SUPPORTING MATERIAL FOR:

Discovery of Naphthyl-Fused 5-Member Lactams as a New class of M1 Positive Allosteric Modulators

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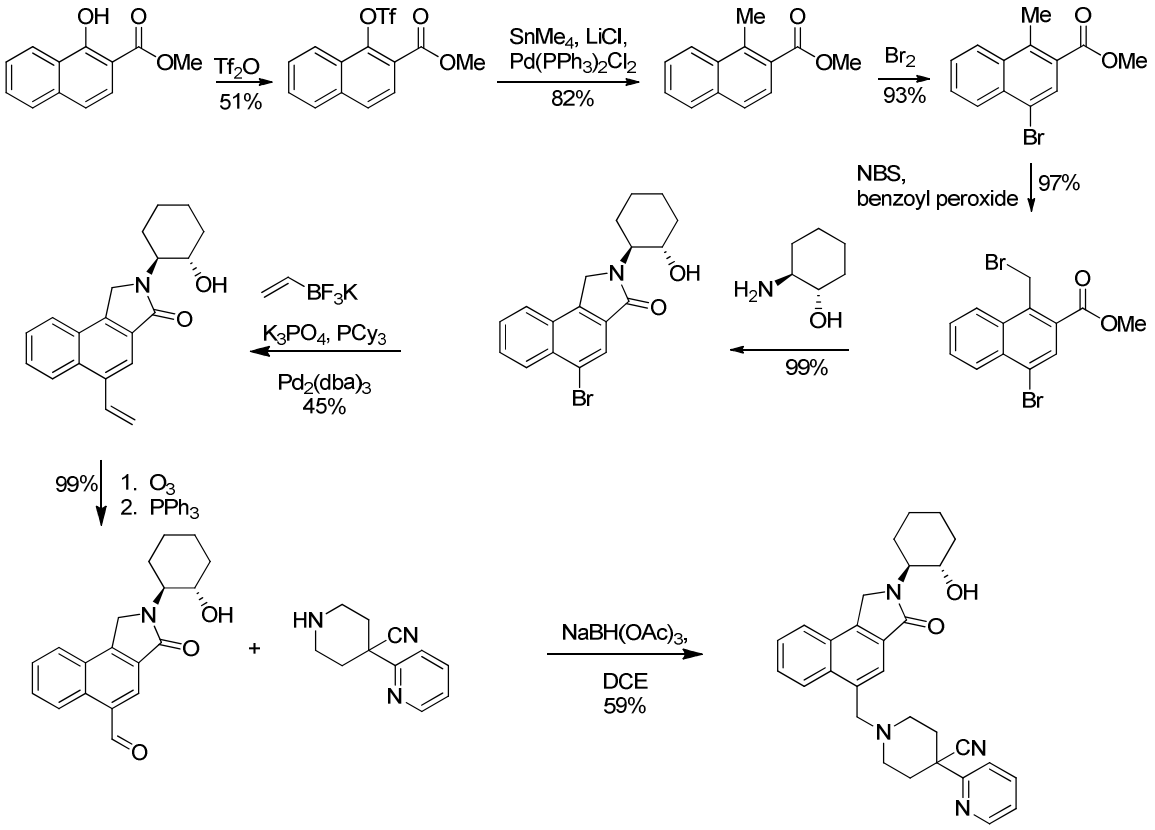
General: All commercially available chemicals and solvents were used without further purification. Automated flash chromatography was performed on an ISCO CombiFlash with peak detection at 254 nm. Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a YMC-Pack Pro or Sunfire C18 column. Peak collection was triggered by UV detection at 214 or 254 nm. ^{1}H (400MHz) NMR spectra for all final compounds were recorded on a Varian VXR 400 spectrometer unless otherwise noted. The chemical shifts are reported in δ (ppm) using the δ 0.00 signal of Me_4Si as an internal standard. HPLC chromatograms on final compounds were recorded on a Hewlett-Packard 1100 with a CombiScreen Pro C-18 column. The purity of compounds was assessed to be >95% by analytical HPLC: i) system 1: linear gradient over 10 min of CH_3CN/0.1% TFA and H_2O/0.1% TFA 10:90 to 95:5 and 2 min at 95:5; flow rate 1.0 mL/min, detection at 215 and 254 nm (YMC-Pack Pro C18, 50 x 4.6 mm column). ii) linear gradient over 3.5 min of CH_3CN/0.1% TFA and H_2O/0.1% TFA 5:95 to 95:5; flow rate 1.5 mL/min, detection at 215 nm (YMC-Pack Pro C18, 50 x 4.6 mm column).

All animal studies described herein were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee.

General Methods for the Preparation of Compounds Illustrated in Scheme 1:

The following compounds were synthesized as described in previously published literature.[1,2]



1-({2-[(1S, 2S)-2-Hydroxycyclohexyl]-3-oxo-2,3-dihydro-1H-benzo[e]isoindol-5-yl}methyl)-4-(pyridine-2-yl)piperidine-4-carbonitrile (4d). To a solution of 1-hydroxy-2-naphthoic acid methyl ester (5.10 g, 25.2 mmol) in 35 mL of pyridine at -5 °C was added trifluoromethanesulfonic anhydride (12.8 mL, 76.0 mmol). After 1 hr, the mixture was poured into 250 mL of ice water and extracted with hexanes and ethyl acetate. The combined organic fractions were washed with water and brine, dried with magnesium sulfate, filtered, and

concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-20% ethyl acetate in hexanes, to provide methyl 1-{[(trifluoromethyl)sulfonyl]oxy}-2-naphthoate that gave a mass ion (ES^+) of 335.1 for $[M+H]^+$.

To a solution of the above compound (4.30 g, 12.9 mmol) in 35 mL of N,N-dimethylformamide under an atmosphere of nitrogen was added lithium chloride (2.73 g, 64.3 mmol), bis(triphenylphosphine)palladium(II) chloride (0.451 g, 0.643 mmol), and tetramethyltin (3.92 mL, 28.3 mmol). The mixture was heated at 110 °C for 3 h, cooled to ambient temperature, and diluted with ethyl acetate. The organic solution was washed with saturated aqueous sodium bicarbonate, water, and brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-10% ethyl acetate in hexanes, to provide methyl 1-methyl-2-naphthoate that gave a mass ion (ES^+) of 201.1 for $[M+H]^+$.

To a solution of the above compound (1.00 g, 4.99 mmol) in 5 mL of acetic acid under an atmosphere of nitrogen was added a 5 mL acetic acid solution of bromine (0.257 mL, 4.99 mmol). The mixture was heated at 90 °C for 4 hr, cooled to ambient temperature and stirred for an additional 15 hr. The mixture was poured into water and extracted with dichloromethane. The organic solution was washed with saturated aqueous sodium bicarbonate, water, and brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-30% ethyl acetate in hexanes, to provide methyl 4-bromo-1-methyl-2-naphthoate that gave a mass ion (ES^+) of 281.1 for $[M+H]^+$.

To a solution of the above compound (1.29 g, 4.62 mmol) in 25 mL of carbon tetrachloride under an atmosphere of nitrogen was added N-bromosuccinimide (0.823 g, 4.62 mmol) and benzoyl peroxide (0.056 g, 0.231 mmoL). The mixture was heated at 90 °C for 1 hr, cooled to ambient temperature, and diluted with ethyl acetate. The organic solution was washed with saturated aqueous sodium bicarbonate, water, and brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-10% ethyl acetate in hexanes, to provide methyl 4-bromo-1-(bromomethyl)-2-naphthoate that gave a proton NMR spectra consistent with theory.

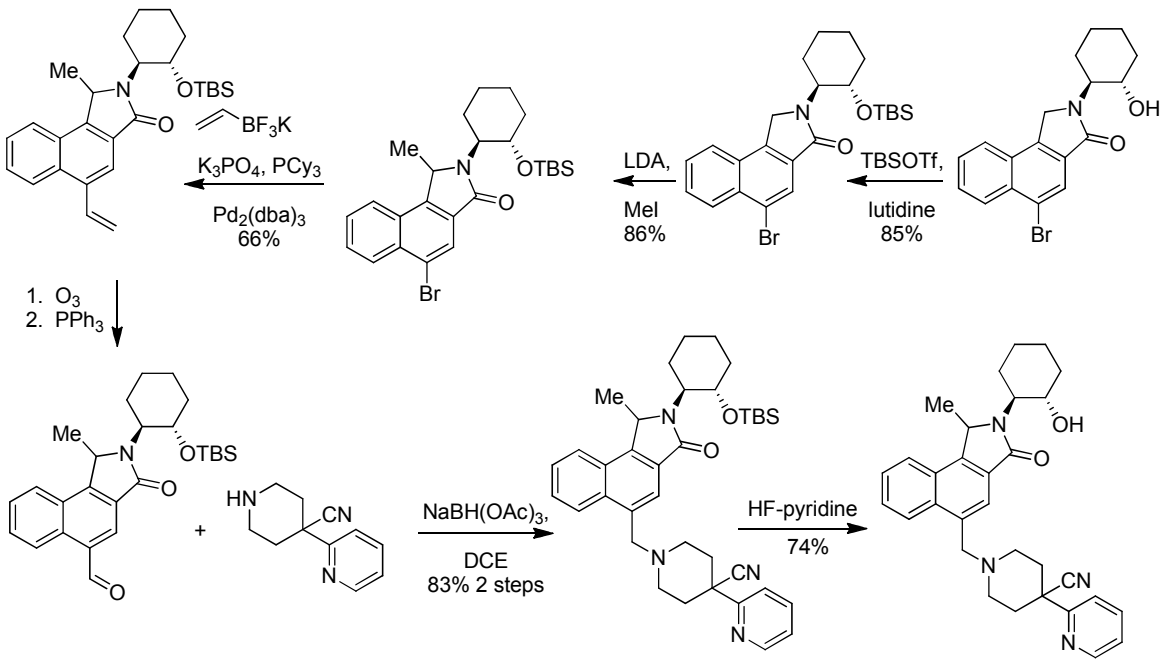
To a solution of the above compound (0.100 g, 0.279 mmol) in 1 mL of THF was added (1S, 2S)-2-aminocyclohexanol (0.161 g, 1.40 mmol). After 15 hr, water (10 mL) was added, the mixture was aged for 30 min and was filtered, providing 5-bromo-2-[(1S, 2S)-2-hydroxycyclohexyl]-1,2-dihydro-3H-benzo[e]isoindol-3-one as a white solid that gave a mass ion (ES^+) of 362.3 for $[M+H]^+$.

To a solution of the above compound (0.225 g, 0.625 mmol) in 2.5 mL of 1,4-dioxane under an atmosphere of nitrogen was added potassium vinyltrifluoroborate (0.167 g, 1.25 mmol), potassium phosphate (1.27 M aqueous, 0.84 mL, 1.1 mmol), tricyclohexylphosphine (4.2 mg, 0.015 mmol), and tris[dibenzylideneacetone]dipalladium (0) (5.7 mg, 0.0062 mmol). The mixture was irradiated in a microwave reactor at 140 °C for 30 min, cooled to ambient temperature, and diluted with saturated aqueous sodium bicarbonate. The mixture was extracted 2x with dichloromethane and the combined organic extracts were washed with brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via preparative

reverse phase HPLC to provide 5-ethenyl-2-[(1S, 2S)-2-hydroxycyclohexyl]-1,2-dihydro-3H-benzo[e]isoindol-3-one that gave a proton NMR spectra consistent with theory.

A solution of the above compound (0.086 g, 0.28 mmol) in 5 mL of dichloromethane was cooled to -78 °C and saturated with ozone. After 5 min, the solution was sparged with nitrogen gas and resin-bound triphenylphosphine (3 mmol/g, 0.600 g, 1.8 mmol) was added. The mixture was warmed to ambient temperature and filtered through Celite. The filtrate was concentrated in vacuo to provide 2-[(1S, 2S)-2-hydroxycyclohexyl]-3-oxo-2,3-dihydro-1H-benzo[e]isoindole-5-carbaldehyde.

To a solution of the above compound (0.072 g, 0.23 mmol) in 4 mL of 1,2-dichloroethane was added 4-(pyridine-2-yl)piperidine-4-carbonitrile (0.048 g, 0.26 mmol), and sodium triacetoxyborohydride (0.099 g, 0.46 mmol). After 15 hr, the mixture was diluted with saturated aqueous sodium bicarbonate and extracted 2x with dichloromethane. The combined organic extracts were washed with brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via preparative reverse phase HPLC to provide the title compound (**4d**) that gave a proton NMR spectra consistent with theory and a mass ion (ES^+) of 481.5 for $[M+H]^+$: ^1H NMR (400 MHz, $CDCl_3$) δ 8.60-8.58 (m, 1H), 8.36-8.34 (m, 1H), 7.88-7.84 (m, 1H), 7.77 (s, 1H), 7.74-7.69 (m, 1H), 7.65-7.56 (m, 3H), 7.25-7.21 (m, 1H), 4.84-4.69 (m, 2H), 4.21-4.15 (m, 1H), 3.95 (s, 2H), 3.77 (br s, 1H), 3.00 (br s, 2H), 2.68-2.56 (m, 2H), 2.34-2.21 (m, 2H), 2.08-1.97 (m, 3H), 1.86-1.83 (m, 2H), 1.71-1.30 (m, 5H) ppm.



1-({2-[(1S, 2S)-2-Hydroxycyclohexyl]-1-methyl-3-oxo-2,3-dihydro-1H-benzo[e]isoindol-5-yl}methyl)-4-(pyridine-2-yl)piperidine-4-carbonitrile (4p)

To a solution of 5-bromo-2-[(1S, 2S)-2-hydroxycyclohexyl]-1,2-dihydro-3H-benzo[e]isoindol-3-one (see Example 1, 2.00 g, 5.55 mmol) in 15 mL of dichloromethane at 0 °C was added 2,6-lutidine (1.29 mL, 11.1 mmol) and tert-butyldimethylsilyl trifluoromethanesulfonate (1.91 mL, 8.33 mmol). The mixture was warmed to ambient temperature and after 15 hr, was treated with water. The mixture was extracted 2x with ethyl acetate and the combined organic extracts were washed with brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-40% ethyl acetate in hexanes, to provide 5-bromo-2-[(1S, 2S)-2-{[tert-butyl(dimethyl)silyl]oxy}cyclohexyl]-1,2-dihydro-3H-benzo[e]isoindol-3-one that gave a mass ion (ES^+) of 475.1 for $[M+H]^+$.

To a solution of diisopropylamine (0.75 mL, 5.3 mmol) in 5 mL of THF at 0 °C was added n-butyllithium (2.5 M tetrahydrofuran solution, 2.32 mL, 5.80 mmol) dropwise, which was added

after 30 min to a solution of the above compound (0.500 g, 1.05 mmol) in 5 mL of THF at −78 °C. After 30 min, iodomethane (0.072 mL, 1.2 mmol) was added dropwise and after 1 h, the mixture was warmed to ambient temperature, treated with saturated aqueous ammonium chloride, and extracted 2x with ethyl acetate. The combined organic extracts were washed with water and brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 0-20% ethyl acetate in hexanes, to provide (1S)- and (1R)-5-bromo-2-[(1S, 2S)-2-{[tert-butyl(dimethyl)silyl]oxy}cyclohexyl]-1-methyl-1,2-dihydro-3H-benzo[e]isoindol-3-one that gave a proton NMR spectra consistent with theory.

1-({2-[(1S, 2S)-2-{[tert-Butyl(dimethyl)silyl]oxy}cyclohexyl]-1-methyl-3-oxo-2,3-dihydro-1H-benzo[e]isoindol-5-yl}methyl)-4-(pyridin-2-yl)piperidine-4-carbonitrile was prepared employing the similar procedures described for the construction of **4d**.

To a solution of 1-({2-[(1S, 2S)-2-{[tert-butyl(dimethyl)silyl]oxy}cyclohexyl]-1-methyl-3-oxo-2,3-dihydro-1H-benzo[e]isoindol-5-yl}methyl)-4-(pyridin-2-yl)piperidine-4-carbonitrile (0.120 g, 0.197 mmol) in 0.1 mL of pyridine was added hydrogen fluoride pyridine (0.098 g, 0.98 mmol). After 15 hr, the mixture was treated with saturated aqueous sodium bicarbonate and extracted 2 x with ethyl acetate. The combined organic extracts were washed with brine, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with 10-100% ethyl acetate in hexanes, to provide the titled compound (**4p**) that gave a proton NMR spectra consistent with theory and a mass ion (ES^+) of 495.5 for $[M+H]^+$: ^1H NMR (400 MHz, CDCl3) δ 8.60-8.58 (m, 1H), 8.45-8.41 (m, 1H), 7.95-

7.90 (m, 1H), 7.83-7.79 (m, 1H), 7.74-7.69 (m, 1H), 7.66-7.56 (m, 3H), 7.25-7.21 (m, 1H), 5.11-5.03 (m, 2H), 4.42 (br s, 1H), 4.03-3.98 (m, 2H), 3.79-3.72 (m, 1H), 3.65-3.59 (m, 1H), 3.06-3.02 (m, 2H), 2.88-2.86 (m, 1H), 2.67-2.59 (m, 2H), 2.35-2.17 (m, 2H), 2.09-2.06 (m, 1H), 2.04-1.81 (m, 2H), 1.42-1.22 (m, 4H), 0.96-0.83 (m, 4H) ppm.

Fluorometric Imaging Plate Reader (FLIPR): CHONFAT cells expressing M1, M2, M3, M4, M5, rhesus M1, dog M1, mouse M1 and rat M1 (in CHOK1 from ATCC) receptors were plated (25,000 cells per well) in clear-bottomed, poly-d-lysine-coated 384-well plates in growth medium by using a Labsystems (Chicago) Multidrop. The plated cells were grown overnight at 37°C in the presence of 6% CO2. The next day, the cells were washed with 3 × 100 μl assay buffer (Hanks' balanced salt solution containing 20 mM Hepes, 2.5 mM probenecid, and 0.1% BSA). The cells were incubated with 1 μM Fluo-4AM (Molecular Probes) for 1 h at 37 °C and 6% CO2. The extracellular dye was removed by washing as described above. Ca2+ flux was measured by using a FLIPR384 fluorometric imaging plate reader (Molecular Devices). Compounds were serially diluted in 100% DMSO and then diluted in assay buffer to a 3X stock at 2% DMSO. This stock was then applied to the cells for a final DMSO concentration of 0.67%. For potency determination, the cells were pre-incubated with various concentrations of compound for 4 min and then stimulated for 4 min with an EC_{20} concentration of agonist (i.e., ACh) for potentiation measurements.

Fold potentiation assay. CHO_{NFAT} cells expressing human mAChR 1 receptor were plated (25,000 cells per well) in clear-bottomed, poly-d-lysine-coated 384-well plates in growth medium by using a Labsystems (Chicago) Multidrop. The plated cells were grown overnight at

37°C in the presence of 6% CO_2. The next day, the cells were washed with 3 × 100 µl assay buffer (Hanks' balanced salt solution containing 20 mM Hepes, 2.5 mM probenecid, and 0.1% BSA). The cells were incubated with 1 µM Fluo-4AM (Molecular Probes) for 1 h at 37 °C and 6% CO_2. The extracellular dye was removed by washing as described above. Ca^{2+} flux was measured by using a $FLIPR_{384}$ fluorometric imaging plate reader (Molecular Devices). Compounds were dissolved in 100% DMSO and then diluted in assay buffer to a 3X stock at 2% DMSO. This stock was then applied to the cells for a final DMSO concentration of 0.67%. For the fold potentiation measurements, the cells were pre-incubated with various concentrations of compound for 4 min and then stimulated for 4 min with a serially diluted agonist (i.e., ACh).

Contextual Fear Conditioning. On day one 10-week old experimentally naïve male B6SJL mice (n = 12-16/group) were dosed IP with **4d** in 5% betacyclodextrin and/or 0.3 mg/kg scopolamine in 0.9% saline 30 min before placement into a chamber (MED-VFC-M, Med Associates) for 2 min before 2 tonefootshock parings (3kHz, 85dB tone for 30 s co-terminated with a 0.5 mA, 1 s shock) 2 min apart. Mice were removed to their home cage 30 s after the last pairing. Twenty-four hours later mice were placed into the same chamber and freezing was measured by Video Freeze (Med Associates).

1. Kuduk, S.D.; Di Marco, C.N.; Yang, Z.-Q. Quinolizidinone M_1 receptor positive allosteric modulators. WO 2012158474 A1 20121122.

2. Chang, R..K.; Di Marco, C.N.; Pitts, D.R.; Greshock, T.J.; Kuduk, S.D. Preparation of 4-heteroaryl-4-cyanopiperidines via S_NAr substitution reactions. *Tetrahedron Lett.* **2009**, *50*, 6303-6306.