Cervix - Endocervix
Colon - Transverse
Colon - Sigmoid
Esophagus - Gastroesophageal Junction
Esophagus - Mucosa
Esophagus - Muscularis
Fallopian Tube
Heart - Atrial Appendage
Heart - Left Ventricle
Kidney - Cortex
Liver
Lung
Minor Salivary Gland
Muscle - Skeletal
Nerve - Tibial
Ovary
Pancreas
Pituitary
Prostate
Skin - Not Sun Exposed (Suprapubic)
Skin - Sun Exposed (Lower leg)
Small Intestine - Terminal Ileum
Spleen
Stomach
Testis
Thyroid
Uterus
Vagina
Whole Blood
Tissue

M3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Expression (rpkm)
1.5
1.0
0.5
0.0
Adipose – Subcutaneous
Adipose – Visceral (Omentum)
Adrenal Gland
Artery – Aorta
Artery – Coronary
Artery – Tibial
Bladder
Brain – Amygdala
Brain – Anterior cingulate cortex (BA24)
Brain – Caudate (basal ganglia)
Brain – Cerebellar Hemisphere
Brain – Cerebellum
Brain – Cortex
Brain – Frontal Cortex (BA9)
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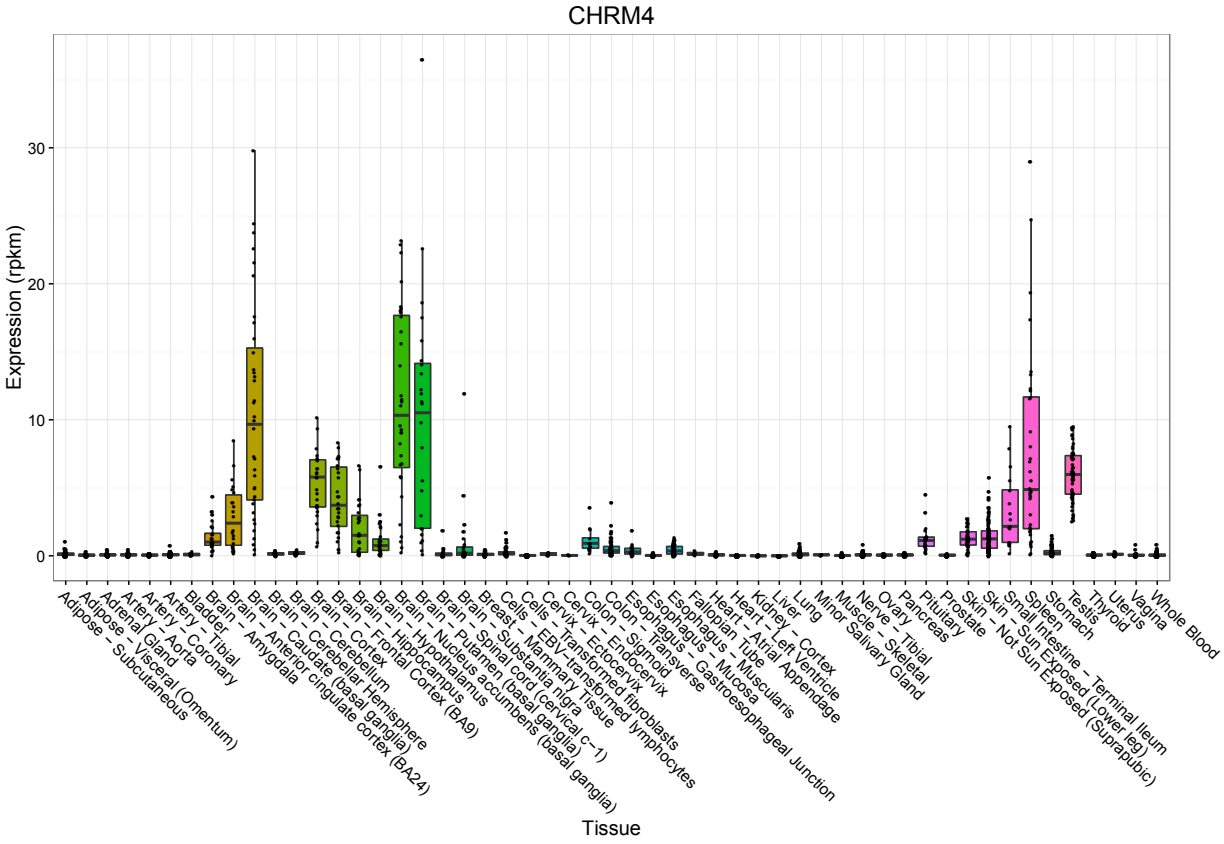
Heart – Left Ventricle
Kidney – Cortex
Liver
Lung
Minor Salivary Gland
Muscle – Skeletal
Nerve – Tibial
Ovary
Pancreas
Pituitary
Prostate
Skin – Not Sun Exposed (Suprapubic)
Skin – Sun Exposed (Lower leg)
Small Intestine – Terminal Ileum
Spleen
Stomach
Testis
Thyroid
Uterus
Vagina
Whole Blood
Tissue

M4


CHRM4
Expression (rpkm)
30
20
10
0
Adipose - Subcutaneous
Adipose - Visceral (Omentum)
Adrenal Gland
Artery - Aorta
Artery - Coronary
Artery - Tibial
Bladder
Brain - Amygdala
Brain - Anterior cingulate cortex (BA24)
Brain - Caudate (basal ganglia)
Brain - Cerebellar Hemisphere
Brain - Cerebellum
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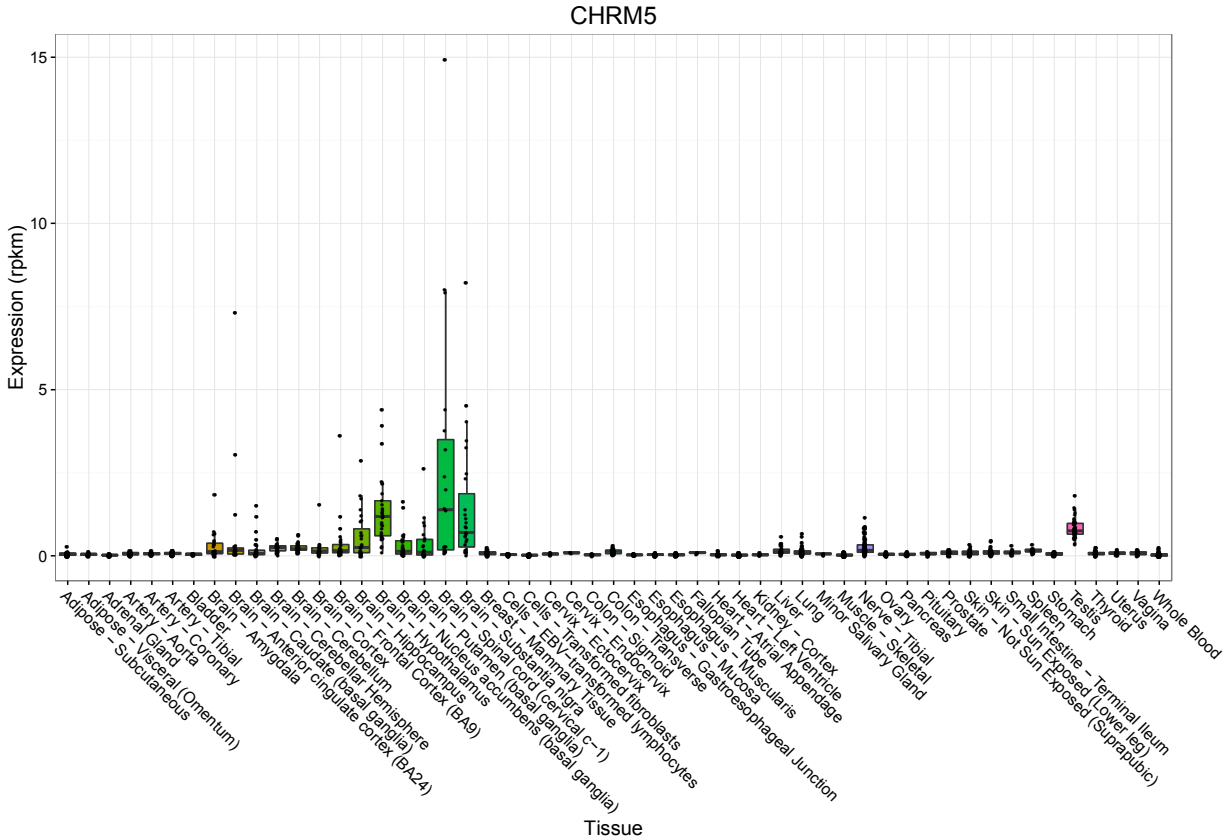
Heart - Atrial Appendage
Heart - Left Ventricle
Kidney - Cortex
Liver
Lung
Minor Salivary Gland
Muscle - Skeletal
Nerve - Tibial
Ovary
Pancreas
Pituitary
Prostate
Skin - Not Sun Exposed (Suprapubic)
Skin - Sun Exposed (Lower leg)
Small Intestine - Terminal Ileum
Spleen
Stomach
Testis
Thyroid
Uterus
Vagina
Whole Blood
Tissue

M5

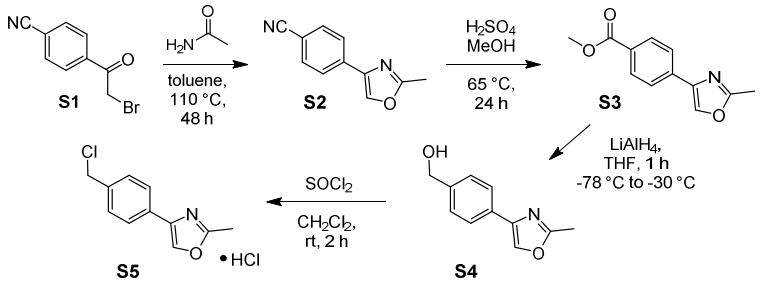


Synthesis Procedures for Relevant Intermediates and Final Compounds

General Information

All solvents and reagents were obtained from commercial sources and were used as received. All reactions were monitored by TLC (TLC plates F254, Merck) or UPLC-MS analysis (Waters Acquity, ESCI +/-, APCI +/-). Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. Mass spectrometry data obtained using a Waters SQ MS (single quad) Tune: ESI-3.5kV Capillary/APCI(in ESCI mode)-0.3µA Corona Pin, 30V Cone, Source 150 °C , Desolvation 475 °C, Desolvation Gas N2 400L/hr. ^{1}H NMR spectra were obtained using deuterated solvent on a Varian 400 MHz instrument. All ^{1}H NMR shifts are reported in δ units (ppm) relative to the signals for chloroform (7.27 ppm), DMSO (2.50 ppm) and MeOH (3.31 ppm). All coupling constants (*J* values) are reported in hertz (Hz). NMR abbreviations are as follows: br, broadened; s, singlet; d, doublet; t, triplet; q, quartet; p, pentuplet; m, multiplet; dd, doublet of doublets; ddd, doublet of doublet of doublets. HPLC purity analysis of the final test compounds was carried out using one of three methods. Method A: UPLC/UV. WuXi AppTec, Shanghai, China. Column: Agilent Xtimate C18, 5 × 30 mm, 3 µm; UV purity detected at 220 nm; Mobile phase A = 0.1% TFA in H_2O; Mobile phase B = 0.1% TFA in CH_3CN. Gradient: 1% B to 100% B in 5.0 min. Flow rate: 1.2 mL/min. Method B: UPLC/UV WuXi AppTec, Shanghai, China. Column: Xbridge C18, 2.1 × 50 mm, 5 µm; UV purity detected at 220 nm; Mobile phase A = 0.0375% TFA in H_2O; Mobile phase B = 0.01875% TFA in CH3CN. Gradient: 1% B to 5% B in 0.6 min, 5%B to 100% B in 4.4 min, 100% B to 1% B for 0.3 min, hold at 1% B for 0.4 min. Flow rate: 0.8 mL/min. Method C: Column: Waters Atlantis C18 4.6 x 50 mm, 5 µm; UV purity detected at 215 nm; Mobile phase A: 0.05% TFA in H_2O (v/v); Mobile phase B: 0.05% TFA in CH_3CN (v/v); Gradient: 95.0% H_2O/5.0% CH_3CN linear to 5.0% H_2O/95.0% CH_3CN in 4.0 min, hold at 5.0% H_2O/95.0% CH_3CN to 5.0 min. Flow rate: 2 mL/min. All final compounds were determined to have a purity of >95% by one of the aforementioned methods unless stated otherwise.

Tail pieces:

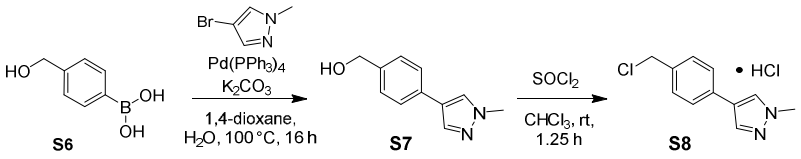


4-(2-Methyl-1,3-oxazol-4-yl)benzonitrile (S2): A mixture of 4-(bromoacetyl)benzonitrile (**S1**) (9.5 g, 42 mmol) and acetamide (6.26 g, 106 mmol) in toluene (200 mL) was heated at reflux for 48 hours, and then it was filtered. After the filtrate was concentrated *in vacuo*, silica gel chromatography (eluent: EtOAc in petroleum ether, 0% to 20%) afforded **S2** as a white solid (7.5 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.81 (br d, J = 8.5 Hz, 2H), 7.68 (br d, J = 8.7 Hz, 2H), 2.54 (s, 3H).

Methyl 4-(2-methyl-1,3-oxazol-4-yl)benzoate (S3): Compound **S2** (6.0 g, 33 mmol) and concentrated sulfuric acid (50 mL) were combined in MeOH (100 mL) and heated at reflux for 24 hours. The reaction mixture was cooled to room temperature and then slowly poured into ice water (300 mL). The resulting mixture was adjusted to a pH = 7 – 8 with solid sodium hydroxide. Upon removal of MeOH *in vacuo*, copious yellow solid precipitated, which was collected via filtration to provide **S3** as a yellow solid (6.5 g, 91%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.4 Hz, 2H), 7.90 (s, 1H), 7.77 (d, J = 8.4 Hz, 2H), 3.92 (s, 3H), 2.53 (s, 3H).

[4-(2-Methyl-1,3-oxazol-4-yl)phenyl]methanol (S4): Lithium aluminum hydride (4.19 g, 110 mmol) was added to a mixture of **S3** (6.00 g, 27.6 mmol) in THF (200 mL) at -78 °C, and the reaction mixture was allowed to stir at −30 °C for 1 hour. Water (4.5 mL) and aqueous sodium hydroxide solution (15%, 4.5 mL) were slowly added to the reaction mixture as it warmed to room temperature. The mixture was then diluted with EtOAc (200 mL) and filtered; the filtrate was dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford **S4** as a white solid (4.0 g, 76%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 4.71 (s, 2H), 2.52 (s, 3H), 2.00-2.14 (br s, 1H).

4-(4-(Chloromethyl)phenyl)-2-methyloxazole, hydrochloride salt (S5): Thionyl chloride (7.55 g, 63.5 mmol) was slowly added to a mixture of **S4** (4.0 g, 21 mmol) in CH_2Cl_2 (150 mL), and the reaction mixture was stirred at room temperature for 2 hours. Removal of solvent *in vacuo* provided **S5** as a yellow solid (4.2 g, 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.90 (d, J=8.2 Hz, 2H), 7.51 (d, J=8.2 Hz, 2H), 4.61 (s, 2H), 2.96 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.5, 139.7, 136.5, 135.7, 129.3, 126.2, 125.8, 44.8, 12.0.



[4-(1-Methyl-1H-pyrazol-4-yl)phenyl]methanol (S7): A mixture of [4-(hydroxymethyl)phenyl]boronic acid (**S6**) (8.00 g, 52.6 mmol), 4-bromo-1-methyl-1*H*-pyrazole (12.7 g, 78.9 mmol), $Pd(PPh_3)_4$ (1.83 g, 1.58 mmol), and potassium carbonate (14.6 g, 106 mmol) in 1,4-dioxane (130 mL) and water (30 mL) was heated at 100 °C for 16 hours. The reaction mixture was filtered, and the filtrate was diluted with water (50 mL) and extracted with EtOAc (5 x 150 mL). The combined organic layers were dried (magnesium sulfate), filtered, and concentrated *in vacuo*; trituration of the residue with EtOAc (100 mL) provided **S7** as a white solid (4.60 g, 46%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.61 (s, 1H), 7.41 (AB quartet, J_{AB} = 8.1 Hz, $\Delta\nu_{AB}$ =40.2 Hz, 4H), 4.70 (br s, 2H), 3.95 (s, 3H).

4-(4-(Chloromethyl)phenyl)-1-methyl-1H-pyrazole, hydrochloride salt (S8): Thionyl chloride (7.77 g, 65.3 mmol) was added dropwise to a mixture of **S7** (4.10 g, 21.8 mmol) in chloroform (150 mL) at 25 °C. The reaction mixture was stirred at 25 °C for 1.25 hours, whereupon it was combined with another similar crude reaction mixture derived from **S7** (2.78 g, 14.8 mmol) and concentrated *in vacuo*. The residue was triturated with EtOAc (150 mL) to afford **S8** as a white solid (8.1 g, 90%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H), 8.43 (s, 1H), 7.56 (AB quartet, J_{AB} = 8.3 Hz, $\Delta\nu_{AB}$ = 63.8 Hz, 4H), 4.66 (s, 2H), 4.11 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 137.8, 132.4, 131.9, 129.5, 129.2, 125.7, 123.8, 45.02, 37.4; LCMS *m/z* $[M+H]^+$ calcd for $C_{11}H_{11}ClN_2$, 207.06; found: 206.8.

Final analogs:

6-(4-(1*H*-Pyrazol-1-yl)benzyl)-2-((3*R*,4*S*)-3-hydroxytetrahydro-2*H*-pyran-4-yl)-5-methylisoindolin-1-one (11a): A mixture of **20** (1.716 g, 4.599 mmol), 1-(4-(bromomethyl)phenyl)-1*H*-pyrazole (1.64 g, 6.90 mmol), Pd(dppf)Cl_2 (336 mg, 0.46 mmol), K_2CO_3 (1.27 g, 9.20 mmol) in 1,4-dioxane (20 mL) and water (2 mL) was degassed with N_2 for 5 min, sealed, and heated to 100 °C for 20h. The mixture was filtered through diatomaceous earth and the filter cake washed with CH_2Cl_2. The filtrate was concentrated *in vacuo* to give a residue that was purified by silica gel chromatography (eluent: EtOAc/petroleum ether 0:1 to 1:0) to give **11a (**680 mg, 37%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 2.5 Hz, 1H), 7.71 (d, *J* = 1.5 Hz, 1H), 7.64-7.55 (m, 3H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.49-6.40 (m, 1H), 4.50-4.41 (m, 1H), 4.41-4.26 (m, 2H), 4.15 (dd, *J* = 5.0, 11.0 Hz, 1H), 4.10-4.00 (m, 3H), 3.81 (dt, *J* = 5.0, 10.0 Hz, 1H), 3.53 (dt, *J* = 2.8, 11.2 Hz, 1H), 3.33-3.21 (m, 1H), 2.32 (s, 3H), 2.02-1.88 (m, 2H), 1.62 (br. s., 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 141.2, 141.00, 140.06, 139.1, 138.6, 137.9, 130.5, 129.6, 126.8, 124.8, 124.6, 119.4, 107.6, 72.1, 68.9, 66.9, 55.6, 46.4, 39.1, 30.0, 20.5; LCMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}N_3O_3$, 404.19; found: 404.0.

2-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-5-methyl-6-(4-(thiazol-4-yl)benzyl)isoindolin-1-one (11b): To a mixture of **20** (802 mg, 2.15 mmol) in toluene (15 mL), 1,4-dioxane (15 mL) and water (1.5 mL) were added 4-(4-(bromomethyl)phenyl)thiazole (1) (451 mg, 2.15 mmol), Pd(dppf)Cl_2 (157 mg, 0.215 mmol), and K_2CO_3 (891 mg, 6.45 mmol). The mixture was stirred at 80 °C for 15 h under N_2. The reaction was concentrated *in vacuo*, and the residue was purified by silica gel chromatography (eluent: (CH_2Cl_2/MeOH 1:0 to 94:6) to give **19b** as a brown oil. The oil was triturated with MTBE (8 mL) and concentrated to give **11b** as a brown solid (370 mg, 41%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.62 (s, 1H), 7.50 (s, 1H), 7.24 (s, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 4.48-4.28 (m, 3H), 4.21-4.12 (m, 1H), 4.12-3.97 (m, 3H), 3.89-3.68 (m, 1H), 3.53 (dt, *J* = 3.3, 11.4 Hz, 1H), 3.39 (d, *J* = 6.0 Hz, 1H), 3.28 (t, *J* = 10.5 Hz, 1H), 2.32 (s, 3H), 2.01-1.85 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 155.5, 154.9, 140.8, 140.6, 140.4, 139.4, 132.5, 131.4, 129.6, 126.7, 125.2, 123.9, 114.2, 72.0, 66.7, 66.5, 55.3, 46.2, 38.7, 30.2, 20.3; LCMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}N_2O_3S$, 421.15; found: 421.2.

General Procedure A: A 3.0 M aqueous solution of Cs_2CO_3 (3.0 equiv.) was added to a mixture of benzyl bromide (1.2 equiv.) and **20** (1.0 equiv.) in anhydrous 1,4-dioxane (0.05 M) at room temperature. $Pd(PPh_3)_4$ (10 mol%) was added and the mixture was heated to 80 °C for 2 h whereupon it was cooled to room temperature, quenched with water and extracted with EtOAc. The combined organic extracts were dried ($MgSO_4$), filtered, then concentrated *in vacuo*.

2-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-5-methyl-6-(4-(2-methyloxazol-4-yl)benzyl)isoindolin-1-one (11c): Following general procedure A with **S5** (196 mg, 0.80 mmol), **11c** was obtained as an orange oil. The residue was purified by silica gel chromatography (eluent: CH_2Cl_2/MeOH 1:0 to 95:5) to afford **11c** as a yellow solid (100 mg, 36%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.40 (s, 1H), 7.44 (s, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 5.08 (d, *J* = 5.7 Hz, 1H), 4.47-4.36 (m, 2H), 4.07 (s, 2H), 4.05-3.96 (m, 1H), 3.86 (dt, *J* = 4.9, 11.8 Hz, 2H), 3.74-3.63 (m, 1H), 3.38 (dt, *J* = 2.4, 12.0 Hz, 1H), 3.07 (t, *J* = 10.5 Hz, 1H), 2.44 (s, 3H), 2.31 (s, 3H), 1.88-1.76 (m, 1H), 1.69-1.61 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 161.8, 140.8, 140.6, 140.1, 140.0, 139.4, 134.8, 131.4, 129.5, 129.4, 125.7, 125.2, 123.9, 72.0, 66.7, 66.5, 55.3, 46.2, 38.7, 30.2, 20.34, 14.0; LCMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{26}N_2O_4$, 419.19; found: 419.5.

2-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-5-methyl-6-(4-(1-methyl-1*H*-pyrazol-4-yl)benzyl)isoindolin-1-one (11e): Following general procedure A with **S8** (195 mg, 0.80 mmol), **11e** was obtained as a yellow residue. The residue was purified by silica gel chromatography (eluent: CH_2Cl_2/MeOH 1:0 to19:1) to afford **11e** as a yellow

solid that was recrystallized from EtOAc/Heptanes to give a white solid (100 mg, 36%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.06 (s, 1H), 7.79 (s, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 5.07 (d, *J* = 5.7 Hz, 1H), 4.47-4.34 (m, 2H), 4.07-3.94 (m, 3H), 3.92-3.81 (m, 5H), 3.76-3.63 (m, 1H), 3.38 (dt, *J* = 1.7, 11.7 Hz, 1H), 3.07 (t, *J* = 10.5 Hz, 1H), 2.32 (s, 3H), 1.89-1.81 (m, 1H), 1.69-1.59 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 168.2, 140.7, 140.5, 139.7, 138.1, 136.4, 131.4, 130.9, 129.6, 128.0, 125.6, 125.1, 123.8, 122.2, 72.0, 66.7, 66.5, 55.3, 46.2, 39.1, 38.6, 30.2, 20.4; LCMS *m/z* [M+H]$^+$ calcd for $C_{25}H_{27}N_3O_3$, 418.21; found: 418.5.

General Procedure B: A 3.0 M aqueous solution of Cs_2CO_3 (3.0 equiv.) was added to a mixture of benzyl bromide (1.0 equiv.) and **21** (1.0 equiv.) in anhydrous THF (0.18 M) at room temperature. Pd(t-Bu$_3$P)$_2$ (20 mol%) was added and the mixture was heated to 60 ^{o}C for 3 h whereupon it was cooled to room temperature, quenched with water and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, then concentrated *in vacuo*.

7-(4-(1*H*-Pyrazol-1-yl)benzyl)-2-((3*R*,4*S*)-3-hydroxytetrahydro-2*H*-pyran-4-yl)-6-methyl-3,4-dihydroisoquinolin-1(2*H*)-one (12a): Following general procedure B with 1-(4-(bromomethyl)phenyl)-1*H*-pyrazole (31 mg, 0.13 mmol), **12a** was obtained as a yellow residue. The residue was dissolved in dimethyl sulfoxide (1 mL) and purified by reversed-phase HPLC (Column: Waters Sunfire C18 19x100, 5u. Mobile phase A: 0.05% TFA in water (v/v); Mobile phase B: 0.05% TFA in MeCN (v/v). Gradient: 70.0% H_2O/30.0% MeCN linear to 30% H_2O/70% MeCN in 8.5min, 30% H_2O/70% MeCN linear to 0% H_2O/100% MeCN in 0.5min, HOLD at 0% H_2O/100% MeCN to 10.0min. Flow: 25mL/min.) to give **12a** as an off-white solid (5.6 mg, 10%): ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 8.37 (d, *J* = 2.6 Hz, 1 H), 7.70 (d, *J* = 8.2 Hz, 1 H), 7.67 (d, *J* = 1.5 Hz, 1 H), 7.62 (s, 1 H), 7.21 (d, *J* = 8.5 Hz, 2 H), 7.06 (s, 1 H), 6.48 (t, *J* = 2.1 Hz, 1 H), 4.90 (d, *J* = 5.6 Hz, 1 H), 4.38 (ddd, *J* = 12.3, 10.3, 4.1 Hz, 1 H), 4.00 (s, 2 H), 3.82 (td, *J* = 11.6, 5.0 Hz, 1 H), 3.60 (tt, *J* = 10.0, 5.3 Hz, 1 H), 3.43 (t, *J* = 6.5 Hz, 1 H,) 3.00 (t, *J* = 10.3 Hz, 1 H), 2.89 (m, *J* = 6.0, 6.0 Hz, 1 H), 2.77-2.84 (m, 1 H), 2.51 (s, 3 H), 2.48-2.56 (m, 2 H), 2.23 (2, 2 H), 1.75 (qd, *J* = 12.6, 4.6 Hz, 1 H), 1.51-1.56 (m, 1 H); LCMS *m/z* [M+H]$^+$ calcd for $C_{25}H_{27}N_3O_3$, 418.21; found: 418.5.

2-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-6-methyl-7-(4-(thiazol-4-yl)benzyl)-3,4-dihydroisoquinolin-1(2*H*)-one (12b): Following general procedure B with 4-(4-(bromomethyl)phenyl)thiazole (1) (130 mg, 0.52 mmol), **12b** was obtained as a brown residue. The residue was purified by silica gel chromatography (eluent: heptanes/EtOAc 7:3 to 0:1) to give **12b** as an off-white solid(111 mg, 50%): ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.87 (dd, *J* = 0.8, 2.0 Hz, 1H), 7.89 (s, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.48 (dd, *J* = 0.8, 2.0 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 6.98 (s, 1H), 4.75 (ddd, *J* = 4.3, 10.3, 12.0 Hz, 1H), 4.14 (dd, *J* = 4.9, 11.2 Hz, 1H), 4.04-3.98 (m, 3H), 3.72 (dt, *J* = 5.1, 10.2 Hz, 1H), 3.60-3.46 (m, 3H), 3.24 (t, *J* = 10.6 Hz, 1H), 3.03-2.87 (m, 2H), 2.24 (s, 3H), 1.91 (dq, *J* = 4.7, 12.4 Hz, 2H), 1.80-1.73 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.7, 156.3, 152.8, 141.5, 140.1, 137.8, 136.1, 132.2, 130.0, 129.1, 129.0, 127.2, 126.6, 112.1, 72.4, 67.7, 67.0, 56.8, 40.6, 39.2, 29.0, 27.9, 19.9; LCMS *m/z* [M+H]$^+$]$^+$ calcd for $C_{25}H_{26}N_2O_3S$, 435.17; found: 435.4.

2-((3*R*,4*S*)-3-Hydroxytetrahydro-2*H*-pyran-4-yl)-6-methyl-7-(4-(2-methyloxazol-4-yl)benzyl)-3,4-dihydroisoquinolin-1(2*H*)-one (12c): Following general procedure B with **S5** (120 mg, 0.49 mmol), the reaction mixture was heated to 60 ^{o}C for 18 h and **12c** was obtained as a brown oil. The residue was purified by silica gel chromatography (eluent: heptanes/EtOAc 7:3 To 0:1) to give **12c** as a white solid(91 mg, 43%): ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.82 (s, 1H), 7.75 (s, 1H), 7.61-7.54 (m, 2H), 7.17-7.10 (m, 2H), 6.92 (s, 1H), 4.73 (ddd, *J* = 4.3, 10.3, 12.0 Hz, 1H), 4.19-4.09 (m, 1H), 4.03-3.94 (m, 3H), 3.73 (dt, *J* = 5.1, 10.0 Hz, 1H), 3.60-3.39 (m, 3H), 3.30-3.18 (m, 2H), 3.02-2.79 (m, 2H), 2.50 (s, 3H), 2.20 (s, 3H), 1.87 (dq, *J* = 4.9, 12.3 Hz, 1H), 1.75 (td, *J*= 2.1, 12.7 Hz, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.6, 161.8, 141.4, 140.5, 139.7, 137.7, 136.1, 132.9, 129.9, 129.04, 128.96, 128.90, 127.1, 125.5, 72.4, 67.5, 67.0, 56.8, 40.6, 39.2, 29.0, 27.9, 19.8, 14.0; LCMS *m/z* [M+H]$^+$ calcd for $C_{26}H_{28}N_2O_4$, 433.20; found: 433.5.

General Procedure C (library protocol): K_2CO_3 (33.1 mg, 0.241 mmol) was added to a mixture of benzyl halide (0.096 mmol) and **17** (30 mg, 0.080 mmol) in dioxane (0.60 mL) and water (0.120 mL) at room temperature. Pd(dppf)Cl$_2$ (5.6 mg, 0.008 mmol) was added and the mixture was heated to 80 ^{o}C for 16 h whereupon it was cooled to room temperature, concentrated *in vacuo*, and purified via prep HPLC to give the final products.

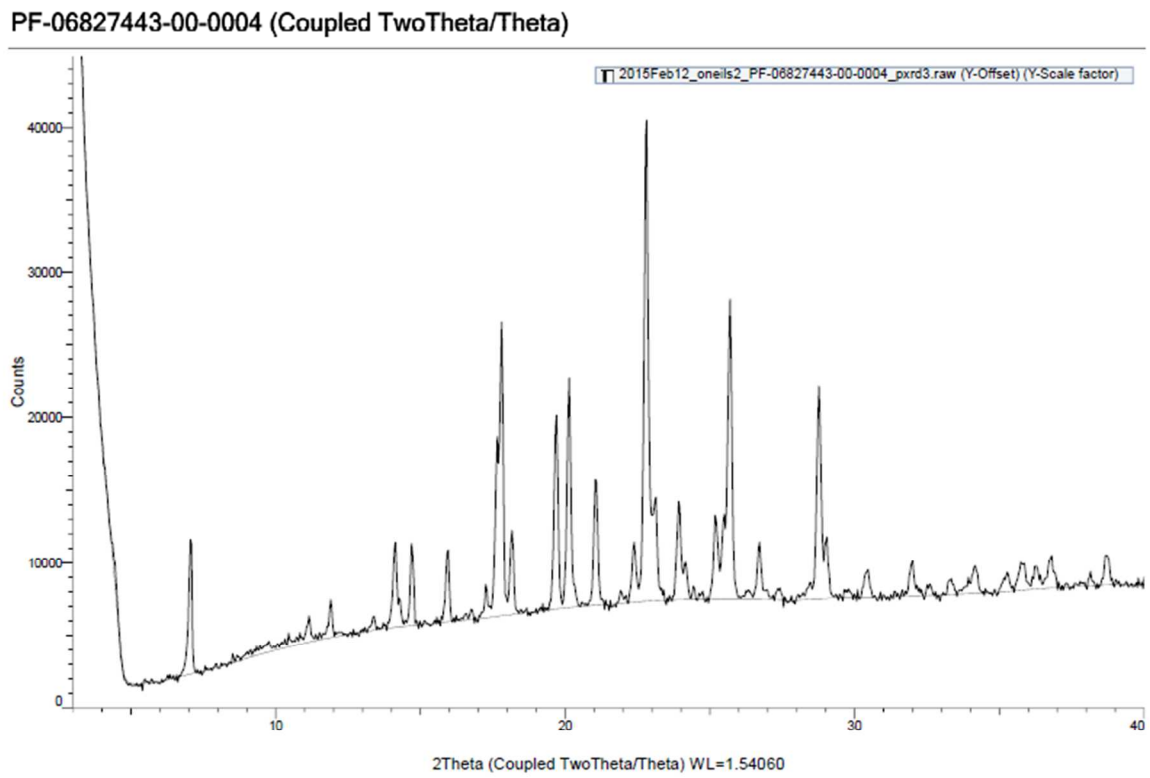
6-(4-(1H-imidazol-1-yl)benzyl)-2-((3S,4R)-3-hydroxytetrahydro-2H-pyran-4-yl)-5-methylisoindolin-1-one (11f): Following general procedure C with 1-(4-(chloromethyl)phenyl)-1*H*-imidazole (18.5 mg, 0.096 mmol), **11f**

was obtained and purified via prep-HPLC (Column: Phenomenex Gemini C18 21.2 x 250 mm, 10u. Mobile phase A: 0.225% formic acid in water (v/v); Mobile phase B: MeCN. Gradient: 1% B to 41%B over 8 min, Flow: 35 mL/min). LCMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}N_3O_3$, 403.19; found: 404 at rt = 2.063 min.

2-((3S,4R)-3-hydroxytetrahydro-2H-pyran-4-yl)-5-methyl-6-(4-(4-methyl-1H-imidazol-1-yl)benzyl)isoindolin-1-one (11g): Following general procedure C with 1-(4-(chloromethyl)phenyl)-4-methyl-1H-imidazole (3) (18.5 mg, 0.096 mmol), **11g** was obtained and purified via prep-HPLC. (Column: Phenomenex Luna C18 25 x 150 mm, 5u. Mobile phase A: 0.225% formic acid in water (v/v); Mobile phase B: MeCN. Gradient: 30% B to 60%B over 10 min, Flow: 35 mL/min). LCMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{27}N_3O_3$, 417.21; found: 418 at rt = 2.128 min.

Additional characterization on compound 1:

PXRD:



Thermodynamic solubility study:

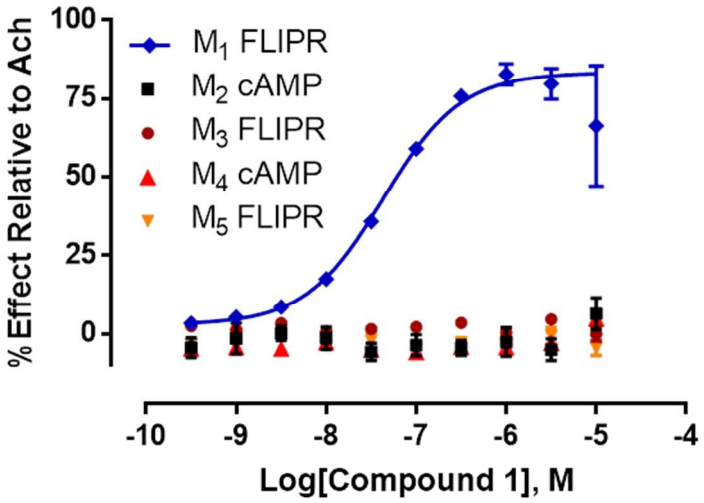
Compound	Lot#	Solubility (mg/mL)			
		SGN	50mM phosphate buffer	Fassif	Fessif
PF-06827443	PF-06827443-00-0004	0.003 pH=1.4	0.0013 pH=6.49	0.0048 pH=6.11	0.0397 pH=6.13

Molecular Formula Strings

Compound Number	SMILES
1	Cc1cc2c(cc1Cc3ccc(cc3)c4cocn4)C(=O)N(C2)[C@H]5CCOC[C@@H]5O
11a	Cc1cc2c(cc1Cc3ccc(cc3)n4cccn4)C(=O)N(C2)[C@H]5CCOC[C@@H]5O
11b	Cc1cc2c(cc1Cc3ccc(cc3)c4cscn4)C(=O)N(C2)[C@H]5CCOC[C@@H]5O
11c	Cc1cc2c(cc1Cc3ccc(cc3)c4coc(n4)C)C(=O)N(C2)[C@H]5CCOC[C@@H]5O
11e	Cc1cc2c(cc1Cc3ccc(cc3)c4cnn(c4)C)C(=O)N(C2)[C@H]5CCOC[C@@H]5O
11f	Cc1cc2c(cc1Cc3ccc(cc3)n4ccnc4)C(=O)N(C2)[C@@H]5CCOC[C@H]5O
11g	Cc1cc2c(cc1Cc3ccc(cc3)n4cc(nc4)C)C(=O)N(C2)[C@@H]5CCOC[C@H]5O
12a	Cc1cc2c(cc1Cc3ccc(cc3)n4cccn4)C(=O)N(CC2)[C@H]5CCOC[C@@H]5O
12b	Cc1cc2c(cc1Cc3ccc(cc3)c4cscn4)C(=O)N(CC2)[C@H]5CCOC[C@@H]5O
12c	Cc1cc2c(cc1Cc3ccc(cc3)c4coc(n4)C)C(=O)N(CC2)[C@H]5CCOC[C@@H]5O
12d	Cc1cc2c(cc1Cc3ccc(cc3)c4cocn4)C(=O)N(CC2)[C@H]5CCOC[C@@H]5O

Composite M_1-M_5 PAM functional dose response curves for compound 1 in the presence of EC_{20} of ACh: $M_{1,3,5}$ used a FLIPR assay while $M_{2,4}$ used a CAMP readout

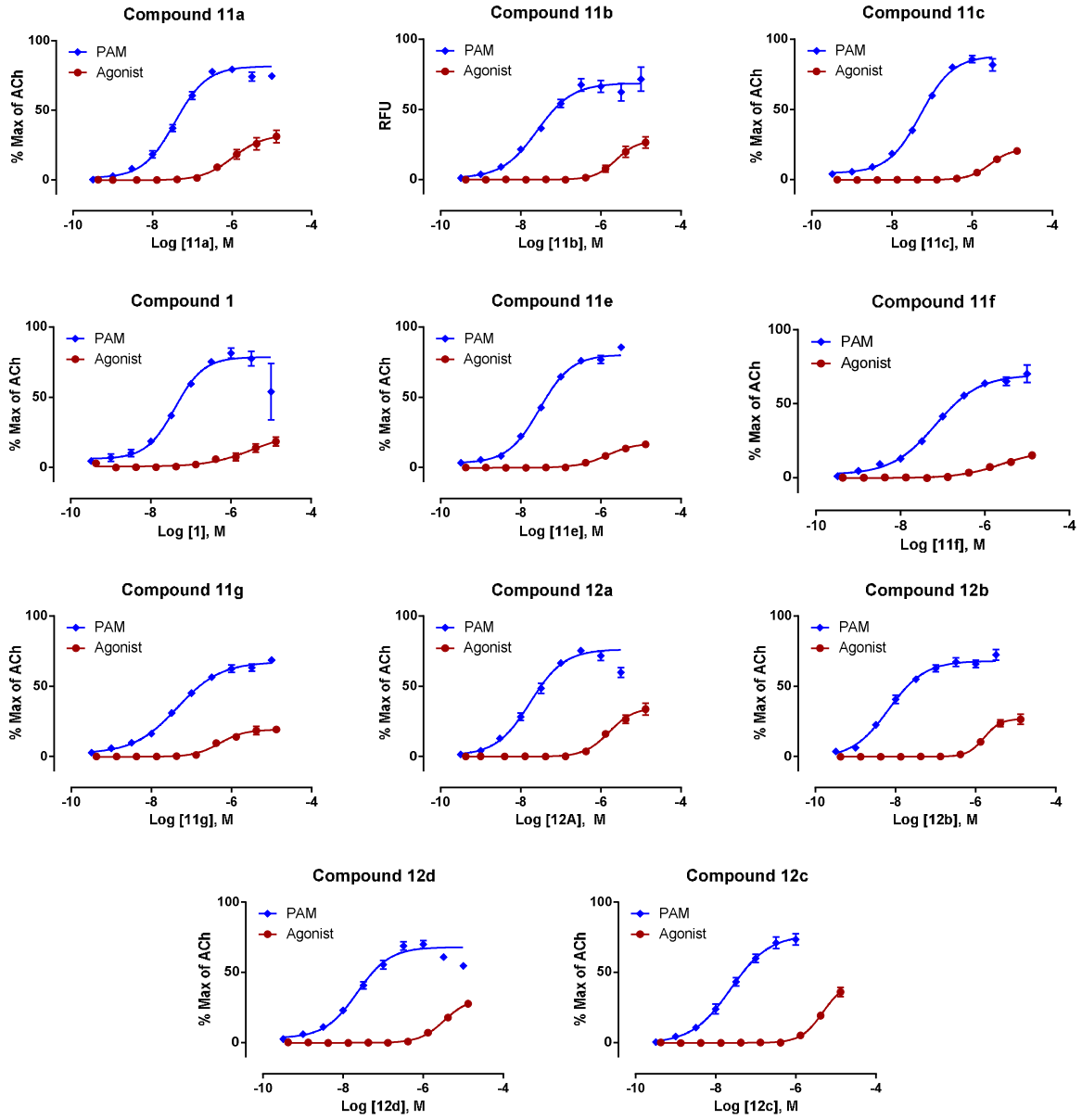
Methods were previously published.[1]



[1] 1. Davoren, J. E.; Lee, C. W.; Garnsey, M.; Brodney, M. A.; Cordes, J.; Dlugolenski, K.; Edgerton, J. R.; Harris, A. R.; Helal, C. J.; Jenkinson, S.; Kauffman, G. W.; Kenakin, T. P.; Lazzaro, J. T.; Lotarski, S. M.; Mao, Y.; Nason, D. M.; Northcott, C.; Nottebaum, L.; O'Neil, S. V.; Pettersen, B.; Popiolek, M.; Reinhart, V.; Salomon-Ferrer, R.; Steyn, S. J.; Webb, D.; Zhang, L.; Grimwood, S., Discovery of the Potent and Selective M1 PAM-Agonist N-[(3R,4S)-3-Hydroxytetrahydro-2H-pyran-4-yl]-5-methyl-4-[4-(1,3-thiazol-4-yl)ben zyl]pyridine-2-carboxamide (PF-06767832): Evaluation of Efficacy and Cholinergic Side Effects. *J. Med. Chem.* **2016,** *59,* 6313-6328.

Functional dose response curves for 1 in the presence (PAM mode, blue diamond) and absence (Agonist mode, red circle) of an EC_{20} of ACh in M_1 FLIPR for compounds in table 1 of main manuscript

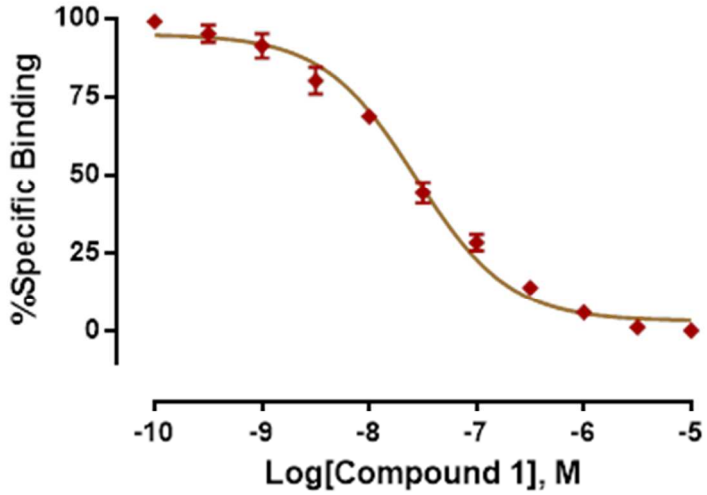
Methods were previously published.[2]



[2] 1. Davoren, J. E.; Lee, C. W.; Garnsey, M.; Brodney, M. A.; Cordes, J.; Dlugolenski, K.; Edgerton, J. R.; Harris, A. R.; Helal, C. J.; Jenkinson, S.; Kauffman, G. W.; Kenakin, T. P.; Lazzaro, J. T.; Lotarski, S. M.; Mao, Y.; Nason, D. M.; Northcott, C.; Nottebaum, L.; O'Neil, S. V.; Pettersen, B.; Popiolek, M.; Reinhart, V.; Salomon-Ferrer, R.; Steyn, S. J.; Webb, D.; Zhang, L.; Grimwood, S., Discovery of the Potent and Selective M1 PAM-Agonist N-[(3R,4S)-3-Hydroxytetrahydro-2H-pyran-4-yl]-5-methyl-4-[4-(1,3-thiazol-4-yl)ben zyl]pyridine-2-carboxamide (PF-06767832): Evaluation of Efficacy and Cholinergic Side Effects. *J. Med. Chem.* **2016,** *59,* 6313-6328.

M_1 binding as measured by inhibition of the M_1 PAM radioligand [^{3}H]PT-1284

Methods were previously published.[3]



Hippocampal Slice Assay Methods

Slice preparation. Adult (8-12 weeks) male Sprague Dawley rats were deeply anesthetized with isofluorane and perfused transcardially with ice-cold high-sucrose artificial cerebrospinal (ACSF) cutting solution containing 206 mM sucrose, 26 mM NaHCO3, 3 mM KCl, 1.25 mM NaH2PO4, 7 mM MgCl2, 0.5 mM CaCl2, 10 mM glucose, 1 mM sodium pyruvate and 0.89 mM L-ascorbate, bubbled with 95% O2 / 5% CO2. Brains were removed into ice cold cutting ACSF, and coronal hippocampal slices were made (300 µM) using a vibrating microtome (Leica VT1000S or 1200S). Slices were incubated at 35 degrees C in recording ACSF (124 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 10 mM Glucose, 1.3 mM MgCl2, 2 mM CaCl2, 1 mM sodium pyruvate, 0.89 mM L-ascorbate) bubbled with 95% O2 / 5% CO2 for at least 1 hour prior to recording.

Extracellular recordings. Slices were placed onto MED-P515A 64-channel multi-electrode arrays (MED64 system, Automate Scientific, Inc.) with the CA1 pyramidal cell layer positioned directly over the array contacts, and held down with a U-shaped platinum horseshoe over a square of nylon mesh. Slices were perfused continuously with recirculating ACSF warmed to 30-32 °C bubbled with 95% O2 / 5% CO2. Electrophysiological signals were high-pass filtered at 0.1 Hz and digitized at 20 kHz using MED64 Mobius acquisition software (WitWerx, Inc). Compound was added stepwise in serially increasing concentrations, with each step lasting 20 min. For assessing PAM activity, compound was applied in the continuous presence of an EC20 concentration of 100 nM carbachol, an orthosteric agonist at cholinergic receptors ("PAM mode").

Data analysis. Extracellular action potentials were detected offline using custom-written Matlab scripts. CA1 spiking activity was measured as the multi-unit firing rate for each electrode contacting the stratum pyramidale. Rates were computed for each channel within a slice, then averaged across channels to generate a single median activity rate for the slice. The median activity during the last 10 min of each step was taken as the activity level for that concentration. For each slice, the effect of the concentration was measured as the median firing rate in the presence of the compound minus the median firing rate under the initial baseline conditions (compound alone for AGO mode, in 100 nM carbachol for PAM mode). Statistical comparisons were done by considering each slice to be an independent N: mean firing rates across slices (n = 4) and SEM are reported.

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[3] Smith, D. L.; Davoren, J. E.; Edgerton, J. R.; Lazzaro, J. T.; Lee, C. W.; Neal, S.; Zhang, L.; Grimwood, S., Characterization of a Novel M1 Muscarinic Acetylcholine Receptor Positive Allosteric Modulator Radioligand, [3H]PT-1284. *Mol. Pharmacol.* **2016,** *90,* 177-187.

In-Vivo IP1 Accumulation Assay Raw Data for Compound 1

Methods for this assay were previously published.[4] Exposure for striatal portion of each animal brain, IP1 accumulation and dose:

$C_{b,u}$ (nM)	IP1 Fold Increase over Baseline	Dose
12.0	1.8	
13.0	1.4	
12.0	1.8	1 mg/kg
15.0	1.2	
19.0	1.9	
50.0	5.2	
37.0	3.3	
50.0	2.1	3 mg/kg
51.0	5.3	
40.0	4.9	
105.0	30.3	
89.0	12.5	
76.0	5.6	10 mg/kg
139.0	39.2	
98.0	3.1	

[4] Popiolek, M.; Nguyen, D. P.; Reinhart, V.; Edgerton, J. R.; Harms, J.; Lotarski, S. M.; Steyn, S. J.; Davoren, J. E.; Grimwood, S., Inositol Phosphate Accumulation in Vivo Provides a Measure of Muscarinic M1 Receptor Activation. *Biochemistry* **2016,** *55,* 7073-7085.

In-Vivo IP1 Accumulation Assay Raw Data for Compound 5

Exposures were estimated per dose group using PK from a satellite group as described[5].

	Study A				Study B		
Dose	3.2 mg/kg	10 mg/kg	32 mg/kg		3.2 mg/kg	10 mg/kg	32 mg/kg
Fold IP1 Over Baseline	1.1	3.7	14.6		2.4		40.8
	0.6	4.9	11.5		0.8	5.9	40.6
	1.8	3.6	12.0		3.3	22.0	25.4
	1.1	4.5	9.4		4.3	17.7	28.1
	1.4	1.3	13.1		3.1	11.9	18.2
average	1.2	3.6	12.1		2.8	14.4	30.6
sd	0.4	1.4	1.9		1.3	7.0	9.9
sem	0.2	0.6	0.9		0.6	3.1	4.4

Locomotor Activity Data for Compound 1

Animals and Equipment

Male C57BL/6J mice at 6-8 weeks of age were delivered from Jackson Laboratory (Bar Harbor, ME) were used for spontaneous and amphetamine-stimulated locomotor studies. Mice were group housed on Innovive IVC racks (San Diego, CA) in an environmentally controlled room under normal 6AM/6PM light/dark cycle conditions with food and water ad libitum. Mice were acclimated to the facility for at least 1 week prior to testing. All testing was performed in Omnitech Open Field monitors running Fusion Software (Omnitech USA; Columbus, OH). Each monitor was enclosed in a sound attenuating box and lights (40 lux) were on for testing. Each monitor consists of sets of 16 infrared (IR) light beams along the horizontal x and y axes to detect animal movement. Data were recorded in 10 min bins. For the locomotor experiment Compound 1 was dissolved in 10% Cremophor/Sterile water; haloperidol in 0.3% Tartaric acid, and amphetamine (corrected for active moiety) dissolved in saline.

Locomotor Data Statistical Analysis

Distance traveled (cm) was recorded in 10 min bins and summed to reflect total distance traveled for the 90 minute test session. Data were analyzed using R 3.0.1 statistical software[6]. The effects of treatment and time and their interaction on distance traveled were assessed using a two-way repeated measure analysis of variance (RM ANOVA) using generalized least squares methods from nonlinear mixed effects models (NLME) library[7]. To account for correlations within subject a first order autoregressive scheme was employed, which assumed that

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[5] Popiolek, M.; Nguyen, D. P.; Reinhart, V.; Edgerton, J. R.; Harms, J.; Lotarski, S. M.; Steyn, S. J.; Davoren, J. E.; Grimwood, S., Inositol Phosphate Accumulation in Vivo Provides a Measure of Muscarinic M1 Receptor Activation. *Biochemistry* **2016,** *55,* 7073-7085.

[6] R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
http://www.R-project.org/

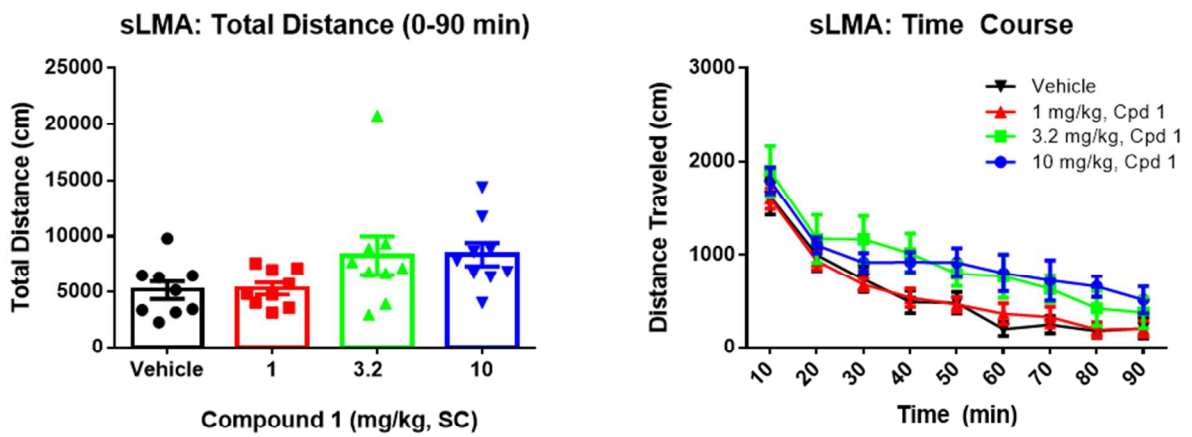
[7] Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2014). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.0.1,
http://CRAN.R-project.org/package=nlme

correlations decay exponentially with the lab between measurements. Time dependence of the variance was also allowed. The model was fitted using the method of restricted maximum likelihood. Significant analysis of variance (ANOVA) results were followed by post-hoc pairwise comparisons of least squared means across treatment arms, separately at each time point[8]. In order to adjust for multiple hypothesis testing a false discovery rate (FDR) method was used which controls the expected proportion of false discoveries amongst the rejected hypotheses[9]. Data were considered statistically significant at a level of p-value (p) <0.05. Longitudinal data were aggregated within each animal over time to yield total distance traveled . A one-way ANOVA was conducted on total distance traveled data with treatment as fixed factor. Bartlett's tests were used to access homogeneity of variances across groups and Shapiro-Wilk test to test normality assumption. Significant ANOVA findings were followed by post-hoc pairwise comparisons of least squared means across treatment arms, with FDR correction for multiple tests. Findings were considered statistically significant at a level of p-value (p) <0.05.

Spontaneous Locomotor Activity (sLMA)

On the test day, mice (n=9/group) were acclimated to the procedure room for 1 h, weighed and dosed with compound 1 (1, 3.2 and 10 mg/kg, SC) or vehicle, then returned to their home cage. Thirty minutes later mice were individually placed in a test chamber for 90 min. Data are shown as mean total distance (cm). There were no statistically significant effects observed but a trend towards increased activity was seen at 3.2 and 10 mg/kg (Fig Xa and b).

Figure: Spontaneous Locomotor Activity Total Distance (A) and Time Course (B)



Amphetamine-Stimulated Locomotor Activity (aLMA)

On the test day, mice (n=10/group) were acclimated t the procedure room for one h prior to testing. Mice were weighed and individually placed in the test chamber for a 1 h habituation period . Next, mice were removed and dosed with compound 1 (0.32, 1 and 3.2 mg/kg, SC), haloperidol (0.1 mg/kg, SC) or vehicle and returned to the chamber for 30 min. Finally, mice were removed and dosed with amphetamine (1.78 mg/kg, IP) or vehicle and returned to the chamber for the 90 min test session. Compound 1 significantly attenuated the hyperactivity induced by amphetamine in C57BL/6J mice at doses that had no significant effects on spontaneous locmotor activity.

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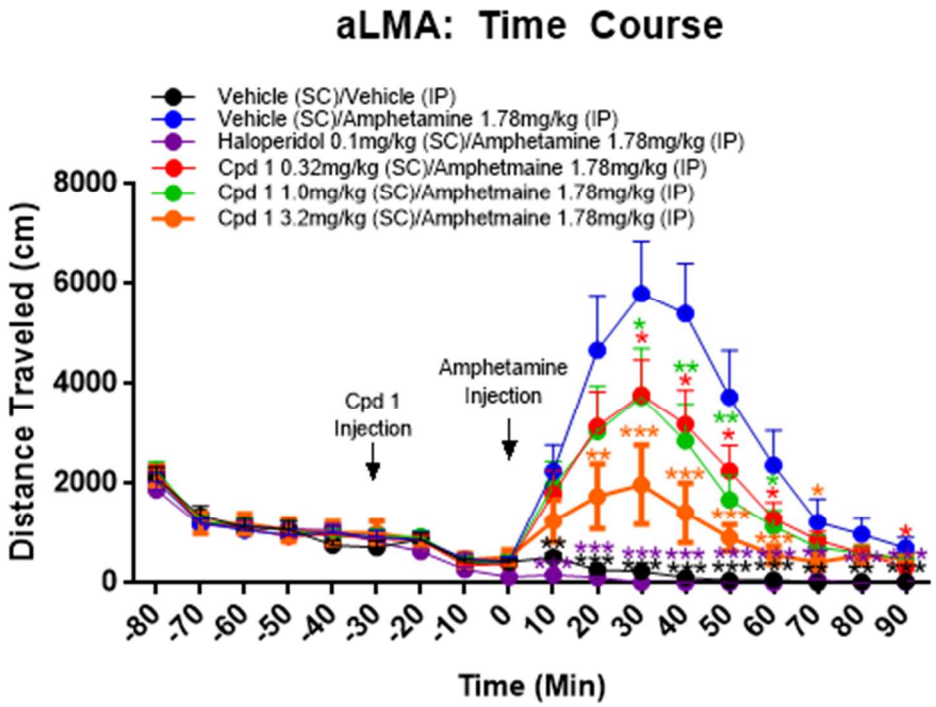
[8] Lenth R.V. (2014). lsmeans: Least-Squares means. R package version 2.11, http://CRAN.R-project.org/package=lsmeans

[9] Benjamini, Y., and Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. Annals of Statistics 29, 1165-1188.

Locomotor Figure: Amphetamine-Stimulated Locomotor Activity Time Course



Scopolamine-Disrupted Morris Water Maze (MWM)

Male Wistar rats (Sino-British SIPPR/BK Lab, Shanghai, China) were acclimated to the facility in HDBiosciences (Shanghai, China) 1 week prior to testing. A circular tank (1.5 m diameter, 60 cm h) was filled with 21-22 ^{o}C water up to 40 cm depth and made opaque with nontoxic black paint. The tank was surrounded by a black curtain to reduce external distractions and large visual cues were hung. Rats (200-220g) were subjected to a visible platform (15 cm diameter; 1 cm above the water) pretest. For training (acquisition) days, the platform was submerged just below the surface of the water. For each individual rat the location of the platform was fixed throughout the 5 training days. For each group of rats, however, the location of platform covered all four quadrants in a counterbalance manner and with the same pattern across groups. Rats received 4 trials each day with a 1h inter-trial interval (ITI). Each trial was a maximum of 60 sec and rats were allowed to remain on the platform for 10 sec once they located it, those that failed to locate the platform were gently guided to it. The release location was randomized daily across all 4 quadrants. The time to locate the platform (escape latency), distance to the platform (path length) and swim speed were automatically recorded by the video-tracking system and data analyzed using R 3.0.1 as described above, where endpoint for MWM include swim distance (cm), escape latency (s) and swim speed (cm/s). . Compound 1 (0.1, 0.32 & 3.2 mg/kg, SC), donepezil (1 mg/kg, SC) or vehicle (20% HPβCD, SC) was administered daily 15 min prior to the first trial each day. Scopolamine (0.032 mg/kg, SC) or vehicle (saline, SC) was delivered 30 min prior to the first trial each day. Twenty-four h after the last training session rats were subjected to a 60 sec probe trial with the platform removed. Compound 1 significantly improved deficits induced by scopolamine with no confounding effects on swim speed in the MWM test.

Scopolamine-Disrupted Morris Water Maze Data