Supporting Information

Discovery of the first highly M5-preferring muscarinic acetylcholine receptor ligand, an M5 positive allosteric modulator derived from a series of 5-trifluoromethoxy *N*-benzyl isatins.

*Thomas M. Bridges, Joy E. Marlo, Colleen M. Niswender, Carrie K. Jones, Satyawan Jadhav, Patrick R. Gentry, Hyekyung C. Plumley, C. David Weaver, P. Jeffrey Conn, and Craig W. Lindsley**

Vanderbilt Program in Drug Discovery, Departments of Pharmacology and Chemistry, Vanderbilt University Medical Center, 1205 LH, Nashville, Tennessee, 37232-0697 USA

Contents of Supporting Information

I. Chemistry

General Experimental. All reactions were carried out employing standard chemical techniques. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. at the highest commercial quality and were used without purification. Microwave-assisted reactions were conducted using a Biotage Initiator-60. All NMR spectra were recorded on a 400 MHz Bruker AMX NMR. ^{1}H chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard in DMSO. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant (Hz), and integration. ^{13}C chemical shifts are reported in δ values in ppm with the DMSO carbon peak set to 39.5 ppm. Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra were recorded on a Waters QToF-API-US plus Acquity system with electrospray ionization. Analytical thin layer chromatography was performed on 250 μm silica gel 60 F_{254} plates. Analytical HPLC was performed on an Agilent 1200 analytical LCMS with UV detection at 214 nm and 254 nm along with ELSD detection. Preparative purification of library compounds was performed on a custom Agilent 1200 preparative LCMS with collection triggered by mass detection. All yields refer to analytically pure and fully characterized materials (^{1}H NMR, ^{13}C NMR analytical LCMS and Hi-Res MS).

General Procedure for Library Synthesis. Compounds **21-112** were synthesized in parallel according to the following procedure. Isatin starting materials (0.34 mmol) were added to vials containing ACN (3 mL), K_2CO_3 (0.68 mmol, 2.0 eq), KI (0.03 mmol, 0.1 eq), and respective benzyl halides (0.85 mmol, 2.5 eq). The reactions were microwave irradiated at 160°C for 10 min. Next, the reactions were partitioned into $CHCl_2$ and H_2O and then passed through disposable phase-separator columns (Biotage Isolute). The organics were concentrated on a heated air-drying block and then analyzed by LCMS. Purification by mass-directed Preparative HPLC afforded desired products as colored solids (20-95%) with >98% purity by ELSD and 214 nM UV analysis.

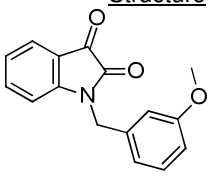
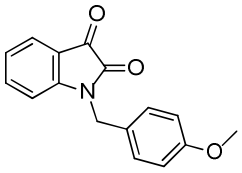
Synthesis of VU0238429 (Compound 42). To a vial containing ACN (15 mL) was added 5-trifluoromethyoxyisatin (1.00 g, 4.33 mmol), K_2CO_3 (8.66 mmol, 2.0 eq), KI (0.43 mmol, 0.1 eq), and 4-methoxybenzyl chloride (4.76 mmol, 1.1 eq). The reaction was stirred for ~24 hours at room temperature while monitoring by TLC. After judging complete, the reaction was partitioned into EtOAc and H_2O, and the combined organics were dried over $MgSO_4$, filtered, and then concentrated *in vacuo* to afford the pure 1-(4-methoxybenzyl)-5-(trifluoromethoxy)indoline-2,3-dione title compound as an orange solid (1.50 g, 4.26 mmol, 98%). ^{1}H-NMR (400MHz, d_6 - DMSO) δ 7.60 (m, 2H), 7.36 (d, J = 8.7, 2H), 7.04 (m, 1H), 6.89 (m, 2H), 4.84 (s, 2H), 3.71 (s, 3H). ^{13}C-NMR (100MHz, d_6 - DMSO) δ 181.99, 158.73, 158.33, 149.00, 143.83, 130.35, 128.88, 126.94, 121.32, 118.79, 117.66, 114.02, 112.36, 55.05, 42.45. LCMS (214 nm) 3.37 min (>98%); *m/z* 352.1 [M+H]. HRMS calcd for $C_{17}H_{12}F_3NO_4$ [M+H] 352.0797 found 352.0795.

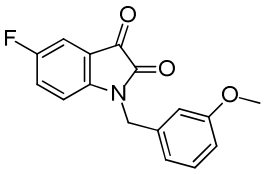
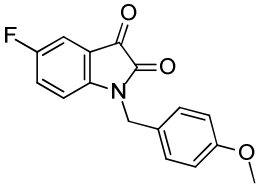
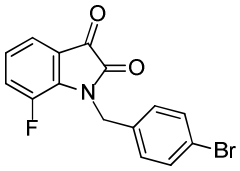
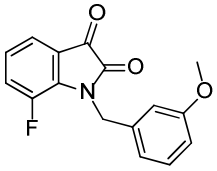
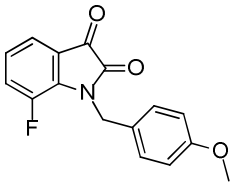
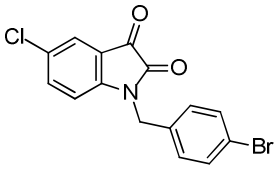
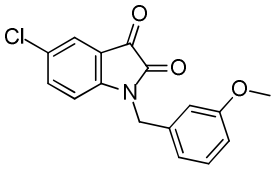
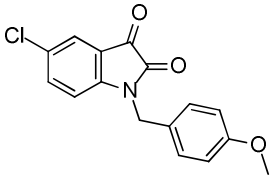
Synthesis of VU0238441 (Compound 56). To a vial containing ACN (3 mL) was added 7-chloroisatin (150 mg, 0.826 mmol), K_2CO_3 (1.652 mmol, 2.0 eq), KI (0.083 mmol, 0.1 eq), and

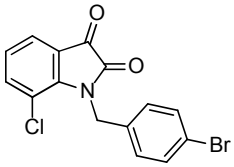
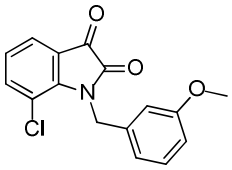
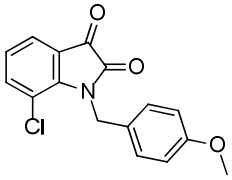
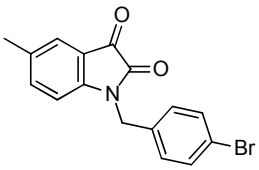
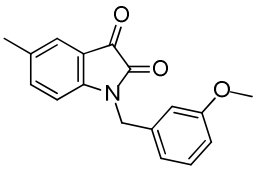
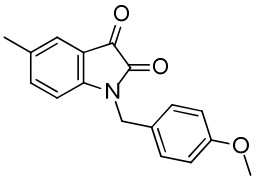
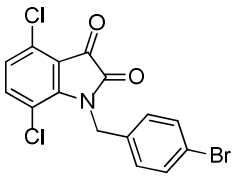
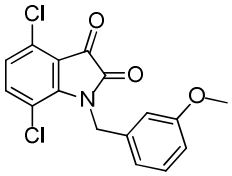
4-trifluoromethylbenzyl bromide (0.909 mmol, 1.1 eq). The reaction was stirred for ~24 hours at room temperature while monitoring by TLC. After judging complete, the reaction was partitioned into EtOAc and H_2O, and the combined organics were dried over $MgSO_4$, filtered, and then concentrated *in vacuo* to afford the pure 7-chloro-1-(4-(trifluoromethyl)benzyl)indoline-2,3-dione title compound as an orange solid (267 mg, 0.786 mmol, 95%). ^{1}H-NMR (400MHz, d_6 - DMSO) δ 7.64 (m, 4H), 7.15 (m, 3H), 5.28 (s, 2H). ^{13}C-NMR (100MHz, d_6 - DMSO) δ 181.56, 159.37, 145.27, 142.25, 139.26, 127.01, 125.30, 124.80, 123.51, 121.40, 115.58, 44.28. LCMS (214 nm) 3.46 min (>98%); *m/z* 340.0 [M+H]. HRMS cald for $C_{16}H_9ClF_3NO_2$ [M+H] 340.0352 found 340.0353.

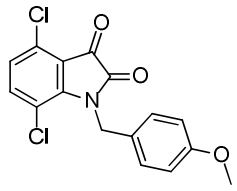
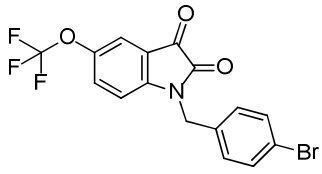
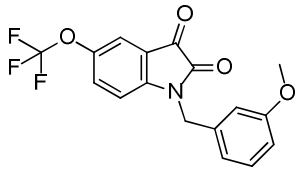
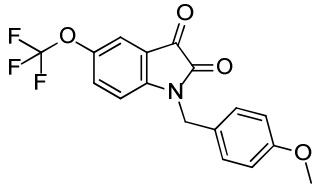
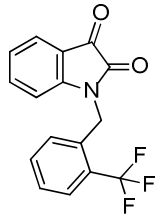
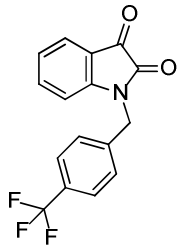
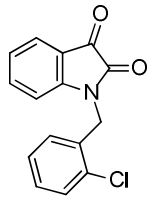
Synthesis of VU0119498 (Compound 113). This HTS screening hit was resynthesized classically as a singleton prior to the library synthesis. To a vial containing ACN (25 mL) was added isatin (1.00 g, 6.80 mmol), K_2CO_3 (13.6 mmol, 2.0 eq), KI (0.68 mmol, 0.1 eq), and 4-bromobenzylbromide (7.48 mmol, 1.1 eq). The reaction was stirred for ~24 hours at room temperature while monitored by TLC. After judging complete, the reaction was partitioned into EtOAc and H_2O, and the combined organics were dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The dried solid was then washed with diethyl ether (3 x 15 mL) to afford the pure 1-(4-bromobenzyl)indoline-2,3-dione title compound as a bright orange solid (1.96 g, 6.18 mmol, 91%). ^{1}H-NMR (400MHz, d_6 - DMSO) δ 7.55 (m, 4H), 7.39 (d, J = 8.5, 2H), 7.11 (t, J = 7.8, 1H), 6.94 (d, J = 8.1, 1H), 4.88 (s, 2H). ^{13}C-NMR (100MHz, d_6 - DMSO) δ 182.91, 158.35, 150.07, 137.87, 135.01, 131.47, 129.64, 124.46, 123.33, 120.65, 117.80, 110.95, 42.27. LCMS (214 nm) 3.25 min (>98%); *m/z* 316.0 [M+H]. HRMS cald for $C_{15}H_{10}BrNO_2$ [M+H] 315.9973 found 315.9974.

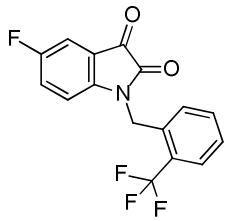
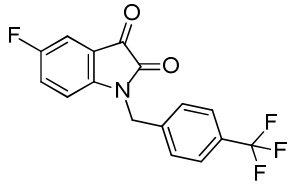
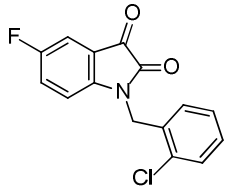
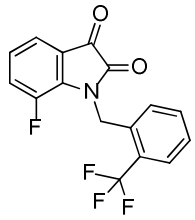
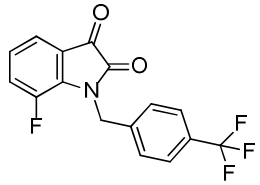
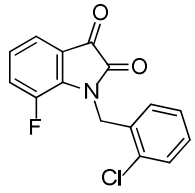
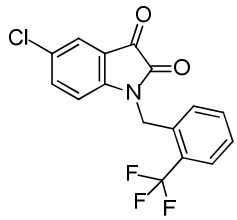
Full Library Structure-Activity Relationship Table. The structures for compounds **21-112** and associated activity data from the initial single concentration (30 μM) potentiator screen against M1 and M5 are shown below as percentage of maximum acetylcholine response (i.e. the degree of potentiation of submaximal acetylcholine). The synthetic method used to generate each compound is shown with the following abbreviations: M, microwave; C, classical. The molecular formula for each compound is also presented.

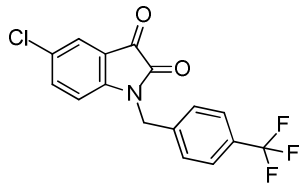
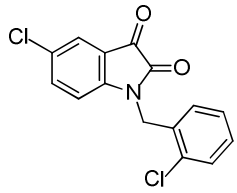
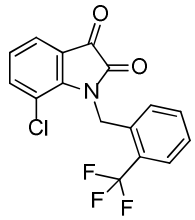
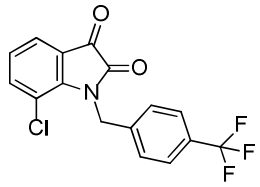
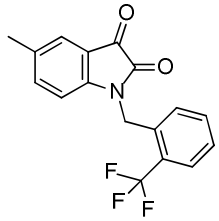
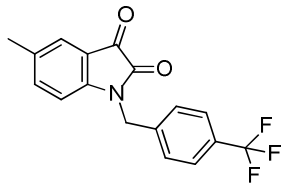
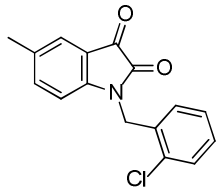
Structure	Cmpd #	Synthesis	Formula	M1 %Max	M5 %Max
	21	M	C16H13NO3	19.40	28.04
	22	M	C16H13NO3	43.58	29.19

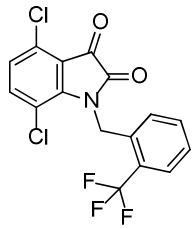
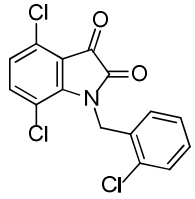
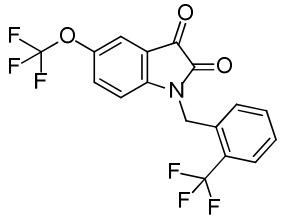
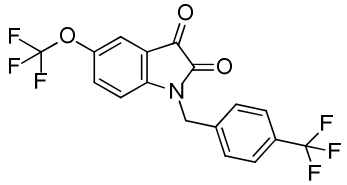
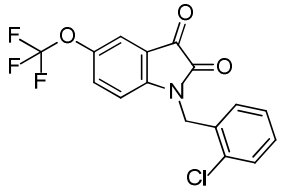
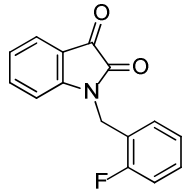
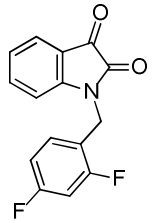
	23	M	C16H12FNO3	32.32	41.61
	24	M	C16H12FNO3	41.14	38.28
	25	M	C15H9BrFNO2	62.67	51.69
	26	M	C16H12FNO3	51.07	51.97
	27	M	C16H12FNO3	54.62	44.40
	28	M	C15H9BrClNO2	26.13	32.03
	29	M	C16H12ClNO3	16.94	27.87
	30	M	C16H12ClNO3	20.31	32.61

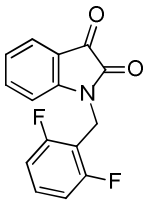
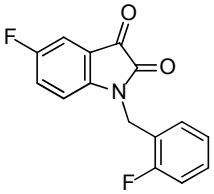
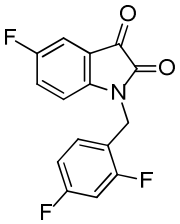
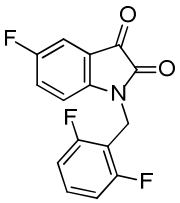
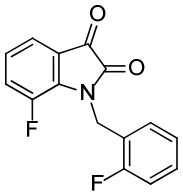
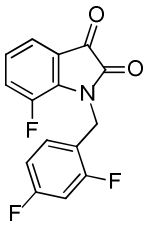
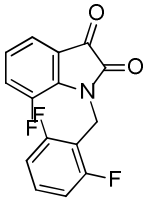
	31	M	C15H9BrClNO2	29.87	44.34
	32	M	C16H12ClNO3	46.88	38.74
	33	M	C16H12ClNO3	49.44	30.32
	34	M	C16H12BrNO2	18.98	24.06
	35	M	C17H15NO3	14.49	29.57
	36	M	C17H15NO3	14.09	21.18
	37	M	C15H8BrCl2NO2	12.08	20.67
	38	M	C16H11Cl2NO3	13.26	35.11

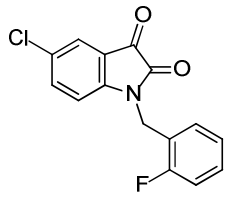
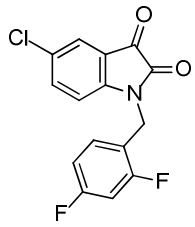
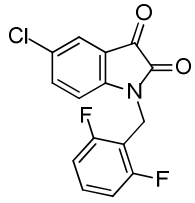
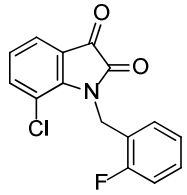
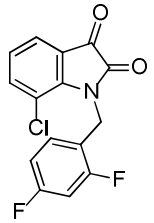
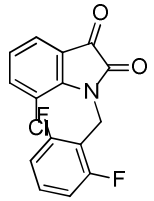
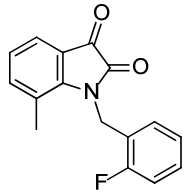
	39	M	C16H11Cl2NO3	18.60	11.73
	40	M	C16H9BrF3NO3	13.21	47.56
	41	M	C17H12F3NO4	28.55	70.08
	42	M, C	C17H12F3NO4	12.69	60.73
	43	M	C16H10F3NO2	37.89	40.23
	44	M	C16H10F3NO2	73.26	61.99
	45	M	C15H10ClNO2	43.42	41.96

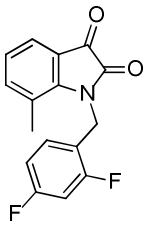
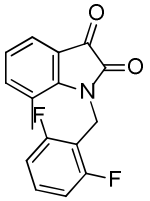
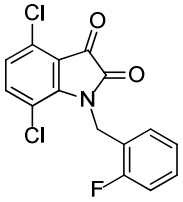
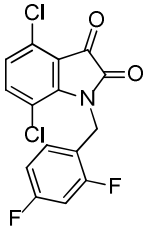
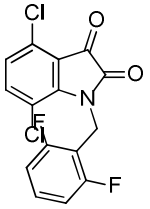
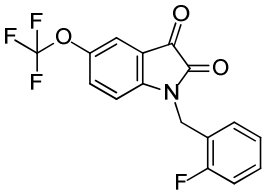
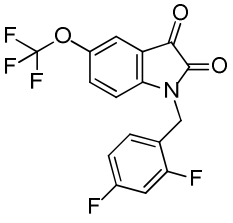
	46	M	C16H9F4NO2	47.78	36.15
	47	M	C16H9F4NO2	67.30	59.76
	48	M	C15H9ClFNO2	41.35	39.90
	49	M	C16H9F4NO2	41.26	30.92
	50	M	C16H9F4NO2	60.86	71.10
	51	M	C15H9ClFNO2	18.53	20.27
	52	M	C16H9ClF3NO2	29.56	48.35

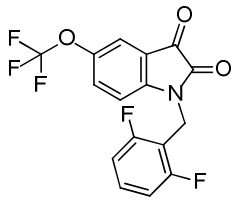
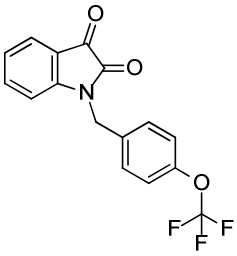
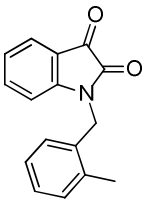
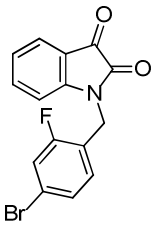
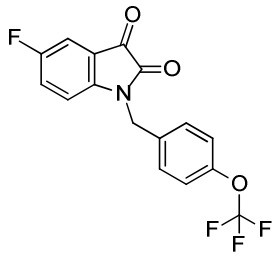
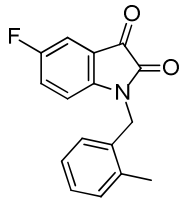
	53	M	C16H9ClF3NO2	44.14	67.39
	54	M	C15H9Cl2NO2	21.27	44.35
	55	M	C16H9ClF3NO2	31.14	13.99
	56	M,C	C16H9ClF3NO2	67.04	70.76
	57	M	C17H12F3NO2	17.81	30.57
	58	M	C17H12F3NO2	22.89	35.45
	59	M	C16H12ClNO2	16.80	24.30

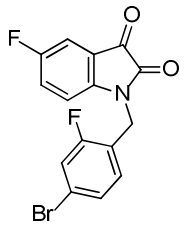
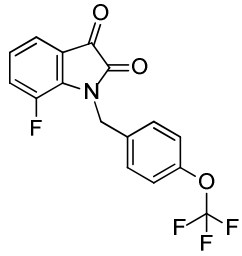
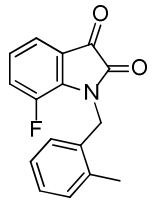
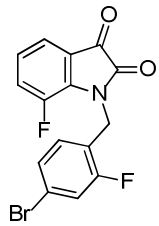
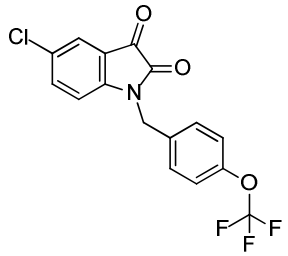
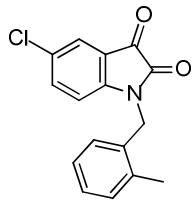
	60	M	C16H8Cl2F3NO2	21.09	16.02
	61	M	C15H8Cl3NO2	13.06	18.94
	62	M	C17H9F6NO3	22.63	56.54
	63	M	C17H9F6NO3	25.69	67.22
	64	M	C16H9ClF3NO3	12.29	51.88
	65	M	C15H10FNO2	38.87	24.49
	66	M	C15H9F2NO2	44.84	40.38

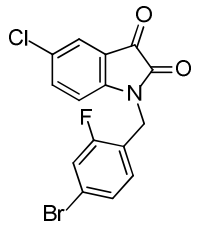
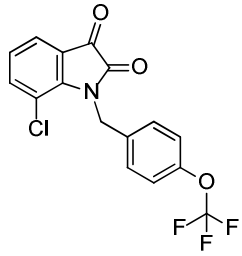
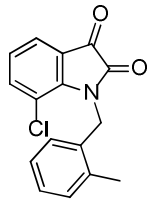
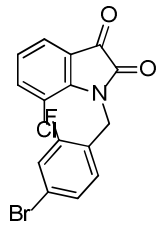
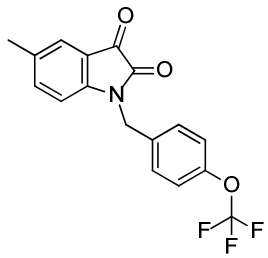
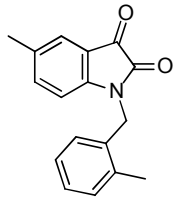
	67	M	C15H9F2NO2	36.65	17.44
	68	M	C15H9F2NO2	40.11	46.18
	69	M	C15H8F3NO2	47.31	60.32
	70	M	C15H8F3NO2	27.59	31.11
	71	M	C15H9F2NO2	42.71	42.40
	72	M	C15H8F3NO2	64.42	68.93
	73	M	C15H8F3NO2	33.10	25.10

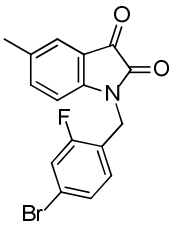
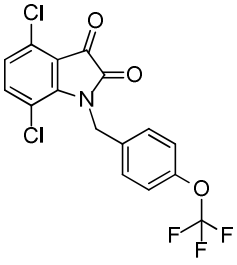
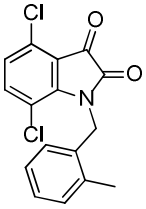
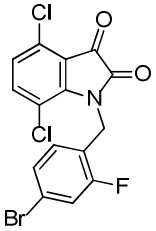
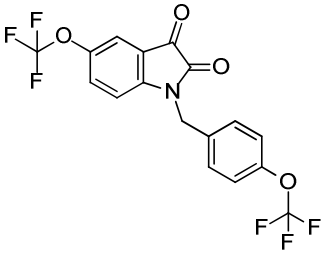
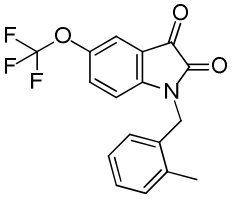
	74	M	C15H9ClFNO2	22.98	36.33
	75	M	C15H8ClF2NO2	32.39	44.90
	76	M	C15H8ClF2NO2	27.34	31.82
	77	M	C15H9ClFNO2	50.42	49.54
	78	M	C15H8ClF2NO2	64.64	61.59
	79	M	C15H8ClF2NO2	38.83	23.59
	80	M	C16H12FNO2	24.85	24.60

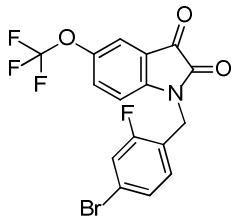
	81	M	C16H11F2NO2	32.56	33.81
	82	M	C16H11F2NO2	17.28	14.40
	83	M	C15H8Cl2FNO2	12.36	14.70
	84	M	C15H7Cl2F2NO2	17.23	19.10
	85	M	C15H7Cl2F2NO2	16.40	12.19
	86	M	C16H9F4NO3	11.30	51.77
	87	M	C16H8F5NO3	25.85	61.90

	88	M	C16H8F5NO3	13.57	29.82
	89	M	C16H10F3NO3	54.72	60.47
	90	M	C16H13NO2	43.16	36.34
	91	M	C15H9BrFNO2	49.20	48.52
	92	M	C16H9F4NO3	59.81	58.39
	93	M	C16H12FNO2	37.72	40.76

	94	M	C15H8BrF2NO2	70.54	63.96
	95	M	C16H9F4NO3	51.78	57.70
	96	M	C16H12FNO2	30.17	27.52
	97	M	C15H8BrF2NO2	39.19	46.34
	98	M	C16H9ClF3NO3	31.28	47.07
	99	M	C16H12ClNO2	27.65	47.26

	100	M	C15H8BrClFNO2	29.89	30.39
	101	M	C16H9ClF3NO3	43.65	72.32
	102	M	C16H12ClNO2	32.83	32.56
	103	M	C15H8BrClFNO2	46.52	60.75
	104	M	C17H12F3NO3	13.83	18.92
	105	M	C17H15NO2	20.19	29.85

	106	M	C16H11BrFNO2	22.32	27.77
	107	M	C16H8Cl2F3NO3	16.03	23.24
	108	M	C16H11Cl2NO2	13.68	16.42
	109	M	C15H7BrCl2FNO2	9.93	12.38
	110	M	C17H9F6NO4	13.82	58.87
	111	M	C17H12F3NO3	26.16	59.97



112	M	C16H8BrF4NO3	11.56	36.36

II. Pharmacology

Calcium Mobilization Assay. All functional cell-based assays were performed essentially as previously described (Marlo *et al.*, 2009; Brady *et al.*, 2008). For the initial 'single-point' potentiator screen of library compounds **21-112**, rM1-CHO cells or hM5-CHO cells (10,000 cells/20 µl/well) were plated in black-walled, clear-bottomed, TC treated, 384 well plates (Greiner Bio-One, Monroe, North Carolina) in Ham's F12 medium supplemented with 10% FBS and 20 mM HEPES. The cells were grown overnight at 37 °C in the presence of 5% CO_2. The next day, the medium was removed and replaced with 20 µl of 2 µM Fluo-4AM in calcium assay buffer (Hank's Balanced Salt Solution supplemented with 20 mM HEPES and 2.5 mM Probenecid) and the cell plates incubated for 60 minutes at 37 °C. Dye solution was removed and replaced with 20 µl of fresh assay buffer. Test compounds were transferred from a 384-well source plate (10 mM DMSO, 30 µl/well) to 384-well daughter plates using an ECHO acoustic plate reformatter (Labcyte) and then diluted into assay buffer to 20 µM stock concentration (2X). Acetylcholine (Aldrich) was diluted in a 384-well plate containing submaximal (~EC_{10}, determined empirically) and maximal (10 µM) stock concentrations (5X). Mobilization of intracellular calcium was measured using the Functional Drug Screening System 6000 (FDSS6000, Hamamatsu). Baseline readings were taken and then test compounds (30 µM final, 20 µl/well) were added using the FDSS's integrated pipettor. After 150 seconds of equilibration, acetylcholine (EC_{10} and maximal concentrations, 10 µl/well) was added using the FDSS's integrated pipettor. Data were obtained as max-min fluorescent ratios and then normalized to percentage of maximum ACh response and represent mean values obtained from three independent determinations (error bars represent +/- SEM). For test compound potency and mAChR subtype-selectivity determination, calcium mobilization assays were performed as previously described (Marlo *et al.*, 2009, Brady *et al.*, 2008) and in a format similar to that described above using the same reagents. CHO cells stably expressing rM1, hM3, hM5, rM4-Gqi5, and rM2-Gqi5 were plated in 100 µl of growth medium at 5 X 10^4 (rM1, hM3, and hM5) or 6 X 10^4 (hM_2, and rM_4) cells per well in Costar 96-well black-walled, TC-treated, clear-bottom plates (Fisher). Cells were incubated overnight at 37°C under 5% CO_2. The next day, medium was removed from the cells, and they were incubated with 50 µl of 2 µM Fluo-4 AM diluted in assay buffer for 1 h at 37°C. Dye was then removed and replaced with 45 µl of fresh assay buffer. Test compounds were diluted in assay buffer at 2X concentration and acetylcholine was diluted in assay at a 10X concentration. FLEXstation II (Molecular Devices) automated plate reader was used for assay execution and measurement of calcium flux. After establishing baseline fluorescence, test compounds (45 µl) were added to the cells using the FLEXstation II's integrated pipettor and allowed to equilibrate for 150 seconds before addition of acetylcholine

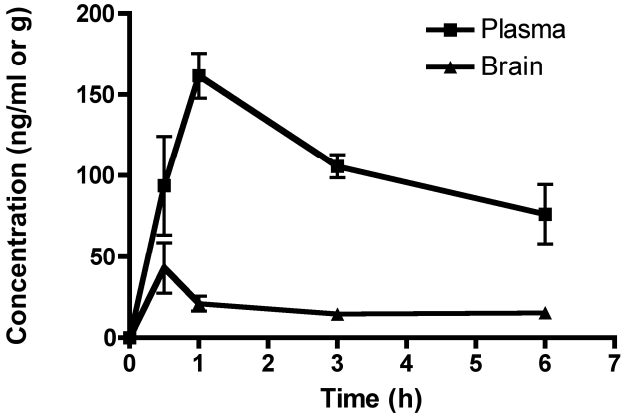
(10 μl). Data were obtained as max-min fluorescent ratios and then normalized to percentage of maximum ACh response. For test compounds exhibiting intrinsic fluorescence (found only at 30 μM), including VU0238429, adjustment of time window for max-min was performed in order to obtain accurate baseline readings. Calculation of potentiation EC_{50} and fold-shift of ACh CRC was performed using the curve-fitting software GraphPad Prism (version 4.0c). Data shown represent mean values obtained from at least three independent determinations performed in duplicate or greater (error bars represent +/- SEM).

[^{3}H-NMS] Competition Binding Assay with CRCs of Compound 113 (VU0119498) and Compound 42 (VU0238429). Membranes were prepared from M5-CHO cells according to a previously described protocol (Marlo *et al.*, 2009, Brady *et al.*, 2008). Binding reactions contained 0.09 nM [^{3}H]-NMS (obtained commercially from Amersham), 15-20 μg of membrane protein, and test compound or atropine in a total volume of 500 μl assay buffer (100 mM NaCl, 10 mM $MgCl_2$, 20 mM HEPES, pH 7.4). 1 μM (final) atropine was used to determine non-specific binding. The K_D of [^{3}H]-NMS was determined empirically to be 0.264 nM. Binding reactions were incubated for 2 hours at room temperature on a Lab-Line Titer plate shaker at setting 7 (~750 rpm). Reactions were stopped and membranes collected onto 96-well Barex microplates with GF/B filter (1μm pore size) using a Brandel harvester and washed 3X with ice-cold harvesting buffer (50mM Tris-HCl, 0.9% NaCl, pH 7.4). Filter plates were dried overnight and counted in a PerkinElmer TopCount scintillation counter (PerkinElmer Life and Analytical Sciences). True [^{3}H]-NMS concentration was back-calculated after counting aliquots of 5X [^{3}H]-NMS used in the reaction. Atropine K_i determined to be 0.21 by Cheng-Prusoff equation. For all assays, radioligand depletion was kept to approximately 10% or less. Data shown represent mean values obtained from at least three independent determinations performed using three or more replicates (error bars represent +/- SEM).

[^{3}H-NMS] Competition Binding Assay with CRCs of Acetylcholine +/- Compound 42 (VU0238429). Membranes were prepared from M5-CHO cells according to a previously described protocol (Marlo *et al.*, 2009, Brady *et al.*, 2008). Binding reactions contained 0.09 nM [^{3}H]-NMS (obtained commercially from Amersham), 20 μg of membrane protein, and ACh plus vehicle or test compound in a total volume of 500 μl assay buffer (100 mM NaCl, 10 mM $MgCl_2$, 20 mM HEPES, pH 7.4). 1 μM (final) atropine was used to determine non-specific binding. The K_D of [^{3}H]-NMS was determined empirically to be 0.264 nM. Binding reactions were incubated for 2 hours at room temperature on a Lab-Line Titer plate shaker at setting 7 (~750 rpm). Reactions were stopped and membranes collected onto 96-well Barex microplates with GF/B filter (1μm pore size) using a Brandel harvester and washed 3X with ice-cold harvesting buffer (50mM Tris-HCl, 0.9% NaCl, pH 7.4). Filter plates were dried overnight and counted in a PerkinElmer TopCount scintillation counter (PerkinElmer Life and Analytical Sciences). True [^{3}H]-NMS concentration was back-calculated after counting aliquots of 5X [^{3}H]-NMS used in the reaction. Radioligand depletion was kept to approximately 10% or less. Data shown represent mean values obtained from at least three independent determinations performed using three or more replicates (error bars represent +/- SEM).

In-vivo Pharmacokinetics Study. Compound **42** (VU0238429) was formulated as 10% Tween 80 micro-suspension in sterile water at the concentration of 5 mg/ml and administered intraperitoneally to male Sprague-Dawley rats weighing 225 to 250 g (Harlan Sprague-Dawley,

Inc., Indianapolis, IN) at the dose of 10 mg/kg. The rat blood and brain were collected at 0.5, 1, 3, and 6 h. Animals were euthanized and decapitated, and the brains were removed, thoroughly washed in cold phosphate buffered saline and immediately frozen on dry ice. Trunk blood was collected in EDTA Vacutainer tubes, and plasma was separated by centrifugation and stored at -80°C until analysis. Three animals were used for each time point. On the day of analysis, frozen whole-rat brains were weighed and homogenized in 1:3 (w/w) volumes of ice-cold phosphate buffered saline (pH 7.4). The sample extraction of plasma (100µl) and brain homogenate (250 µl) was performed by a method based on protein precipitation, using three volumes of cold acetonitrile containing 0.1% formic acid and an internal standard (VU-178) having final concentration of 50 ng/ml. Extracts were vortex mixed for 5 min. followed by centrifugation at 14000 rpm for 10 min. The supernatants of plasma and brain homogenate extracts were analyzed by means of HPLC/MS/MS, using a ThermoFinnigan TSQ Quantum Ultra (Thermo Fisher Scientific, Waltham, MA) mass spectrometer in positive ion mode. The chromatographic separation was achieved on an Acquity UPLC BEH C18 column (1.7um; 2.1x50mm) at a flow rate of 0.8 ml/min. The gradient program was used with the mobile phase, combining solvent A (95: 5: 0.1% formic acid in water: acetonitrile) and solvent B (95: 5: acetonitrile: 0.1% formic acid in water) as follows: 20% B (0.5 min), 20–95% B (0.5 min), 95% B (1 min), 95–20% B (0.2 min), 20% B (2.8 min). The column temperature was set at 50°C. The software Xcalibur version 2.0 was used to control the instrument and collect data. The electrospray ionization source was fitted with a stainless steel capillary (100 µm i.d.). Nitrogen was used as both the sheath gas and the auxiliary gas. The ion transfer tube temperature was 300°C. The spray voltage, tube lens voltage, and pressure of sheath gas and auxiliary gas were optimized to achieve maximal response using the test compounds mixing with the mobile phase A (50%) and B (50%) at a flow rate of 0.8 ml/min. Collision-induced dissociation was performed on compound **42** (VU0238429) and internal standard under 1.0 mTorr of argon. Selected reaction monitoring was carried out using the transitions from *m/z* 352 to 121 for compound **42** (VU0238429), and *m/z* 310 to 223 for VU-178 (internal standard). The calibration curves were constructed and linear response was obtained in the range of 10- 2000 ng/ml by spiking known amounts of compound **42** (VU0238429) in blank brain homogenates and plasma. Brain concentrations were corrected for dilution in PBS and for residual blood volume using 15µl/g as the vascular space (Brown et al., 1986). The final PK parameters were calculated by noncompartmental analysis using WinNonlin software (version 5.1, Pharsight Inc.).



Plasma and brain homogenate concentration time profile of 42 (VU0238429)

PK Parameter	Plasma	Brain
C_{max} (ng/ml or g) (mean±SD)	161.7 ± 13.9	43.08 ± 15.68
T_{max} (h)	1	0.5
Elimination half life (h)	4.7	3.69
$AUC_{(0\text{-}6h)}$ (ng.h/ml or g) (mean±SD)	621.1 ± 39.9	158.62 ± 47.23
CL/F (ml/min)	145	
V/F (L/kg)	59.6	
AUC_{brain}/AUC_{plasma}	0.25	

Abbreviations: PK, pharmacokinetic; C_{max}, maximum concentration; T_{max}, time at which maximum concentration is reached; AUC, area under the curve; CL, clearance; F, bioavailable fraction; V, volume.