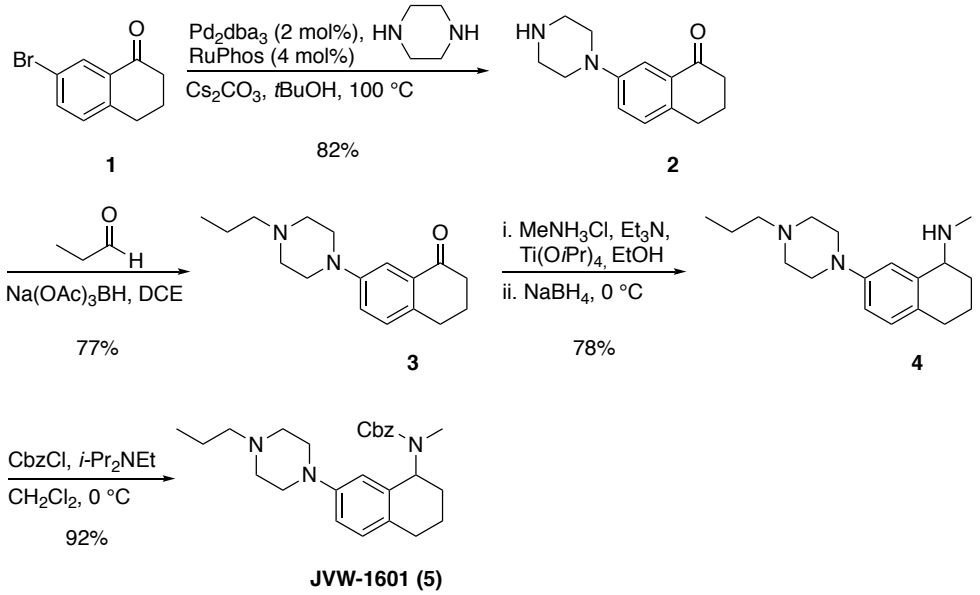
Supporting Information Appendix

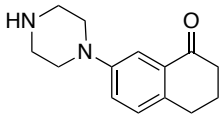
Synthetic Chemistry Methods

General methods

Methylene chloride (CH_2Cl_2), *N*,*N*-diisopropylethylamine (*i*-Pr_2NEt), triethylamine (Et_3N), *tert*-butanol (*t*BuOH), and ethanol (EtOH) were distilled from calcium hydride (CaH_2) immediately prior to use. All reagents were reagent grade and used without purification unless otherwise noted. Where required, solvents were degassed by sparging with nitrogen prior to use. All reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of nitrogen or argon in glassware that was flame dried. Reaction temperatures refer to the temperature of the cooling/heating bath. Volatile solvents were removed under reduced pressure using a Büchi rotary evaporator. Thin-layer chromatography (TLC) was performed on EMD 60 F254 glass-backed pre-coated silica gel plates and were visualized using one or more of the following methods: UV light (254 nm) and staining with basic potassium permanganate ($KMnO_4$) or acidic *p*-anisaldehyde (PAA). Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained at the indicated field as solutions in $CDCl_3$ unless otherwise indicated. Chemical shifts are referenced to the deuterated solvent and are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS, δ = 0.00 ppm). Coupling constants (*J*) are reported in Hz and the splitting abbreviations used are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; comp, overlapping multiplets of magnetically nonequivalent protons; br, broad; app, apparent.

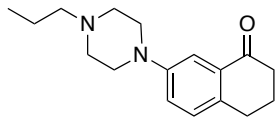


Scheme 1. Synthesis of JVW-1601 (5).



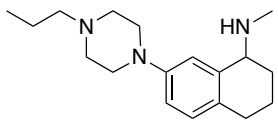
2

7-(Piperazin-1-yl)-3,4-dihydronaphthalen-1(2*H*)-one (2). A resealable tube was charged with tetralone **1** (0.500 g, 2.22 mmol), piperazine (1.913 g, 22.2 mmol), Cs_2CO_3 (1.086 g, 3.33 mmol) and degassed *t*BuOH (11.1 mL). The suspension was stirred at 45 °C for 15 min, whereupon a freshly prepared *t*BuOH solution (0.67 mL) containing Pd_2dba_3 (40.6 mg, 0.044 mmol) and RuPhos (41.5 mg, 0.088 mmol) that had been stirred at 60 °C for 30 min was added. The tube was sealed, and the reaction was stirred at 100 °C for 3 h. After cooling to room temperature, the mixture was filtered through Celite, the filter cake was washed with CH_2Cl_2 (200 mL), and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated aq. $NaHCO_3$ (2 x 50 mL), and extracted with 1 N HCl (4 x 30 mL). The combined acidic aqueous extracts were made basic with 6 N NaOH and extracted with CH_2Cl_2 (4 x 50 mL), after which the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude material was purified via flash chromatography (SiO_2) eluting with CH_2Cl_2/MeOH/Et_3N (97:2:1) affording 0.418 g (82%) of **2** as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 2.8 Hz, 1 H), 7.08 (d, *J* = 8.4 Hz, 1 H), 7.02 (dd, *J* = 8.4, 2.8 Hz, 1 H), 3.14 – 3.06 (comp, 4 H), 3.00 – 2.93 (comp, 4 H), 2.79 (t, *J* = 6.1 Hz, 2 H), 2.70 (br s, 1 H), 2.54 (m, 2 H), 2.01 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.5, 150.3, 135.8, 132.8, 129.4, 122.0, 112.8, 50.0, 45.8, 39.1, 28.7, 23.4; HRMS (ESI) *m/z* calcd for $C_{14}H_{18}N_2O$ $(M+H)^+$, 231.1492; found 231.1497



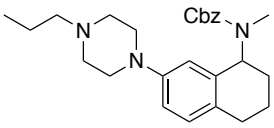
3

7-(4-Propylpiperazin-1-yl)-3,4-dihydronaphthalen-1(2*H*)-one (3). A solution of propionaldehyde (0.65 g, 0.81 mL, 11.2 mmol) in dichloroethane (25 mL) was added dropwise to a solution of amine **2** (2.363 g, 10.3 mmol) and $Na(OAc)_3BH$ (4.349 g, 20.5 mmol) in dichloroethane (103 mL), and the reaction was stirred at room temperature for 3 h. The reaction mixture was then washed with saturated aq. $NaHCO_3$ (2 x 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude material was purified via flash chromatography (SiO_2) eluting with hexanes/EtOAc/Et_3N (74:25:1) affording 2.165 g (77%) of **3** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 2.7 Hz, 1 H), 7.14 (d, *J* = 8.4 Hz, 1 H), 7.08 (dd, *J* = 8.5, 2.7 Hz, 1 H), 3.24 – 3.19 (comp, 4 H), 2.86 (t, *J* = 6.1 Hz, 2 H), 2.64 – 2.55 (comp, 6 H), 2.38 – 2.31 (m, 2 H), 2.09 (m, 2 H), 1.60 – 1.48 (m, 2 H), 0.92 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.9, 150.1, 135.8, 133.0, 129.6, 122.0, 113.0, 60.8, 53.2, 49.1, 39.3, 28.9, 23.6, 20.1, 12.1; HRMS (ESI) *m/z* calcd for $C_{17}H_{24}N_2O$ $(M+H)^+$, 273.1961; found 273.1965



4

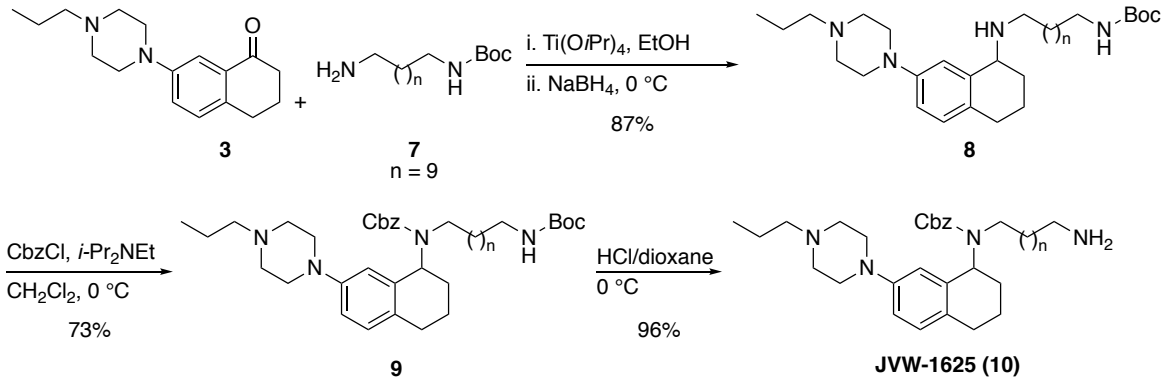
***N*-Methyl-7-(4-propylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-amine (4).** In a modification of a literature procedure(1), tetralone **3** (0.102 g, 0.375 mmol) was dissolved in EtOH (2.5 mL) in a resealable tube, whereupon $Ti(O\textit{i}Pr)_4$ (1.44 g, 1.5 mL, 3.75 mmol), Et_3N (0.19 g, 0.26 mL, 1.9 mmol) and $MeNH_3Cl$ (0.127 g, 1.88 mmol) were sequentially added. The tube was sealed, and the reaction was stirred at room temperature for 7 h. The solution was cooled to 0 °C, and $NaBH_4$ (0.028 g, 0.75 mmol) was added in one portion. Stirring was continued at 0 °C for 1 h, and the mixture was added to 2 M aq. NH_4OH (10 mL). The suspension was filtered through a pad of Celite, and the filter cake was washed with hot EtOAc (150 mL). The filtrate was concentrated under reduced pressure and partitioned between CH_2Cl_2 (15 mL) and saturated aq. $NaHCO_3$ (10 mL). The organic layer was separated and extracted with 1 M HCl (3 x 15 mL). The combined aqueous extracts were adjusted to pH 10 with 6 M NaOH and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified via flash chromatography (SiO_2) eluting with CH_2Cl_2/MeOH/Et_3N (98:1:1) affording 0.0763 g (78%) of **4** as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (d, *J* = 8.4 Hz, 1 H), 6.92 (d, *J* = 2.6 Hz, 1 H), 6.77 (dd, *J* = 8.4, 2.7 Hz, 1 H), 3.61 (t, *J* = 4.9 Hz, 1 H), 3.20 – 3.14 (comp, 4 H), 2.77 – 2.62 (comp, 2 H), 2.62 – 2.57 (comp, 4 H), 2.49 (s, 3 H), 2.38 – 2.32 (comp, 2 H), 1.96 – 1.81 (comp, 3 H), 1.75 – 1.66 (m, 1 H), 1.60 – 1.49 (comp, 2 H), 1.37 (br s, 1 H), 0.92 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.7, 139.6, 129.7, 128.8, 116.4, 115.4, 60.8, 57.6, 53.4, 49.8, 34.1, 28.6, 27.9, 20.1, 19.2, 12.1; HRMS (ESI) *m/z* calcd for $C_{18}H_{29}N_3$ $(M+H)^+$, 288.2434; found 288.2438



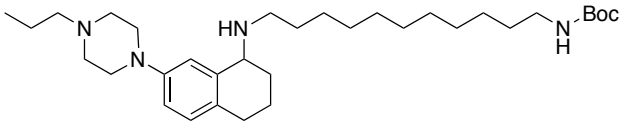
JVW-1601 (5)

Benzyl methyl(7-(4-propylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate (5). *i*-Pr_2NEt (0.0519 g, 70 μL, 0.377 mmol) and CbzCl (0.0478 g, 40 μL, 0.276 mmol) were added with stirring to a solution of amine **4** (0.0721 g, 0.251 mmol) in CH_2Cl_2 (1.3 mL) cooled to 0 °C. The solution was stirred at 0 °C for 4 h and then diluted with CH_2Cl_2 (10 mL). The solution was washed with 1 N HCl (2 x 10 mL), 1 N NaOH (2 x 10 mL), saturated aqueous $NaHCO_3$ (1 x 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified via flash chromatography (SiO_2) eluting with hexanes/EtOAc/Et_3N (74:25:1) affording 0.0969 (92%) g of **5** as a colorless oil. ^{1}H NMR (499 MHz, $CDCl_3$) (mixture of rotamers) δ 7.45 – 7.27 (comp, 5 H), 7.02 – 6.96 (m, 1 H), 6.81 – 6.75 (m, 1 H), 6.66 – 6.60 (m, 1 H), 5.51 – 5.11 (comp, 3 H), 3.16 – 3.05 (comp, 4 H), 2.75 – 2.61 (comp, 5 H), 2.61 – 2.54 (comp, 4 H), 2.39 – 2.33 (comp, 2 H), 2.08 – 1.92 (comp, 2 H), 1.83 – 1.68 (comp, 2 H), 1.61 – 1.51 (comp, 2 H), 0.94 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) (mixture of rotamers) δ 157.2, 157.0, 150.1, 150.0, 137.2, 137.1, 135.8, 135.7, 130.0, 129.9, 129.8, 129.6,

128.5, 127.9, 127.8, 127.8, 115.6, 115.4, 114.6, 114.2, 67.2, 67.1, 60.8, 55.6, 55.4, 53.3, 53.3, 49.6, 49.5, 30.3, 29.7, 28.8, 28.7, 28.2, 27.7, 22.3, 22.1, 20.1, 12.1; HRMS (ESI) *m/z* calcd for $C_{26}H_{35}N_3O_2$ $(M+H)^+$, 422.2802; found 422.2816

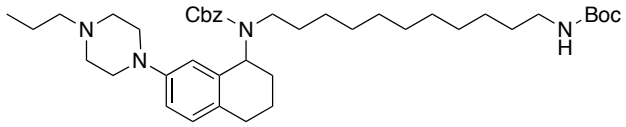


Scheme 2. Synthesis of JVW-1625 (10).



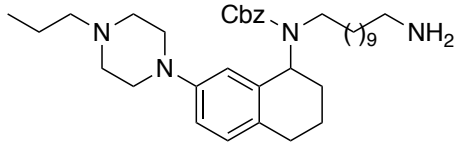
8

***tert*-Butyl (11-((7-(4-propylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)amino)undecyl)carbamate (8).** Tetralone **3** (0.0508 g, 0.186 mmol) was dissolved in EtOH (1.2 mL) in a screw cap vial, whereupon the known amine **7(2)** (0.0801 g, 0.279 mmol) and Ti(O*i*Pr)$_4$ (0.5 g, 0.6 mL, 1.9 mmol) were sequentially added. The vial was sealed, and the reaction was stirred at 45 °C for 22 h. The solution was cooled to 0 °C and $NaBH_4$ (0.014 g, 0.37 mmol) was added in one portion. Stirring was continued at 0 °C for 1 h, and the mixture was added to 2 M aq. NH_4OH (10 mL). The suspension was filtered through a pad of Celite, and the filter cake was washed with hot EtOAc (250 mL). The filtrate was concentrated under reduced pressure and partitioned between CH_2Cl_2 (20 mL) and 1 M NaOH (15 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 x 15). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified via flash chromatography (SiO_2) eluting with hexanes/acetone/Et_3N (84:15:1) affording 0.0876 g (87%) of **8** as a colorless oil. ^{1}H NMR (499 MHz, $CDCl_3$) δ 6.96 (d, *J* = 8.4 Hz, 1 H), 6.93 (d, *J* = 2.6 Hz, 1 H), 6.76 (dd, *J* = 8.4, 2.6 Hz, 1 H), 4.56 (br s, 1 H), 3.69 (t, *J* = 5.0 Hz, 1 H), 3.20 – 3.13 (m, 4 H), 3.08 (comp, *J* = 6.8 Hz, 2 H), 2.75 – 2.62 (comp, 4 H), 2.61 – 2.57 (comp, 4 H), 2.37 – 2.32 (comp, 2 H), 1.97 – 1.87 (m, 1 H), 1.86 – 1.79 (comp, 2 H), 1.72 – 1.64 (m, 1 H), 1.60 – 1.40 (comp, 15 H), 1.38 – 1.22 (m, 15 H), 0.92 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.1, 149.7, 140.1, 129.7, 129.0, 116.4, 115.5, 79.1, 60.9, 56.0, 53.4, 49.9, 47.4, 40.7, 30.7, 30.2, 29.7, 29.7, 29.7, 29.6, 29.4, 28.7, 28.6, 28.5, 27.6, 26.9, 20.2, 19.4, 12.1; HRMS (ESI) *m/z* calcd for $C_{33}H_{58}N_4O_2$ $(M+H)^+$, 543.4633; found 543.4651



9

Benzyl (11-((*tert*-butoxycarbonyl)amino)undecyl)(7-(4-propylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate (9). *i*-Pr_2NEt (0.038 g, 52 μL, 0.30 mmol) and CbzCl (0.038 g, 32 μL, 0.22 mmol) were added with stirring to a solution of amine **8** (0.0804 g, 0.148 mmol) in CH_2Cl_2 (0.75 mL) cooled to 0 °C. The solution was stirred at 0 °C for 1 h and then diluted with CH_2Cl_2 (20 mL). The solution was washed with 1 N HCl (2 x 10 mL), 1 N NaOH (2 x 20 mL), saturated aqueous $NaHCO_3$ (1 x 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified via flash chromatography (SiO_2) eluting with hexanes/EtOAc/Et_3N (74:25:1) affording 0.0806 g (80%) of **9** as a pale yellow oil. ^1H NMR (499 MHz, $CDCl_3$) (mixture of rotamers) δ 7.43 – 7.19 (comp, 5 H), 6.99 – 6.95 (m, 1 H), 6.76 (dd, *J* = 8.4, 2.5 Hz, 1 H), 6.60 (d, *J* = 2.5 Hz, 1 H), 5.42 – 4.98 (m, 3 H), 4.51 (br s, 1 H), 3.29 – 3.01 (comp, 7 H), 2.88 – 2.50 (comp, 7 H), 2.41 – 2.31 (comp, 2 H), 2.10 – 1.38 (comp, 19 H), 1.32 – 1.07 (comp, 14 H) 0.93 (t, *J* = 7.4 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) (mixture of rotamers) δ 157.3, 156.6, 156.1, 150.0, 149.9, 137.3, 137.2, 137.1, 136.7, 130.0, 129.8, 129.5, 128.6, 128.5, 128.0, 127.9, 127.9, 115.7, 115.4, 114.5, 114.0, 79.1, 67.1, 67.0, 60.8, 56.5, 53.3, 49.6, 49.5, 45.0, 40.8, 30.6, 30.5, 30.2, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 28.9, 28.8, 28.6, 27.3, 26.9, 22.7, 22.5, 20.2, 12.1; HRMS (ESI) *m/z* calcd for $C_{41}H_{64}N_4O_4$ $(M+H)^+$, 677.5000; found 677.4994



JVW-1625 (10)

Benzyl (11-aminoundecyl)(7-(4-propylpiperazin-1-yl)-1,2,3,4-tetrahydronapthalen-1-yl)carbamate (10). Carbamate **9** (0.0373 g, 0.0551 mmol) was dissolved in a solution of dioxane (0.4 mL) containing HCl (4 M) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and then diluted with CH_2Cl_2 (20 mL). The solution was washed with 1 N NaOH (1 x 20 mL), saturated aqueous $NaHCO_3$ (1 x 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford 0.0306 g of **10** (96%) as a pale yellow oil. ^1H NMR (499 MHz, $CDCl_3$) (mixture of rotamers) δ 7.44 – 7.18 (comp, 5 H), 7.01 – 6.94 (m, 1 H), 6.76 (dd, *J* = 8.4, 2.5 Hz, 1 H), 6.63 – 6.56 (m, 1 H), 5.44 – 5.01 (comp, 3 H), 3.29 – 2.99 (comp, 5 H), 2.86 – 2.60 (comp, 5 H), 2.58 – 2.50 (comp, 4 H), 2.38 – 2.31 (comp, 2 H), 2.07 – 1.41 (comp, 12 H), 1.32 – 1.08 (comp, 14 H), 0.93 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) (mixture of rotamers) δ 157.2, 156.5, 149.8, 149.7, 137.2, 136.9, 136.5, 129.8, 129.7, 129.3, 128.4, 128.4, 127.8, 127.8, 127.7, 115.6, 115.2, 114.3, 113.8, 66.9, 66.8, 60.7, 56.3, 53.2, 53.2, 49.5, 44.8, 42.1, 33.5, 30.4, 29.6, 29.5, 29.5, 29.4, 29.2, 29.1, 28.7, 28.6, 27.2, 26.9, 22.6, 22.4, 20.1, 12.0; HRMS (ESI) *m/z* calcd for $C_{36}H_{56}N_4O_2$ $(M+H)^+$, 577.4476; found 577.4484

References

1. A. Neidigh K, A. Avery M, S. Williamson J, & Bhattacharyya S (1998) Facile preparation of N-methyl secondary amines by titanium(IV) isopropoxide-mediated reductive amination of carbonyl compounds. *Journal of the Chemical Society, Perkin Transactions 1* (16):2527-2532.
2. Morrell A*, et al.* (2007) Investigation of the lactam side chain length necessary for optimal indenoisoquinoline topoisomerase I inhibition and cytotoxicity in human cancer cell cultures. *J Med Chem* 50(9):2040-2048.

Supplementary Figures

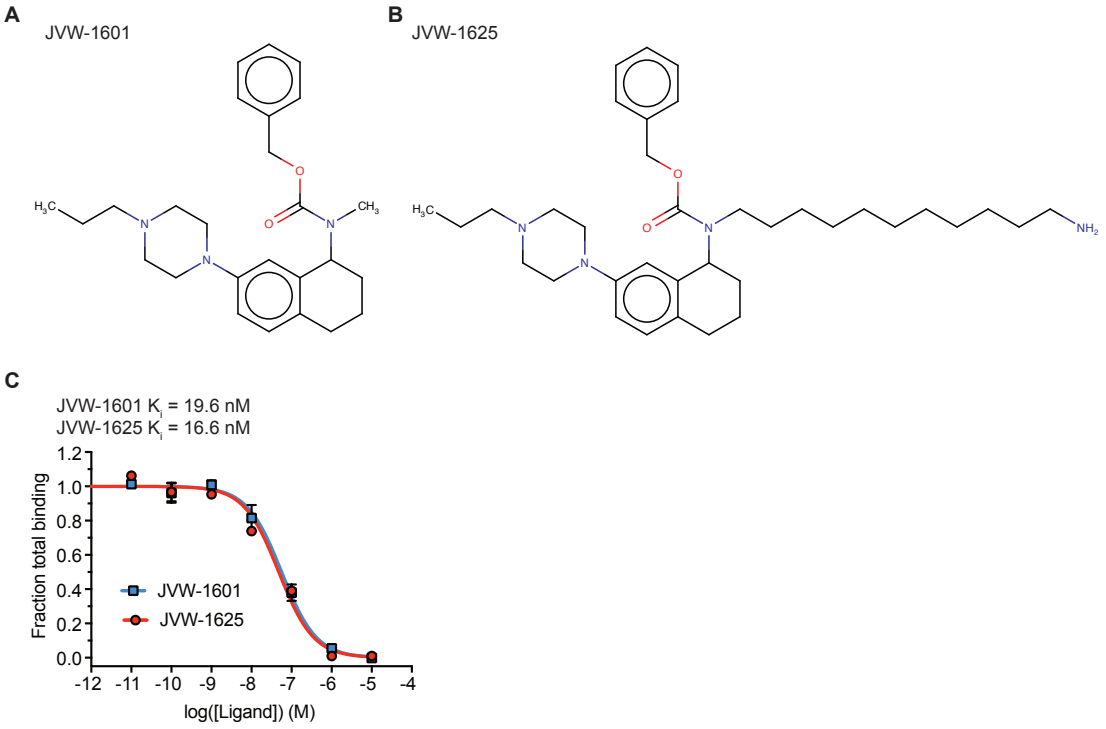


Fig. S1

Evaluation of immobilizable σ_2 ligands. (**A**) The structure of the compound JVW-1601, which was the parent compound for JVW-1625. (**B**) The full structure of JVW-1625, the final ligand that was ultimately attached to the column and used in the pull down to identify candidates for σ_2 receptor. (**C**) Full σ_2 receptor competition binding curve in PC-12 membranes confirming the high affinity of the JVW compounds for the σ_2 receptor.

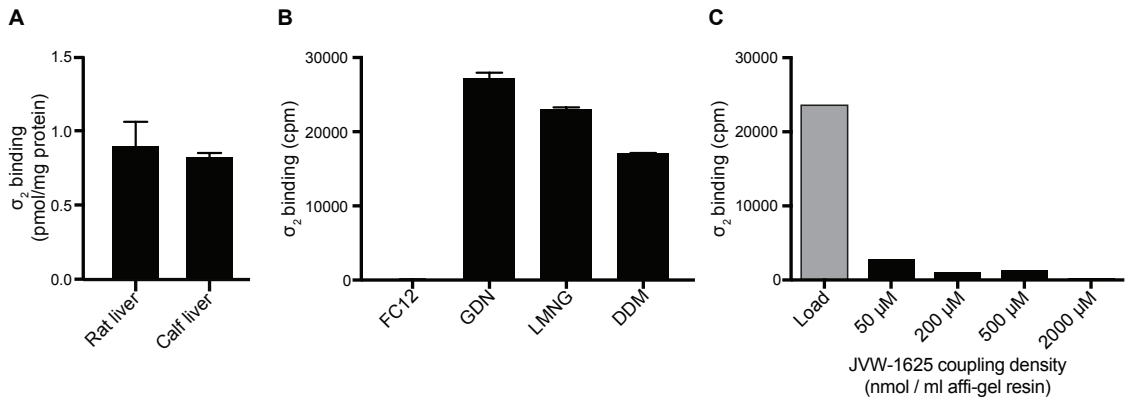


Fig. S2

Optimization of liver tissue extraction and capture. (**A**) Binding to rat and calf liver membranes was assessed by DTG binding at 300 nM, using a 1:10 isotopically dilution of [^{3}H]DTG. Values were normalized by membrane protein content. Data are shown as mean ± SEM from an experiment performed in duplicate. (**B**) Calf liver membranes were solubilized with the following detergents at 1% (w/v): fos-choline-12 (FC12), glyco-diosgenin (GDN), lauryl maltose neopentyl glycol (LMNG), and n-dodecyl-β-D-maltopyranoside (DDM). Extraction efficiency was assessed by single-point radioligand binding at 25 nM [^{3}H]DTG. Data are shown as mean ± SEM from an experiment performed in duplicate. (**C**) JVW-1625 was coupled to affi-gel 10 resin at indicated coupling densities. Calf liver membrane extract was flowed onto the column and capture of σ_2 was assessed by radioligand binding of the flow through using 25 nM [^{3}H]DTG.

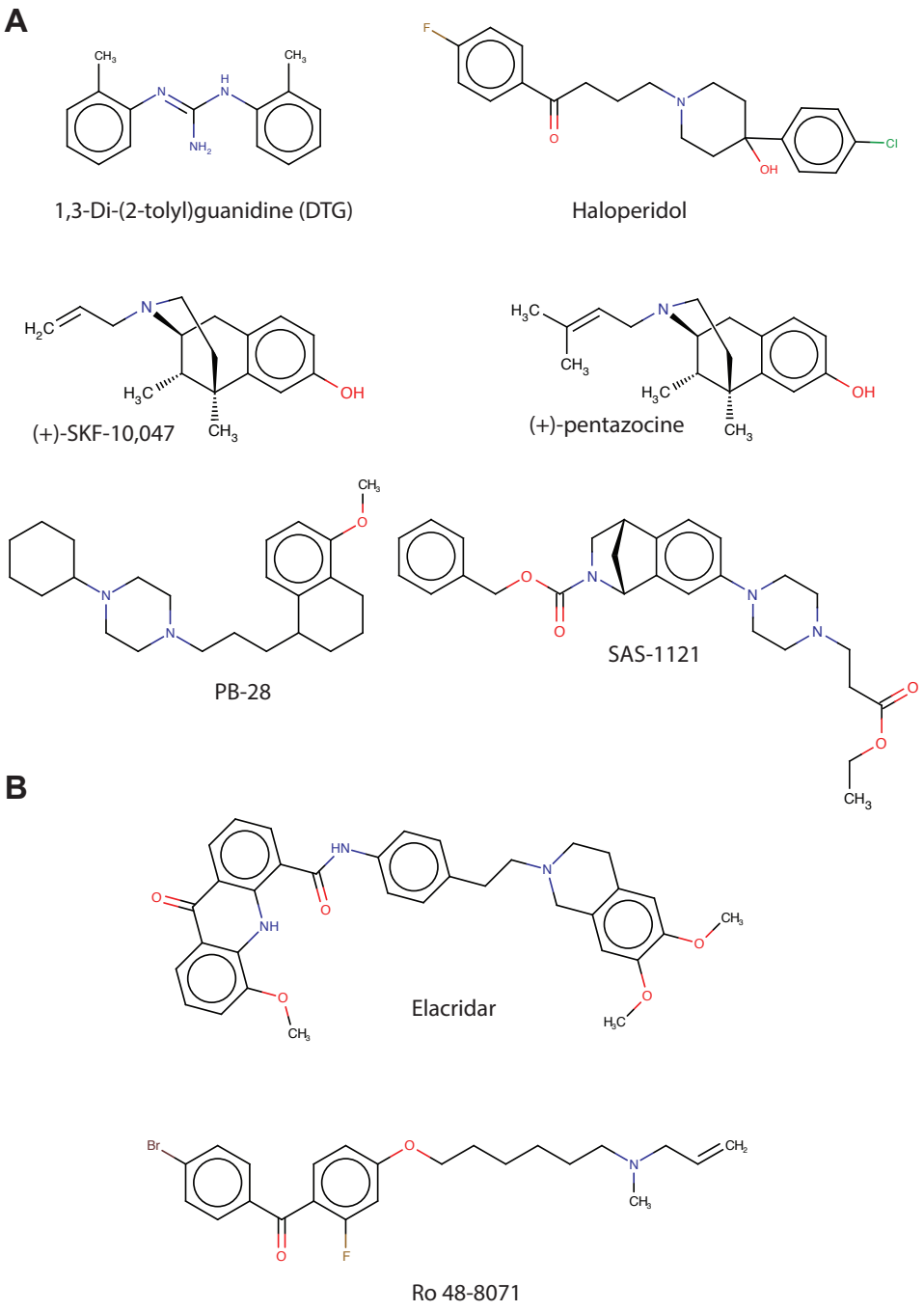


Fig. S3

Ligand structures. (**A**) The chemical structures of the six σ receptor ligands assessed in the [^{3}H]DTG competition binding assay with *Sf*9 membranes expressing human TMEM97. (**B**) The chemical structures of the two TMEM97 ligands assessed in parallel [^{3}H]DTG binding assays performed on *Sf*9 membranes expressing human TMEM97 and MCF-7 cell membranes.

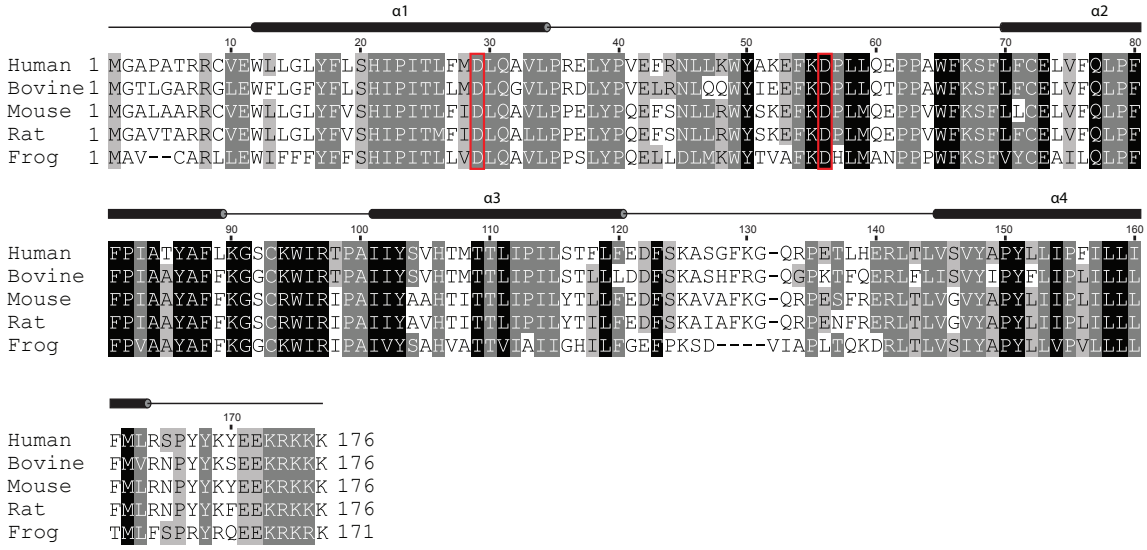


Fig. S4

Sequence conservation. The results of an alignment of 81 TMEM97 sequences from vertebrates with sequences from human (*Homo sapiens*), bovine (*Bos Taurus*), mouse (*Mus musculus)*, rat (*Rattus norvegicus)*, and frog (*Xenopus tropicalis)* displayed. Residues with 98%, 80%, and 60% similarity across all 81 species are shown in black, grey, and light grey respectively. TMHMM-predicted transmembrane helices are shown above the alignment. Numbering is according to the human TMEM97 gene. Red squares indicate residues essential for DTG binding (main text Figure 3).

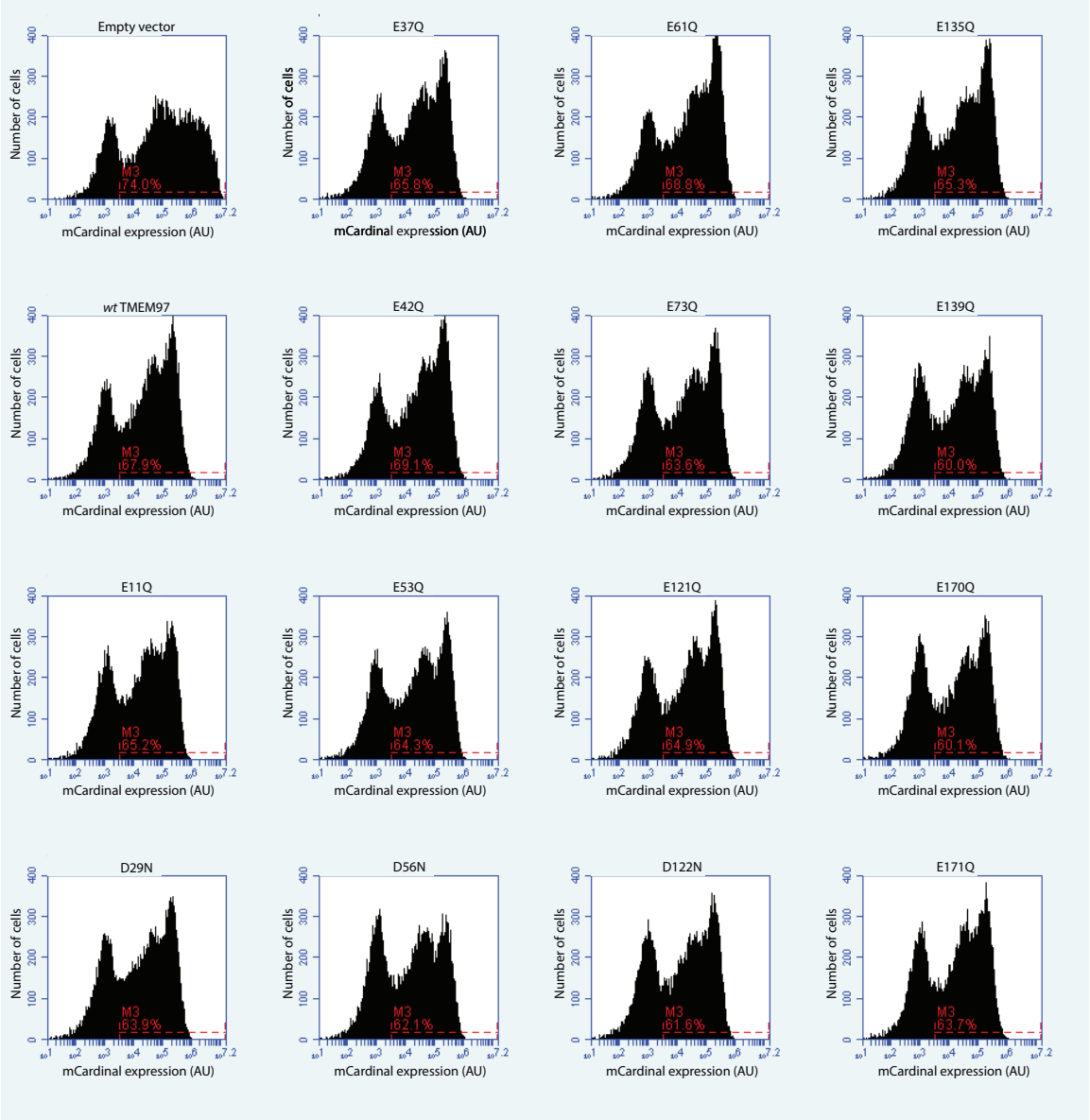


Fig. S5

Expression assessment of TMEM97 point mutants. Expi293 cells transfected with a vector that harbors empty vector, wild-type TMEM97, or TMEM97 mutants were assessed for expression using the fluorescent protein mCardinal as a reporter (see methods).