Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information
Synthesis, Functional and Binding Profile of (*R*)-Apomorphine Based Homobivalent Ligands Targeting the Dopamine D_2 Receptor

Jeremy Shonberg,[†] J. Robert Lane,[‡] Peter J. Scammells,[†,*] Ben Capuano[†,*]

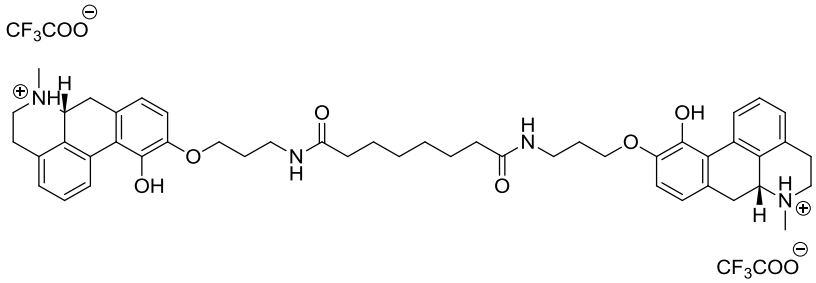
[†]Medicinal Chemistry and [‡]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052 Australia

GENERAL EXPERIMENTAL

Morphine was kindly donated by GlaxoSmithKline Australia. All other reagents were purchased from Aldrich or Alfa Aesar, and used without purification with the exception of methanesulfonic acid which was distilled prior to use. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 Ultrashield Plus spectrometer at 400.13 and 100.62 MHz respectively. Results were recorded as follows: chemical shift values are expressed as δ units acquired in $CDCl_3$, CD_3OD or CD_3CN with tetramethylsilane (0.00 ppm) as reference, multiplicity (*s* = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet), coupling constants (*J*) in Hertz and integration. Flash Chromatography was performed using Merck Silica Gel 60, 230-400 mesh. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE (TOF) using electrospray ionization (ESI) at a cone voltage of 50 V. Liquid chromatography–mass spectrometry (LCMS) data was obtained on an Agilent 1200 series LC coupled directly to a photodiode array detector and an Agilent 6100 Quadrupole MS, using a Phenomenex column (Luna 5 µm C8, 50 mm × 4.60 mm ID). Analytical reverse-phase high-performance liquid chromatography (HPLC) was performed on a Waters HPLC system fitted with a Phenomenex® Luna C8 (2) 100Å column (150 mm × 4.6 mm, 5 µm) using a binary solvent system; solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/80% ACN/H_2O. Gradient elution was achieved using 100% solvent A to 100% solvent B over 20 min at a flow rate of 1 mL/min. Preparative reverse phase-HPLC was performed using a Waters liquid chromatography system (Model 600 Controller and Waters 486 Tunable Absorbance Detector) with a Phenomonex Axia 10u Luna C8(2) 100A column (50 x 21.20 mm, 10 micron). Microwave reactions were performed in a CEM Discover® System at specified temperatures. All compounds were >95% purity by HPLC analysis at two wavelengths (λ = 254, 214 nm) prior to biological testing.

CHEMICAL SYNTHESIS OF BIVALENT LIGANDS

N^1,*N*^{8}-bis(3-(6a*R*)-(Apomorphin-*O*^{10}-yl)propyl)octanediamide ditrifluoroacetate (**7c**)



4 (102 mg, 316 µmol) was taken up in DCM (3 mL) and to the brown solution at rt was added *N,N*-diisopropylethylamine (55 µL, 316 µmol), octanedioic acid (25 mg, 144 µmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (140 mg, 316 µmol) under a nitrogen atmosphere. After stirring for 2 h, the mixture was evaporated to dryness and the product purified by flash column chromatography ($CHCl_3$/CH_3OH, 10:1 + 0.1% NH_4OH) to give a brown oil, then further purified by preparatory HPLC (100 – 30% Solvent A [99.9% water, 0.01% trifluoroacetic acid] in Solvent B [80% CH_3CN, 19.9% water, 0.01% trifluoroacetic acid]) to give the title compound as a white amorphous solid as the di-TFA salt (32 mg, 28%).

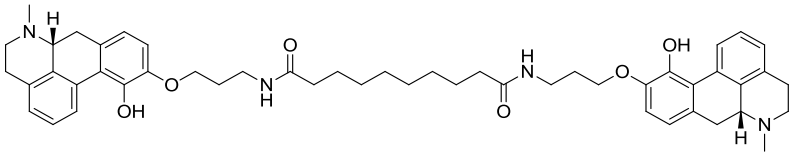
^{1}H NMR (400 MHz, CD_3CN) δ 8.30 (d, *J* = 7.9 Hz, 2H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.12 (d, *J* = 7.6 Hz, 2H), 6.82 (d, *J* = 8.1 Hz, 2H), 6.74–6.62 (m, 4H), 4.10–3.95 (m, 6H), 3.64 (dd, *J* = 11.7, 5.1 Hz, 2H), 3.46–3.16 (m, 10H), 3.05–2.90 (m, 8H), 2.83 (t, *J* = 13.6 Hz, 2H), 2.04 (t, *J* = 7.3 Hz, 4H), 1.90 (m, 4H), 1.47–1.35 (m, 4H), 1.18-1.15 (d, *J* = 7.1 Hzm, 4H).

^{13}C NMR (101 MHz, CD_3CN) δ 174.6 (C), 161.2 (q, $^2J_{CF}$ = 35.2 Hz, C), 147.1 (C), 145.3 (C), 133.0 (C), 131.0 (C), 129.3 (C), 128.9 (CH), 128.3 (CH), 128.2 (CH), 127.3 (C), 120.5 (C), 120.0 (CH), 117.5 (q, $^1J_{CF}$ = 292.1 Hz, C), 113.5 (CH), 67.2 (CH_2), 63.1 (CH), 53.1 (CH_2), 42.4 (CH_3), 36.8 (CH_2), 36.1 (CH_2), 32.0 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 26.6 (CH_2), 26.3 (CH_2).

HPLC t_R = 8.44 min, 99 % purity.

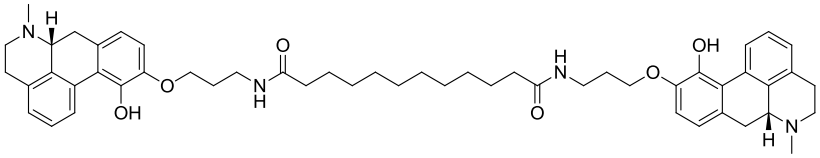
HRMS (*m/z*): $[M+2H]^{2+}/2$ calcd. for $C_{48}H_{58}N_4O_6$, 394.2251; found 394.2242.

N^{1},*N*^{10}-bis(3-(6a*R*)-(Apomorphin-*O*^{10}-yl)propyl)decanediamide (**7d**)



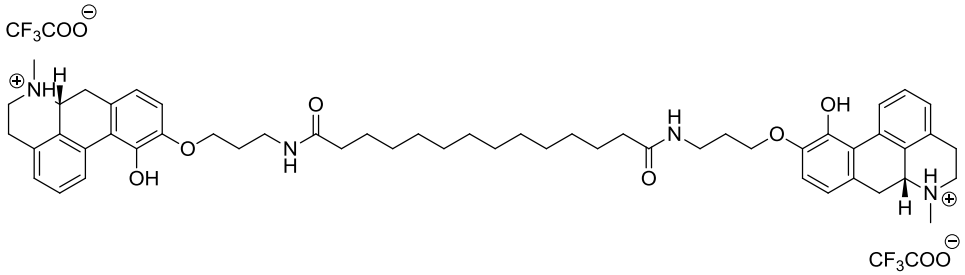
Prepared according to General Procedure. Decanedioc acid used as carboxylic acid. Isolated as the free base as a white amorphous solid (57 mg, 33%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.8 Hz, 2H), 7.16 (t, *J* = 7.7 Hz, 2H), 6.97 (d, *J* = 7.5 Hz, 2H), 6.69–6.61 (m, 4H), 5.84 (t, *J* = 6.0 Hz, 2H), 4.00 (td, *J* = 6.2, 1.4 Hz, 4H), 3.37 (dd, *J* = 12.8, 6.4 Hz, 4H), 3.18–2.92 (m, 8H), 2.71–2.62 (m, 2H), 2.53–2.40 (m, 10H), 2.08–2.00 (m, 4H), 1.90 (td, *J* = 6.2 Hz, 4H), 1.54–1.43 (m, 4H), ~~1.14~~1.31–1.16 (~~s~~m, 8H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8 (C), 145.2 (C), 144.0 (C), 134.51 (C), 132.7 (C), 131.7 (C), 130.4 (C), 127.6 (CH), 126.4 (CH), 126.3 (CH), 121.1 (C), 118.7 (CH), 111.4 (CH), 67.05 (CH_2), 62.6 (CH), 53.2 (CH_2), 44.1 (CH_3), 36.8 (CH_2), 36.4 (CH_2), 34.8 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 25.7 (CH_2).
HPLC t_R = 8.92 min, 95 % purity.
HRMS (*m/z*): $[MH]^+$ calcd. for $C_{50}H_{62}N_4O_6$, 815.4742; found 815.4764.

N^{1},*N*^{12}-bis(3-(6a*R*)-(Apomorphin-*O*^{10}-yl)propyl)dodecanediamide (**7e**)



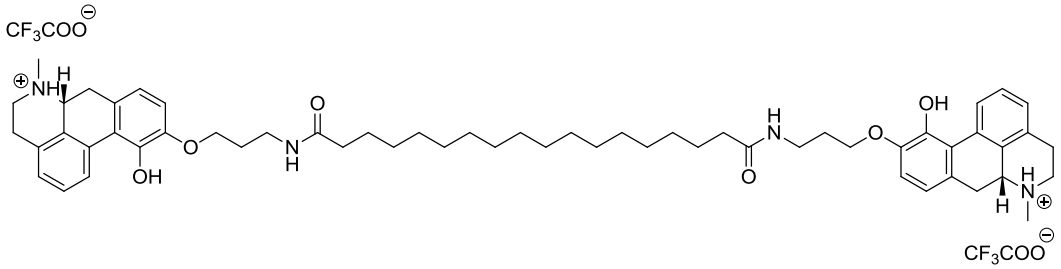
Prepared according to General Procedure. Dodecanedioic acid used as carboxylic acid. Isolated originally as the free base without preparatory HPLC for purification (16 mg, 8%), and converted later to the di-HCl salt to give a pale green amorphous solid.
^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (d, *J* = 7.7 Hz, 2H), 7.19 (t, *J* = 7.7 Hz, 2H), 7.02 (d, *J* = 7.4 Hz, 2H), 6.79 (d, *J* = 8.2 Hz, 2H), 6.70 (d, *J* = 8.1 Hz, 2H), 4.07 (t, *J* = 6.1 Hz, 4H), 3.40 (t, *J* = 6.7 Hz, 4H), 3.21–3.03 (m, 8H), 2.80–2.70 (m, 2H), 2.59–2.48 (m, 10H), 2.15 (t, *J* = 7.4 Hz, 4H), 2.04–1.95 (m, 4H), 1.60–1.50 (m, 4H), 1.~~18~~28–1.17 (~~dd, *J* = 14.7, 7.6 Hz~~m, 12H).
^{13}C NMR (101 MHz, CD_3OD) δ 176.5 (C), 147.3 (C), 145.4 (C), 134.8 (C), 133.4 (C), 133.1 (C), 130.3 (C), 128.2 (CH), 127.8 (CH), 127.3 (CH), 121.9 (C), 119.5 (CH), 112.3 (CH), 67.8 (CH_2), 64.0 (CH), 54.1 (CH_2), 44.0 (CH_3), 37.5 (CH_2), 37.2 (CH_2), 35.3 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 29.6 (CH_2), 27.0 (CH_2).
HPLC t_R = 10.16 min, 99 % purity.
HRMS (*m/z*): $[M-H]^-$ calcd. for $C_{52}H_{66}N_4O_6$, 841.4910; found 841.4945.

N^{1},*N*^{14}-bis(3-(6a*R*)-(Apomorphin-*O*^{10}-yl)propyl)tetradecanediamide ditrifluoroacetate (**7f**)



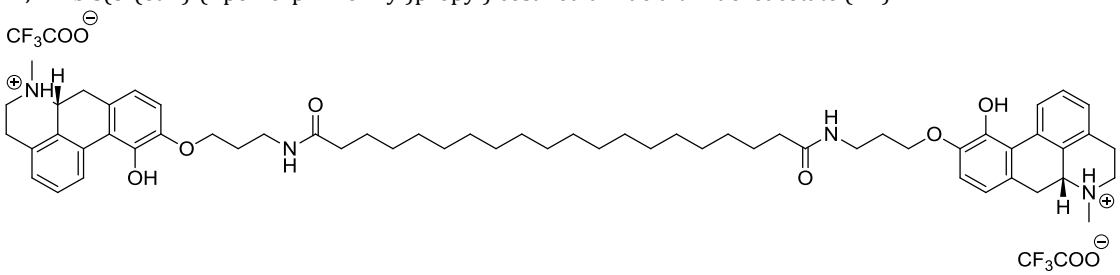
Prepared according to General Procedure. Tetradecanedioic acid used as carboxylic acid. Isolated as a white amorphous solid (21 mg, 25%).
^{1}H NMR (400 MHz, CD_3CN) δ 8.31 (d, *J* = 7.9 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.13 (d, *J* = 7.6 Hz, 2H), 6.85 (d, *J* = 8.1 Hz, 2H), 6.74 (d, *J* = 8.1 Hz, 2H), 6.69 (t, *J* = 4.9 Hz, 2H), 4.12–3.99 (m, 6H), 3.66 (dd, *J* = 11.6, 4.8 Hz, 2H), 3.46–3.20 (m, 10H), 3.08–2.91 (m, 8H), 2.85 (t, *J* = 13.6 Hz, 2H), 2.10 (t, *J* = 7.2 Hz, 4H), 1.98–1.87 (m, 4H), 1.53–1.42 (m, 4H), 1.24–1.07 (m, ~~5 Hz,~~16H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.8 (C), 161.3 (q, $^{2}J_{CF}$ = 35.2 Hz, C), 147.1 (C), 145.4 (C), 133.0 (C), 131.0 (C), 129.3 (C), 128.9 (CH), 128.4 (CH), 128.2 (CH), 127.4 (C), 120.5 (C), 120.0 (CH), 117.5 (q, $^{1}J_{CF}$ = 292.1 Hz (C), 113.6 (CH), 67.3 (CH_2), 63.2 (CH), 53.2 (CH_2), 42.5 (CH_3), 36.9 (CH_2), 36.1 (CH_2), 32.1 (CH_2), 30.1 (CH_2), 30.1 (CH_2), 30.0 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 26.6 (CH_2), 26.5 (CH_2).
HPLC t_R = 10.10 min, >99 % purity.
HRMS (*m/z*): $[MH]^+$ calcd. for $C_{54}H_{70}N_4O_6$, 871.5368; found 871.5411

N[1],*N*[18]-bis(3-(6a*R*)-(Apomorphin-*O*[10]-yl)propyl)octadecanediamide ditrifluoroacetate (**7g**)



Prepared according to General Procedure. Octadecanedioic acid used as carboxylic acid. Isolated as a white amorphous solid (45 mg, 42%).
^{1}H NMR (400 MHz, CD_3CN) δ 8.30 (d, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 2H), 7.13 (d, *J* = 7.5 Hz, 2H), 6.84 (d, *J* = 8.2 Hz, 2H), 6.73 (d, *J* = 7.7 Hz, 4H), 4.13–3.96 (m, 6H), 3.66 (dd, *J* = 11.2, 4.5 Hz, 2H), 3.45–3.21 (m, 10H), 3.07–2.90 (m, 8H), 2.84 (t, *J* = 13.7 Hz, 2H), 2.11 (t, *J* = 7.3 Hz, 4H), 1.93–1.88 (m, 4H), 1.59–1.43 (m, 4H), 1.20–1.17 (d, *J* = 10.6 Hz, 24H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.9 (C), 161.2 (q, $^2J_{CF}$ = 35.1 Hz, C), 147.2 (C), 145.4 (C), 133.1 (C), 131.0 (C), 129.3 (C), 129.0 (CH), 128.4 (CH), 128.3 (CH), 127.3 (C), 120.6 (C), 120.1 (CH), 117.4 (q, $^1J_{CF}$ = 292.5 Hz, C), 113.6 (CH), 67.4 (CH_2), 63.4 (CH), 53.3 (CH_2), 42.6 (CH_3), 37.0 (CH_2), 36.3 (CH_2), 32.1 (CH_2), 30.29 (CH_2), 30.28 (CH_2), 30.24 (CH_2), 30.16 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 26.7 (CH_2), 26.6 (CH_2).
HPLC t_R = 11.07 min, 99 % purity.
HRMS (*m/z*): $[MH]^+$ calcd. for $C_{58}H_{78}N_4O_6$, 927.5994; found 927.6023.

N[1],*N*[20]-bis(3-(6a*R*)-(Apomorphin-*O*[10]-yl)propyl)icosanediamide ditrifluoroacetate (**7h**)



Prepared according to General Procedure. Icosanedioic acid used as carboxylic acid. Isolated as a white amorphous solid (14 mg, 14%).
^{1}H NMR (400 MHz, CD_3CN) δ 8.32 (d, *J* = 7.8 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 7.5 Hz, 2H), 6.87 (d, *J* = 8.2 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 2H), 6.65 (br s, 2H), 4.12–3.99 (m, 6H), 3.73–3.64 (m, 2H), 3.49–3.15 (m, 10H), 3.09–2.93 (m, 8H), 2.86 (t, *J* = 13.8 Hz, 2H), 2.11 (t, *J* = 7.3 Hz, 4H), 1.95–1.87 (m, 4H), 1.47–1.56 (m, 4H), 1.21 (mbr s, 28H).
^{13}C NMR (101 MHz, CD_3CN) δ 174.8 (C), 161.2 (q, $^2J_{CF}$ = 35.2 Hz, C), 147.1 (C), 145.5 (C), 133.1 (C), 131.0 (C), 129.3 (C), 129.0 (CH), 128.4 (CH), 128.3 (CH), 127.4 (C), 120.6 (C), 120.0 (CH), 117.4 (q, $^1J_{CF}$ = 292.2 Hz, C), 113.6 (CH) 67.3 (CH_2), 63.4 (CH), 53.3 (CH_2), 42.6 (CH_3), 37.0 (CH_2), 36.2 (CH_2), 32.1 (CH_2), 30. (6 × CH_2), 30.22 (CH_2), 30.15 (CH_2), 30.0 (CH_2), 29.84 (CH_2), 29.81 (CH_2), 26.7 (CH_2), 26.6 (CH_2).
HPLC t_R = 11.56 min, 99% purity.
HRMS (*m/z*): $[MH]^+$ calcd. for $C_{60}H_{82}N_4O_6$, 955.6307; found 955.6317.

BIOLOGICAL ASSAYS

Cell Culture

Chinese hamster ovary (CHO) FlpIn cells were stably transfected with the human D_2(long) dopamine receptor (D_2-CHOFlpIn). Cells were grown and maintained in DMEM containing 20mM HEPES, 5% fetal bovine serum and 200 µg/mL Hygromycin-B. Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2, 95% O_2. For ERK1/2 phosphorylation assays, cells were seeded into 96-well silicon coated plates at a density of 50,000 cells/well. After 4 hours, cells were washed twice with PBS and then maintained in DMEM containing 20 mM HEPES for at least 16 hours before assaying.

ERK1/2 phosphorylation

Dose-response experiments in the absence or presence of ligand were performed at 37 °C in a 200 µL total volume of DMEM containing 20 mM HEPES and 0.1 % ascorbic acid. Kinetic experiments were performed to determine the time of peak pERK1/2 response for each ligand tested. For all compounds dose-response experiments were performed using a stimulation time of 5 min. Stimulation of cells was terminated by the removal of media and the addition of 100 µL of SureFire™ lysis buffer to each well. The plate was agitated for 1-2 min. A 4:1 v/v dilution of Lysate:SureFire™ activation buffer was made in a total volume of 50 µL. A 1:100:120 v/v dilution of AlphaScreen™ beads:activated lysate mixture:SureFire™ reaction buffer in an 11 µL total volume was then transferred to a white opaque 384-well Proxiplate™ in the dark. This plate was then incubated in the dark at 37 °C for 1.5 hours after which time the fluorescence signal was measured by a Fusion-™ plate reader (PerkinElmer), using standard AlphaScreen™ settings.

Data analysis

In the functional ERK1/2 assay, agonist concentration response curves were fitted to the following four-parameter Hill equation using Prism 5;

$$response = \frac{(top - bottom)}{1 + (10^{\log EC_{50}} / x)^{n_H}} \quad (1)$$

where top represents the maximal asymptote of the concentration response curves, bottom represents the lowest asymptote of the concentration-response curves, logEC_{50} represents the logarithm of the agonist EC_{50}, x represents the concentration of the agonist and n_H represents the Hill slope.

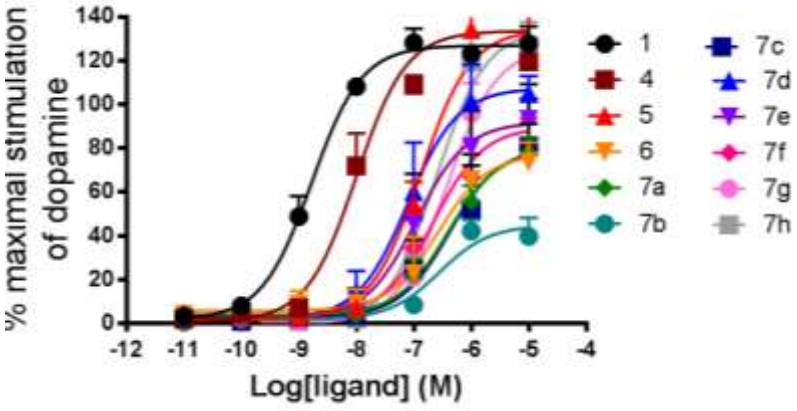


Figure SI-1. Ability of increasing concentrations of (*R*)-apomorphine (**1**), monovalent ligands (**4-6**) and homobivalent ligands (**7a-h**) to stimulate ERK1/2 phosphorylation in a SureFire AlphaScreen™ assay using CHO cells stably expressing the D_2R. Data represents three separate experiments performed in duplicate.

[^{3}H]Spiperone Binding Assay

Cell membranes of FlpIN CHO cells expressing the D_2R (3 µg) were incubated with varying concentrations of test compound in binding buffer (20 mM HEPES, 6 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA; pH 7.4) containing 0.05 nM of [^{3}H]spiperone to a final volume of 1 mL and incubated at 37°C for 3 hours. Binding was terminated by fast flow filtration over GF/B membranes using a brandel harvester followed by three washes with ice-cold 0.9% NaCl. Bound radioactivity was measured in a Tri-Carb 2900TR liquid scintillation counter (Perkin Elmer).

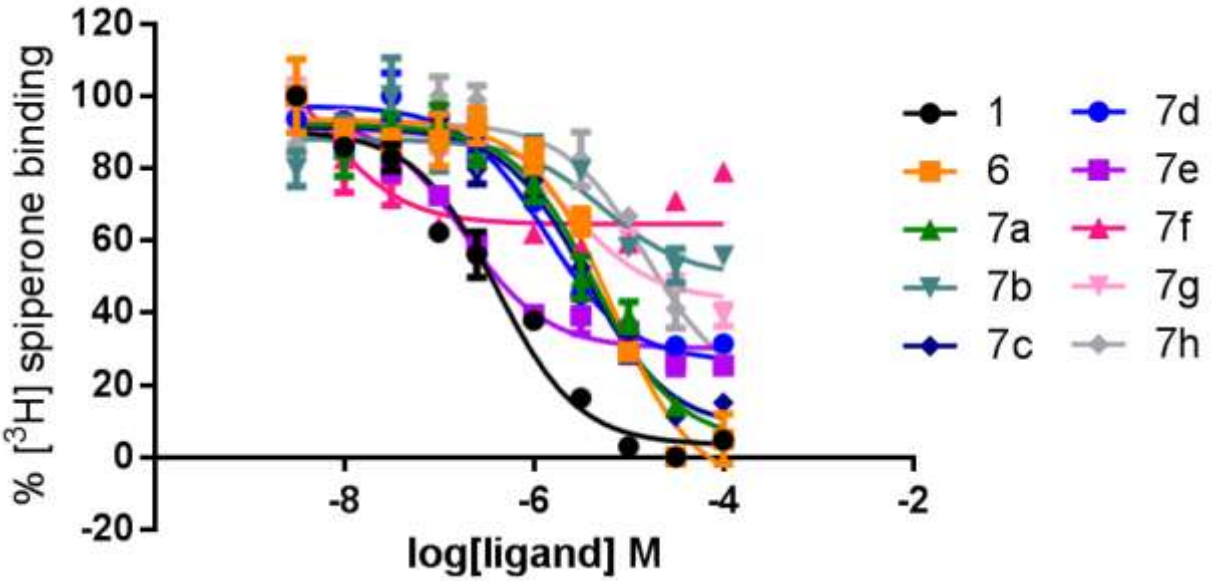
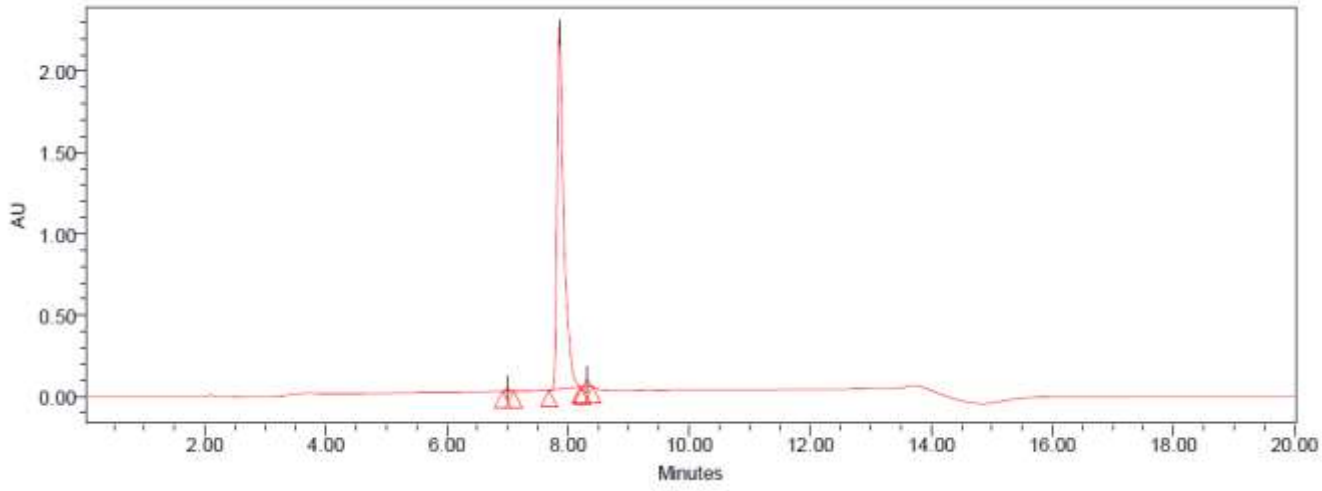


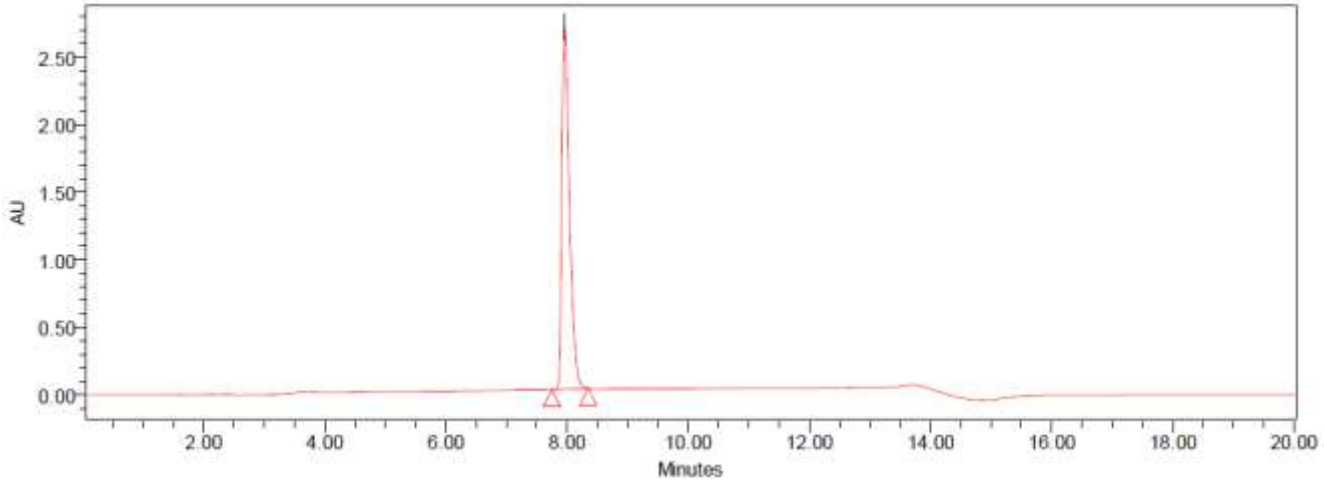
Figure SI-2. Ability of increasing concentrations of (*R*)-apomorphine (**1**), monovalent ligand (**6**) and homobivalent ligands (**7a-h**) to displace the radiolabeled antagonist [^{3}H]spiperone at human $D_{2L}R$ expressed in FlpIn CHO cell membranes.

Analytical HPLCs for Bivalent Ligands (λ = 214 nm)

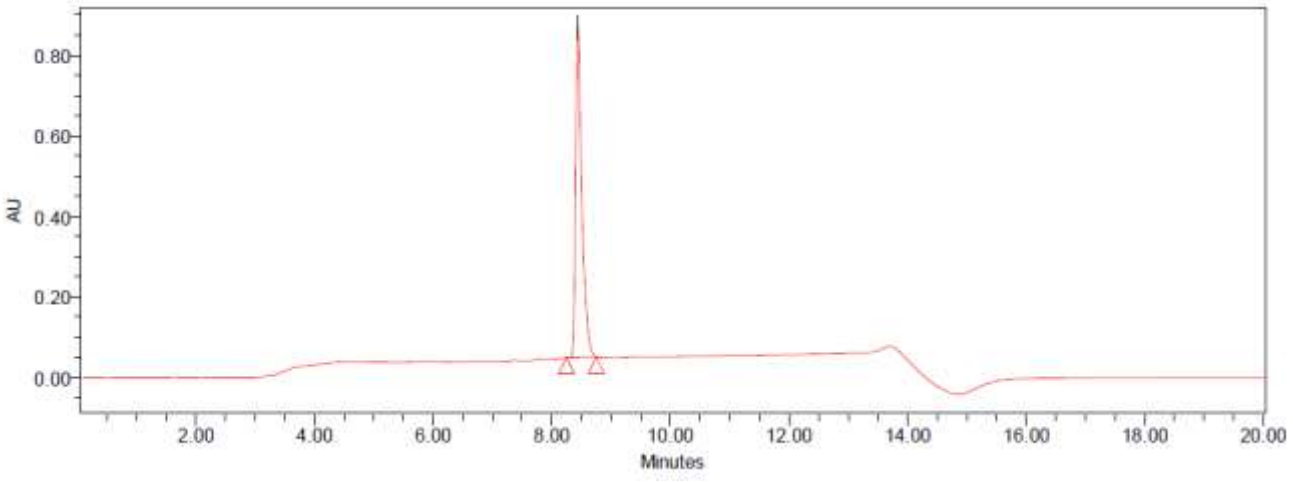
7a



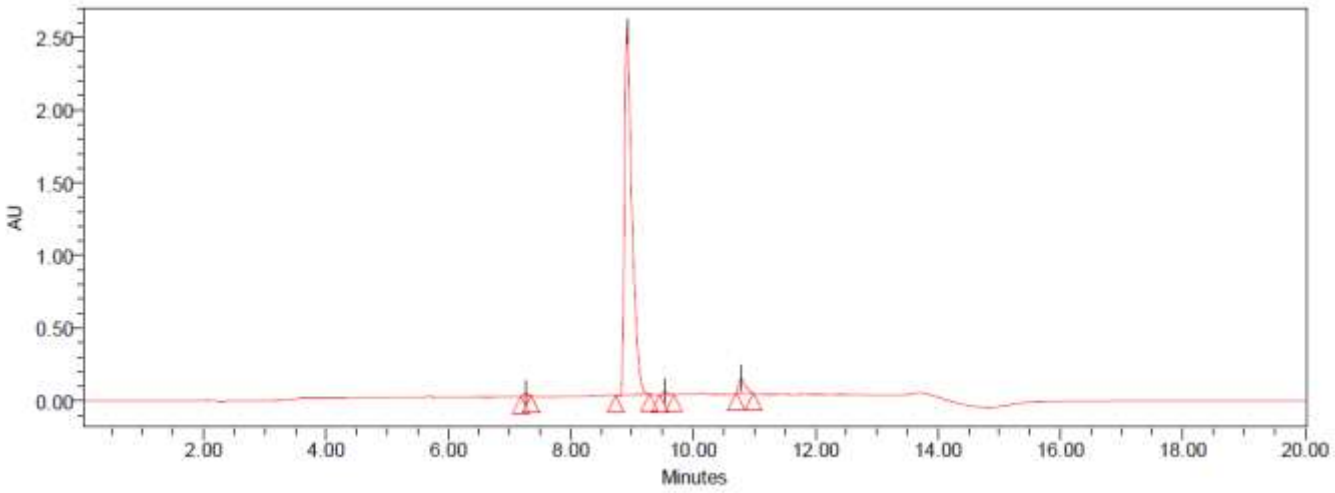
7b



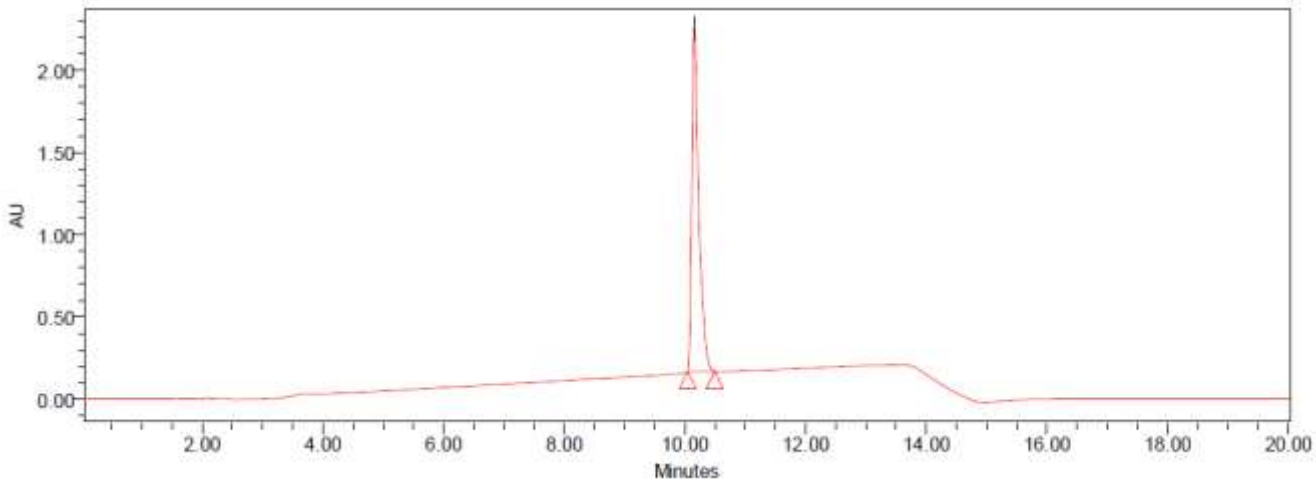
7c



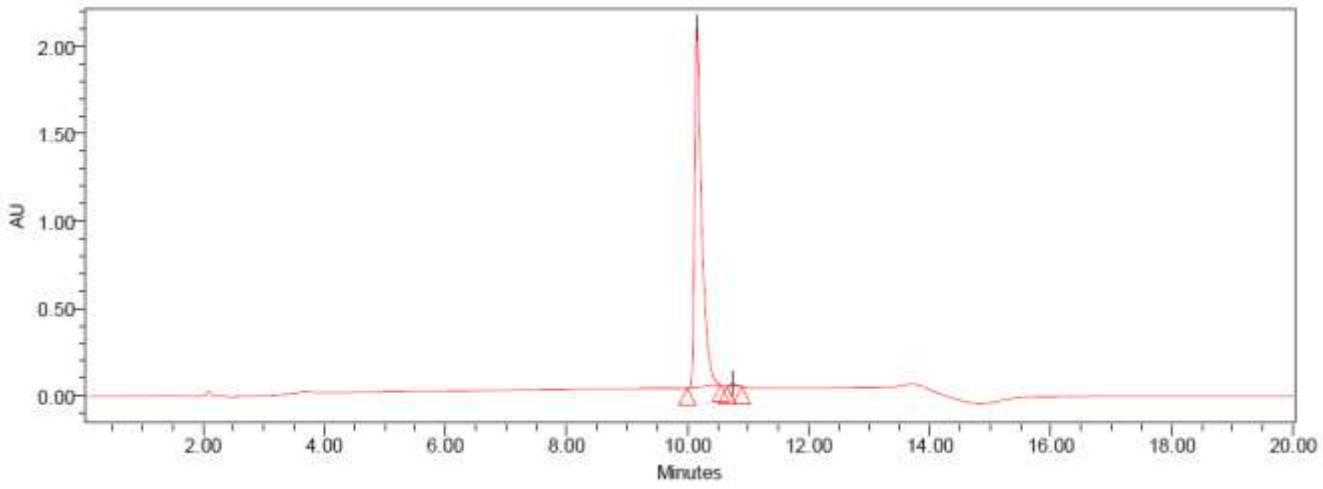
7d



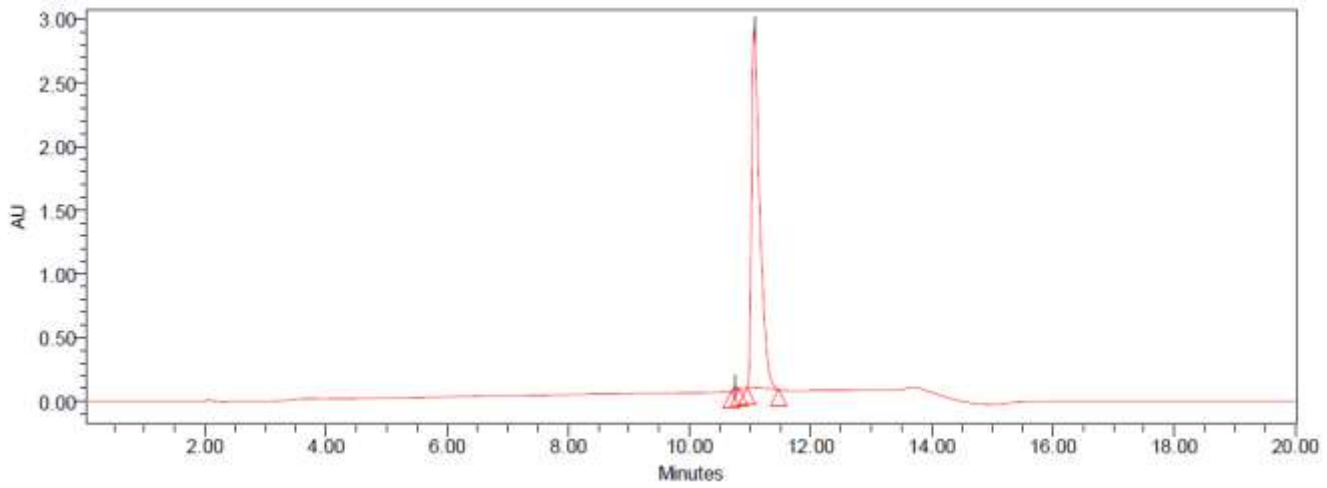
7e



7f



7g



7h