Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.


# Investigation of novel ropinirole analogues: Synthesis, pharmacological evaluation and computational analysis of dopamine $D_2$ receptor functionalized congeners and homobivalent ligands

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## Experimental Section

### General Information

All reactions were stirred magnetically in oven-dried glassware. Anhydrous solvents were transferred via oven-dried syringe or cannula. Technical grade solvents used for extraction and column chromatography were distilled prior to use. Absolute solvents were used without further purification. Ropinirole hydrochloride was purchased from Betapharma Shanghai Co., Ltd. and 4-(2-hydroxyethyl)indolin-2-one was purchased from China Langchem Inc. All other reagents were purchased from Merck, TCI Chemicals, Aldrich or AK Scientific in the highest available grade and used without further purification. Phase separation funnels of the type ISOLUTE ® SPE Accessories from Biotage were used to dry organic layers after extractions. Analytical thin layer chromatography (TLC) plates from Merck were used for reaction control (silica gel 60 on aluminium sheets). Silica gel 60 (Fluka) was used for silica gel flash chromatography.

### Analytical Methods

Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on Bruker spectrometers Avance 400 (400 MHz for $^1$H and 101 MHz for $^{13}$C) at ambient temperature in the solvents indicated and referenced to tetramethylsilane (TMS). $^{13}$C NMR spectra were routinely run with broadband decoupling.

Distortionless enhancement by polarization transfer (DEPT) experiments were routinely used for $^{13}C$ NMR spectra. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Diffusion ordered spectroscopy (DOSY) spectra were acquired on a Bruker AVANCE 600 MHz NMR spectrometer using a pseudo 2D version of a stimulated echo sequence with a 50 ms longitudinal echo gradient delay and bipolar gradient pulses of 1 ms.[1] Each pseudo 2D spectrum consisted of 12 1D spectra where the gradient strength was varied linearly from 5 to 95%. Spectra were processed using Topspin 1.3. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE (TOF) spectrometer fitted with an electrospray ion source. Mass signals are given in mass units per charge (*m/z*). The fragments and intensities are written in brackets. Liquid Chromatography Mass Spectra (LCMS) were measured on either one of the two instruments. Instrument 1 (LCMS-1): Agilent 6100 Series Single Quad LC/MS, Agilent 1200 Series HPLC. (Pump: 1200 Series G1311A Quaternary pump, Autosampler: 1200 Series G1329A Thermostatted Autosampler, Detector: 1200 Series G1314B Variable Wavelength Detector). Gradient takes 4 minutes to get to 100% acetonitrile; maintain for 3 minutes and a further 3 minutes to return to the original 5% acetonitrile. Instrument 2 (LCMS-2): Waters Acquity system with a Waters column (Acquity UPLC BEH C18 2.1 × 5.0 mm, 1.7 μm) running a water/acetonitrile (0.05% formic acid or ammonium hydroxide) gradient over 2 minutes with a flow rate of 1 mL/min. The detectors used were a PDA (210 – 450 nm), MS+ (100 – 1700 m/z), MS- (100 – 1700 *m/z*) and ELS detector. The gradients maintains 10 seconds at 97% water, takes 100 seconds to get to 3% water, maintains 40 seconds and a further 10 seconds to back to the original 3% acetonitrile. Preparative HPLC was performed on a Waters MDAP (Mass Directed Auto Preparative) system with a Waters column (XBridge C18 19 × 150 mm or 30 × 100 mm, 5 μm) running a generic water/acetonitrile (with 0.2% diethylamine) gradient over 15 minutes with a flow rate of 20 or 40 mL/min. The detectors used were a PDA (210 – 450 nm), MS+ (100 – 1700 *m/z*), MS- (100 – 1700 *m/z*) and optional ELS detector. Analytical reverse-phase HPLC was carried out on a Waters Millenium 2690 system, fitted with a Phenomenex Luna C8, 100 Å, 5 μm (50 × 4.60 mm i.d.) column. A binary solvent system was used (solvent A, 0.1% TFA/ water; solvent B, 0.1% TFA/19.9% water/ 80% acetonitrile), with UV detection at 214 and 254 nm. Used gradient elution, beginning with 100% solvent A and going to 20% solvent A/ 80% solvent B over 20 min

at a flow rate of 1 mL/min. Melting Points were measured with a MP50 Melting Point System from Mettler Toledo.

Biological assay

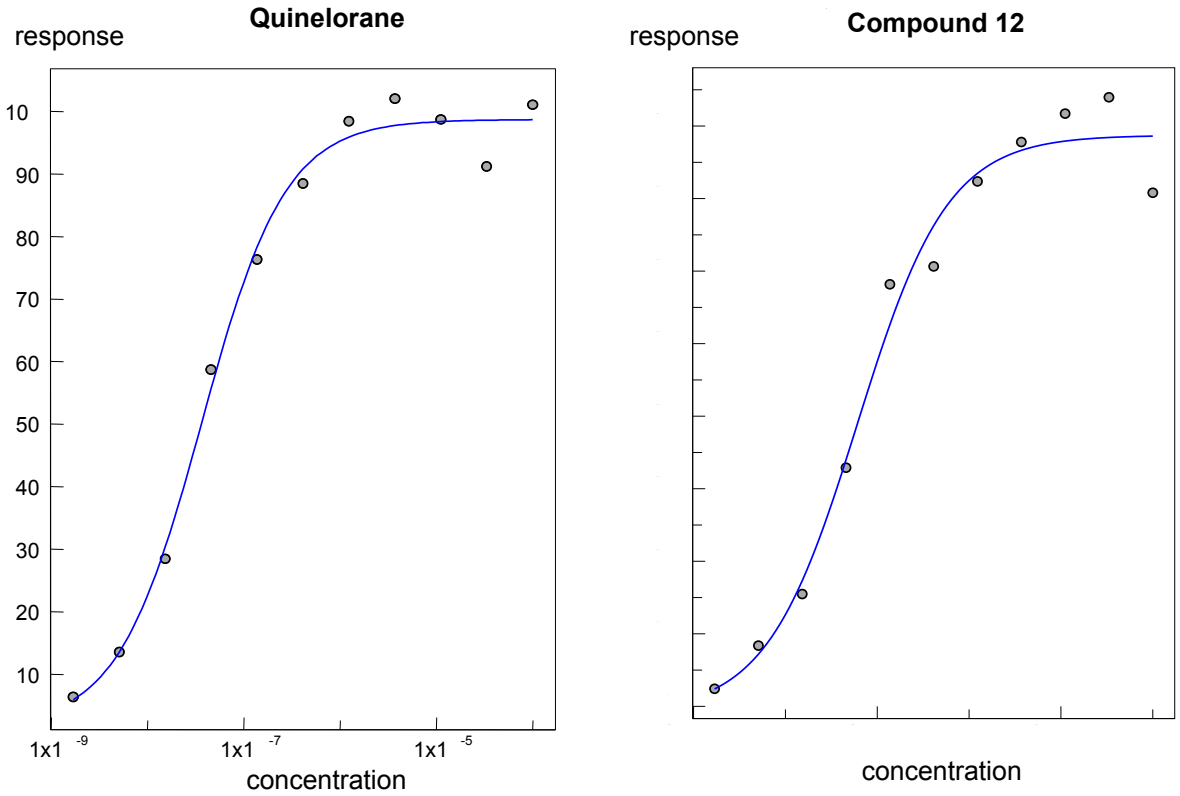
**Cell culture:** Chinese hamster ovary (CHO) cells stably expressing human $D_{2L}$ dopamine receptors (Wilson *et al.*, 2001) were grown in Dulbecco's modified Eagle's medium containing 5% foetal bovine serum and 400 mg/ml active geneticin (to maintain selection pressure). Cells were grown at 37 °C in a humidified atmosphere of 5% $CO_2$.

**Membrane preparation:** Membranes were prepared from CHO cells expressing $D_{2L}$ dopamine receptors as described previously (Castro and Strange, 1993). Briefly, confluent 175 $cm^2$ flasks of cells were washed once with 10 ml 4-(2-hydroxyethyl)-1-piperazineethyl-sulphonic acid (HEPES) buffer (11.9 g/L HEPES, 2 ml/L ethylenediaminetetraacetic acid ((EDTA), 47 mg/L leupeptin, 25 mg/mL bacitracin; pH 7.4 using KOH). The cell pellet was resuspended in 10 volumes of buffer. Pepstatin ($2 \times 10^{-6}$ M) and PMSF (1 mM) were added to Waring blender and homogenized for 15 secs (full speed). Homogenate was left to stand on ice for 5 mins and homogensized for further 15 secs and let it stand for another 30 mins (500 ml blender for volumes up to 200 ml, 1 L blender for volumes 200 ml – 500 ml. Volumes larger than 500 ml are split and processed in smaller aliquots). The liquid had settled after 30 mins and was transfered to 500 ml centrifuge tubes and centrifuged at 1200 rpm, 4 °C for 10 mins. Supernatant was transferred to Beckman 70 ml ultracentrifuge tubes and centrifuged at 24,800 rpm for 36 mins. Supernatant was discarded and pellet was resuspend in approx 4 volumes of buffer using a 20ml syringe. Once the membrane pellet was an even suspension it was passed through a 0.6 x 25 mm needle and dispensed into 1 ml aliquots and stored at -80 °C until use. Protein concentration was determined using a BCA assay (determined by the method of Lowry *et al.*, 1951).

**[$^{35}$S]GTPγS binding assays:** Cell membranes (20 µg/ml) were pre-incubated for 30 min at room temperature in 20 mM HEPES buffer containing 1 µM GDP, 5 mM NaCl, 95 mM NMDG and 10 mM $MgCl_2$, pH 7.4. Also 30 µg/ml Saponin, 0.01% ploronic F-127 and 5 mg/ml of PS-WGA beads were also added before the incubation. After the addition of ligands (in duplicated, 1/3 dilution serial response) the assay was initiated by adding [$^{35}$S]GTPγS to give a final concentration of 500 pM. The assay was incubated for 1 hour and then plates were centrifuged at 1200 rpm for 2 min before to read them in a ViewLux reader from PerkinElmer.

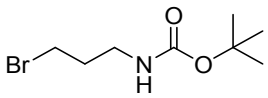
**Data analysis:** Results in the text are shown as means ± SEM, along with the number of experiments. Quinelorane was used as pharmaceutical standard compound as well as dopamine and (-)-3PPP (preclamol) in order to check the assay robustness, variability and validate the data arisen. Data from [$^{35}$S]GTPγS binding experiments were fitted to a sigmoidal concentration/ response curve with a Hill coefficient of one which provided the best fit to the data in all cases ($P$<0.05). Data were fitted well by mono-exponential equations from which the apparent first-order rate constant ($k$, min$^{-1}$) and maximal binding ($B_{max}$, fmol mg$^{-1}$) values could be extracted. The initial rate of [$^{35}$S]GTPγS binding was calculated as $k.B_{max}$ in fmol mg$^{-1}$ min$^{-1}$. Observed was a typical fold increase of about 3 over basal binding (signal to background), from 180 to 540 cpm approximately. The maximum response values are calculated by normalizing to 0% activation (DMSO, solvent) and 100% activation (quinelorane at 100 µM, $hD_2$ agonist).

**Representative curves:** Dose response curve (n= 1) of quinelorane (reference compound) and compound **12** in [$^{35}$S]GTPγS assay.



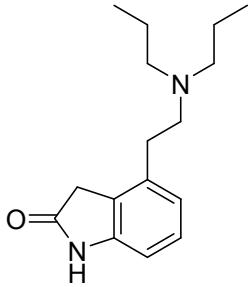
Synthesis

tert-*Butyl (3-bromopropyl)carbamate* ($C_8H_{16}BrNO_2$)



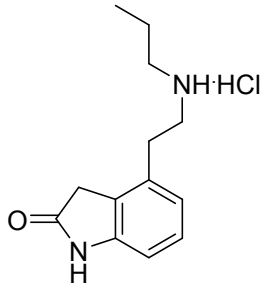
3-Bromopropan-1-amine hydrobromide (2.00 g, 9.14 mmol) and di-*tert*-butyl dicarbonate (1.83 g, 8.40 mmol) were dissolved in $CH_2Cl_2$ (30 mL). $Et_3N$ (1.27 mL, 9.14 mmol) was added dropwise and the reaction was stirred at room temperature for 1.5 h. After this time, further $CH_2Cl_2$ (20 mL) was added and the reaction mixture was washed with saturated $KHSO_4$ solution. The organic layer was dried with a phase separating funnel and the solvent was removed under reduced pressure. Purification by column chromatography (petroleum spirits → petroleum spirits: EtOAc 7:3) afforded the title compound (1.71 g, 86%) as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$): δ 4.36 (br s, 1H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.27 (q, *J* = 6.4 Hz, 2H), 2.05 (m, 2H), 1.44 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$): δ 156.0 (C), 66.8 (C), 39.0 ($CH_2$), 32.7 ($CH_2$), 30.8 ($CH_2$), 28.4 ($CH_3$).

*4-(2-(Dipropylamino)ethyl)indolin-2-one (ropinirole)(**1**)* ($C_{16}H_{24}N_2O$)



4-(2-Dipropylamino)ethyl)indolin-2-one hydrochloride (**2**) (1.05 g, 3.54 mmol) was dissolved in 1 M aq. NaOH solution (100 mL) and stirred for 15 min. $CH_2Cl_2$ (100 mL) was added and the reaction mixture was stirred for a further 15 min. The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 × 50 mL). The combined organic layers were washed with water, dried with anhydrous $Na_2SO_4$, filtered and the solvent removed under reduced pressure to afford the title compound **1** (867 mg, 94%) as a light-purple oil. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.84 (s, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.4 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 3.50 (s, 2H), 2.67 (m, 4H), 2.45 (m, 4H), 1.46 (m, 4H), 0.88 (t, *J* = 7.4 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.2 (C), 142.7 (C), 137.1 (C), 128.0 (CH), 124.1 (C), 122.8 (CH), 107.7 (CH), 56.1 ($CH_2$), 54.2 ($CH_2$), 35.2 ($CH_2$), 30.7 ($CH_2$), 20.2 ($CH_2$), 12.0 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 261.2 ($MH^+$, 100).

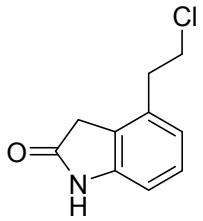
*4-(2-(Propylamino)ethyl)indolin-2-one hydrochloride (**4** )* ($C_{13}H_{19}ClN_2O$)




**Method A:** 1-Chloroethyl chloroformate (207 µL, 1.92 mmol) was added to a mixture of 4-(2-(dipropylamino)ethyl)indolin-2-one (**1**) (100 mg, 384 µmol) and $NaHCO_3$ (161 mg, 1.92 µmol) in 1,2-dichloromethane (1.5 mL). The reaction mixture was heated to 85 °C and stirred for 17 h, then cooled to room temperature and filtered. The filtrate was evaporated to dryness under reduced pressure and the resultant residue dissolved in MeOH (10 mL) and stirred at reflux for 18 h. The MeOH was removed under reduced pressure and the crude material was purified by column chromatography ($CH_2Cl_2$ → $CH_2Cl_2$: MeOH 5:2) to furnish the title compound **4** (55 mg, 43%) as a white solid, mp: 288-292 °C.

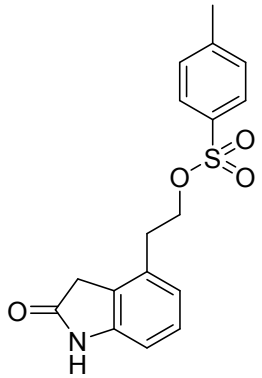
**Method B:** 2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**6**) (2.00 g, 60.4 mmol) was dissolved in propylamine (3.57 g, 60.4 mmol). The reaction mixture was stirred at reflux for 1.5 h then partitioned between EtOAc and 1 M aq. $K_2CO_3$ solution. The aqueous layer was removed and the organic layer was extracted with 1 M aq. HCl solution. The aqueous layer was concentrated under reduced pressure and the resultant residue suspended in MeOH, then filtered and washed with additional MeOH. The filter cake was dried on high vacuum overnight affording the title compound **4** (1.13 g, 74%) as a yellow solid. $^1$H NMR (400 MHz, $d_6$-DMSO) δ 10.43 (s, 1H), 9.05 (br s, 2H), 7.14 (t, $J$ = 7.7 Hz, 1H), 6.82 (d, $J$ = 7.7 Hz, 1H), 6.73 (d, $J$ = 7.7 Hz, 1H), 3.52 (s, 2H), 3.07 (m, 2H), 2.97 – 2.80 (m, 4H), 1.65 (m, 2H), 0.92 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $d_6$-DMSO): δ 176.1 (C), 143.7 (C), 133.2 (C), 127.8 (CH), 124.9 (C), 121.4 (CH), 107.7 (CH), 48.2 ($CH_2$), 46.3 ($CH_2$), 34.6 ($CH_2$), 29.0 ($CH_2$), 18.9 ($CH_2$), 11.0 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 219.2 ($MH^+$, 100). HRMS ($C_{13}H_{18}N_2O$): Calcd. 219.1492 $[M+H]^+$, Found 219.1497. HPLC: $t_R$ 7.34 min, 98% (214 nm), 97% (254 nm).

*4-(2-Chloroethyl)indolin-2-one (**3**)* ($C_{10}H_{10}ClNO$)




Obtained byproduct when using method A to synthesize compound **4**. $^1$H NMR (400 MHz, $d_6$-DMSO) δ 10.36 (s, 1H), 7.12 (t, $J$ = 7.8 Hz, 1H), 6.85 (d, $J$ = 7.7 Hz, 1H), 6.70 (d, $J$ = 7.6 Hz, 1H), 3.84 (t, $J$ = 7.2 Hz, 2H), 3.50 (s, 2H), 2.96 (t, $J$ = 7.2 Hz, 2H). $^{13}$C NMR (101 MHz, $d_6$-DMSO) δ 176.2 (C), 143.5 (C), 134.1 (C), 127.5 (CH), 124.97 (C), 121.9 (CH), 107.5 (CH), 44.2 ($CH_2$), 35.5 ($CH_2$), 34.5 ($CH_2$). LCMS-1: *m/z* (ESI 20 V) 196.1 ($MH^+$, 100).

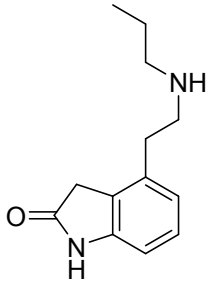
*2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**6**)*[2] ($C_{17}H_{17}NO_4S$)




To a cooled (5-10 °C) suspension of 4-(2-hydroxyethyl)indolin-2-one (**5**) (6.30 g, 35.6 mmol) and pyridine (14.1 g, 177.8 mmol), a solution of *p*-toluenesulfonyl chloride (8.13 g, 42.7 mmol) in $CH_2Cl_2$ (32 mL) was added portion-wise over 30 min. The reaction mixture was stirred at 5-10 °C for 4 h. 6M aq. HCl (35 mL) was added so that the temperature was maintained below 15 °C. $CH_2Cl_2$ was added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$. The combined organic layers were washed with water, then dried with anhydrous $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. The organic layer was concentrated under reduced pressure until a residual volume of about 50 mL was obtained. Petroleum spirits (about 50 mL) was added to induce the product to crystallize. The suspension was filtered and the filter cake was washed with $CH_2Cl_2$: petroleum spirits 1:1. The filter cake was dried on the high vacuum overnight to give the title compound **6** (9.39 g, 80%) as a yellowish-white solid, mp: 128-130 °C (lit.[2] 130-131 °C). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 7.68 – 7.60 (m, 2H), 7.30 – 7.22 (m, 2H), 7.13 (t, $J$ = 7.8 Hz, 1H), 6.78 (d, $J$ = 2.5 Hz, 1H),

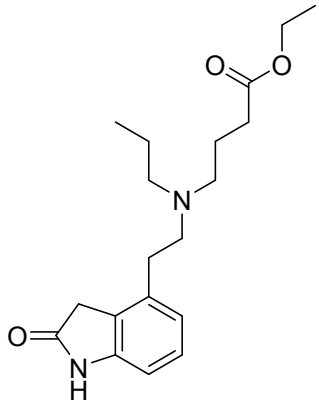
6.76 (d, *J* = 2.6 Hz, 1H), 4.23 (t, *J* = 6.7 Hz, 2H), 3.33 (s, 2H), 2.89 (t, *J* = 6.7 Hz, 2H), 2.43 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.4 (C), 144.9 (C), 142.6 (C), 132.8 (C), 132.6 (C), 129.8 (CH), 128.4 (CH), 127.7 (CH), 124.5 (C), 122.9 (CH), 108.5 (CH), 69.3 ($CH_2$), 34.9 ($CH_2$), 32.6 ($CH_2$), 21.6 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 332.2 ($MH^+$, 65), 663.3 ($2M\text{-}H^+$, 100).

*4-(2-(Propylamino)ethyl)indolin-2-one* ($C_{13}H_{18}N_2O$)



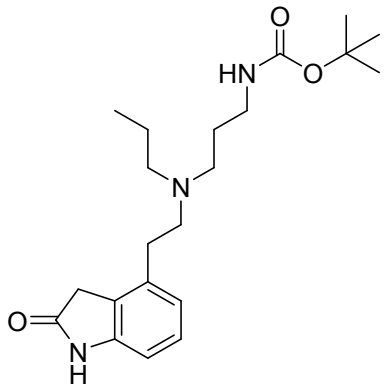

4-(2-(Propylamino)ethyl)indolin-2-one hydrochloride (1.00 g, 3.93 mmol) was dissolved in 1 M aq. NaOH solution (100 mL) and stirred for 5 min. $CH_2Cl_2$ (100 mL) was added and the reaction mixture was stirred for another 15 min. The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 × 50 mL). The combined organic layers were washed with water, dried with a phase separating funnel and the solvent was removed under reduced pressure to afford the title compound (788 mg, 92%) as a yellowish oil. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.08 (br s, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 7.6 Hz, 1H), 3.49 (s, 2H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.65 (br s, 1H), 1.49 (m, 2H), 0.90 (t, *J* = 7.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): 177.1 (C), 142.3 (C), 136.6 (C), 128.1 (CH), 124.2 (C), 122.7 (CH), 107.6 (CH), 51.8 ($CH_2$), 49.6 ($CH_2$), 35.1 ($CH_2$), 33.7 ($CH_2$), 23.2 ($CH_2$), 12.0 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 219.2 ($MH^+$, 100).

*Ethyl 4-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)butanoate (7)* ($C_{19}H_{28}N_2O_3$)



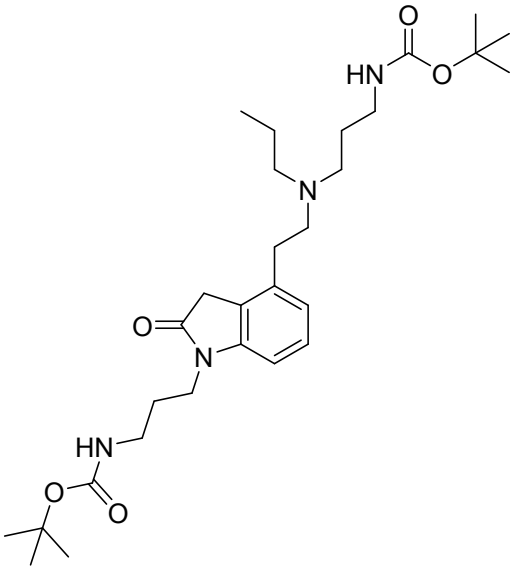
4-(2-(Propylamino)ethyl)indolin-2-one (70.3 mg, 322 μmol) was suspended in acetone (5 mL). $K_2CO_3$ (44.5 mg, 322 μmol) and ethyl 4-bromobutyrate (46.0 μL, 322 μmol) were added at room temperature and the reaction mixture was heated at reflux for 21 h. Following this time, another portion of ethyl 4-bromobutyrate (46.0 μL, 322 μmol) was added and the reaction mixture was stirred at reflux for a further 6 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ and washed with 1 M aq. $K_2CO_3$ solution and water. The organic layer was dried with anhydrous $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography ($CH_2Cl_2$ 100% → $CH_2Cl_2$: EtOH 8:2) affording the title compound **7** (30 mg, 28%) as a white solid. $^1$H NMR (400 MHz, $d_6$-DMSO) δ 10.32 (s, 1H), 7.07 (t, $J$ = 7.7 Hz, 1H), 6.83 (d, $J$ = 7.2 Hz, 1H), 6.64 (d, $J$ = 7.3 Hz, 1H), 4.04 (q, $J$ = 7.1 Hz, 2H), 3.43 (s, 2H), 2.58 (m, 4H), 2.47 – 2.34 (m, 4H), 2.26 (t, $J$ = 7.2 Hz, 2H), 1.62 (m, 2H), 1.39 (m, 2H), 1.17 (t, $J$ = 7.1 Hz, 3H), 0.83 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $d_6$-DMSO) δ 176.2 (C), 172.9 (C), 143.4 (C), 136.5 (C), 127.4 (CH), 124.4 (C), 121.7 (CH), 106.8 (CH), 59.6 ($CH_2$), 55.2 ($CH_2$), 53.5 ($CH_2$), 52.1 ($CH_2$), 34.5 ($CH_2$), 31.1 ($CH_2$), 30.0 ($CH_2$), 22.1 ($CH_2$), 19.9 ($CH_2$), 14.1 ($CH_3$), 11.7 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 333.2 ($MH^+$, 100). HRMS ($C_{19}H_{28}N_2O_3$): Calcd. 333.2173 $[M+H]^+$, Found 333.2189. HPLC: $t_R$ 6.58 min, 97% (214 nm), 97% (254 nm).

tert-*Butyl (3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)carbamate (**9**)* ($C_{21}H_{33}N_3O_3$)



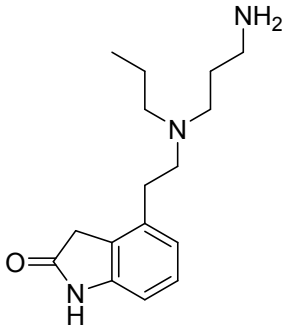
4-(2-(Propylamino)ethyl)indolin-2-one (788 g, 3.61 mmol), *tert*-butyl (3-bromopropyl)-carbamate (860 mg, 3.61 mmol) and $K_2CO_3$ (549 mg, 3.97 mmol) were dissolved in ACN (35 mL). The reaction mixture was heated at reflux and stirred for 19 h, cooled down to room temperature and the solvent removed under reduced pressure. Purification by column chromatography ($CH_2Cl_2$ → $CH_2Cl_2$: MeOH 9:1) gave the title compound **9** (522 mg, 38%) as a purple oil which was immediately stored under $N_2$ to minimize decomposition. $^1$H NMR (400 MHz, $d_6$-DMSO) δ 10.32 (s, 1H), 7.07 (t, $J$ = 7.7 Hz, 1H), 6.77 (d, $J$ = 7.1 Hz, 2H), 6.64 (d, $J$ = 7.3 Hz, 1H), 3.44 (s, 2H), 2.93 (m, 2H), 2.59 (m, 4H), 2.45 (m, 2H), 2.40 (m, 2H), 1.50 (m, 2H), 1.41 (m, 2H), 1.37 (s, 9H), 0.83 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $d_6$-DMSO) δ 176.2 (C), 155.5 (C), 143.4 (C), 136.5 (C), 127.4 (CH), 124.40 (C), 121.7 (CH), 106.8 (CH), 77.3 (C), 55.2 ($CH_2$), 53.5 ($CH_2$), 50.8 ($CH_2$), 38.3 ($CH_2$), 34.5 ($CH_2$), 29.7 ($CH_2$), 28.2 ($CH_3$), 26.9 ($CH_2$), 19.9 ($CH_2$), 11.7 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 376.5 ($MH^+$, 100). HRMS ($C_{21}H_{33}N_3O_3$): Calcd. 376.2595 $[M+H]^+$, Found 376.2612. HPLC: $t_R$ 4.88 min, 97% (214 nm), 97% (254 nm).

tert-*Butyl (3-((2-(1-(3-((*tert-*butoxycarbonyl)amino)propyl)-2-oxoindolin-4-yl)ethyl)(propyl) amino)propyl)carbamate (**10**)* ($C_{29}H_{48}N_4O_5$)



Byproduct isolated when greater than 1 equivalent of *tert*-butyl (3-bromopropyl)carbamate was used. The title compound **10** was obtained as a pale-brown colored liquid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.14 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.62 (d, *J* = 7.7 Hz, 1H), 5.28 (br s, 1H), 5.21 (br s, 1H), 3.71 (t, *J* = 6.5 Hz, 2H), 3.42 (s, 2H), 3.15 – 2.99 (m, 4H), 2.61 (m, 4H), 2.48 (m, 2H), 2.38 (m, 2H), 1.76 (m, 2H), 1.55 (m, 2H), 1.44 (m, 2H), 1.37 (s, 18H), 0.83 (t, *J* = 7.4 Hz, 3H). LCMS-1: *m/z* (ESI 20 V) 533.4 ($MH^+$, 100). HRMS ($C_{29}H_{48}N_4O_5$): Calcd. 533.3697 $[M+H]^+$, Found 533.3718. HPLC: $t_R$ 9.06 min, 95% (214 nm), 95% (254 nm).

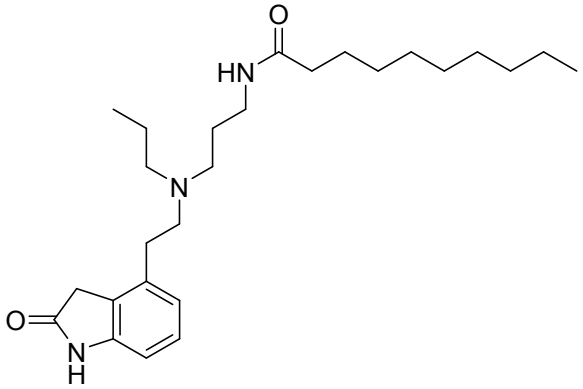
*4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**11**)* ($C_{16}H_{25}N_3O$)



To a stirred solution of *tert*-butyl (3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl) carbamate (**9**) (250 mg, 666 mmol) in MeOH (2 mL) was added 4 M HCl in dioxane (2 mL). The reaction mixture was stirred at room temperature for 1 h before the solvent was removed under reduced pressure. The residue was diluted with $CH_2Cl_2$ and 2 M aq. NaOH solution was added until pH-13. The water layer was extracted with $CH_2Cl_2$. The organic layers were

combined, dried via phase separation funnel and the solvent was removed under reduced pressure to give the title compound **11** (154 mg, 84%) as a yellow oil. Due to stability, the title compound **11** was stored under nitrogen atmosphere in the fridge. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.50 – 7.80 (br s, 1H), 7.14 (t, *J* = 7.7 Hz, 1H), 6.84 (d, *J* = 7.7 Hz, 1H), 6.71 (d, *J* = 7.7 Hz, 1H), 3.48 (s, 2H), 2.72 (t, *J* = 6.8 Hz, 2H), 2.56, (m, 4H), 2.54 (m, 2H), 2.43 (m, 2H), 1.20 – 2.20 (br s, 2H), 1.60 (m, 2H), 1.45 (m, 2H), 0.88 (t, *J* = 7.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): δ 177.8 (C), 142.6 (C), 136.9 (C), 128.0 (CH), 124.1 (C), 122.8 (CH), 107.7 (CH), 56.0 ($CH_2$), 54.0 ($CH_2$), 52.3 ($CH_2$), 40.7 ($CH_2$), 35.1 ($CH_2$), 30.6 ($CH_2$), 29.7 ($CH_2$), 20.2 ($CH_2$), 11.9 ($CH_3$). LCMS-1: *m/z* (ESI 20 V) 276.3 ($MH^+$, 100). HRMS ($C_{16}H_{25}N_3O$): Calcd. 276.2070 $[M+H]^+$, Found 276.2080. HPLC: $t_R$ 4.52 min, 95% (214 nm), 98% (254 nm).

N-*(3-((2-(2-Oxoindolin-4-yl)ethyl)(propyl)amino)propyl)decanamide (**12**)* ($C_{26}H_{43}N_3O_2$)




Decanoyl chloride (58 µL, 280 µmol) and DIPEA (30.8 µL, 335 µmol) were added to a solution of 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (**11**) (77 mg, 280 µmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred at room temperature for 15 min before another portion of decanoyl chloride (58 µL, 280 µmol) was added. The reaction was stirred for an additional 30 min before it was quenched with 1 M aq. HCl. The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$. The combined organic layers were washed with 1 M aq. NaOH solution, then dried via phase separation funnel and the solvent was removed under reduced pressure. Purification by preparative HPLC gave the title compound **12** (20 mg, 17%) as pinkish coloured oil. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.3 Hz, 1H), 6.78 – 6.74 (m, 2H), 3.48 (s, 2H), 3.33 (q, *J* = 5.9 Hz, 2H), 2.72 (m, 4H), 2.62 (t, *J* = 6.4 Hz, 2H), 2.49 (m, 2H), 2.11 (m, 2H), 1.68 (m, 2H), 1.61 (m, 2H), 1.51 (m, 2H), 1.34 – 1.20 (m, 12H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.88 (t, *J* = 6.9 Hz, 3H). $^{1}$H NMR (101 MHz, $CDCl_3$) δ 176.9 (C), 173.8 (C), 142.9 (C), 134.6 (C), 128.4 (CH), 124.1 (C), 122.4 (CH), 108.3 (CH), 54.9 ($CH_2$),

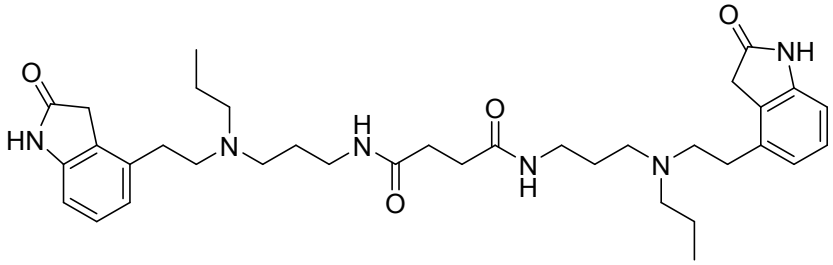
53.3 ($CH_2$), 52.1 ($CH_2$), 38.0 ($CH_2$), 36.8 ($CH_2$), 35.0 ($CH_2$), 31.9 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.0 ($CH_2$), 25.4 ($CH_2$), 25.2 ($CH_2$), 22.7 ($CH_2$), 18.6 ($CH_2$), 14.1 ($CH_3$), 11.6 ($CH_3$). LCMS-2: *m/z* (ESI 20 V) 430.7 ($MH^+$, 100); (basic) $t_R$ 1.31 min, >99%; (acidic) $t_R$ 0.97 min, 95%. HRMS ($C_{26}H_{43}N_3O_2$): Calcd. 430.3434 $[M+H]^+$, Found 430.3426.

*General procedure for the synthesis of homobivalent ligands*

The dicarboxylic acid (**14a-e**) (93.4 µmol) was dissolved/ suspended in dry $CH_2Cl_2$ (2 mL). Oxalyl chloride (2.2 equivalent) and DMF (1 drop) were added to the solution. The reaction mixture was stirred at room temperature for 1 h. After this time, 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (**11**) (2.0 equivalents) dissolved in $CH_2Cl_2$ (2 mL) and DIPEA (2.5 equiv) was added to the formed diacid chloride. The reaction mixture was stirred at room temperature for 1 h, then partitioned between $CH_2Cl_2$ and 1 M aq. NaOH solution. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$ . The combined organic layers were dried via phase separation funnel and the solvent was removed under reduced pressure. The crude material was purified by preparative HPLC to give the title compounds **15a-e**.

Attention! 4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**11**) degraded within a very short time (about 5 days). The degradation caused lower yields and in one case just the monovalent ligand (compound **16**) was isolated.

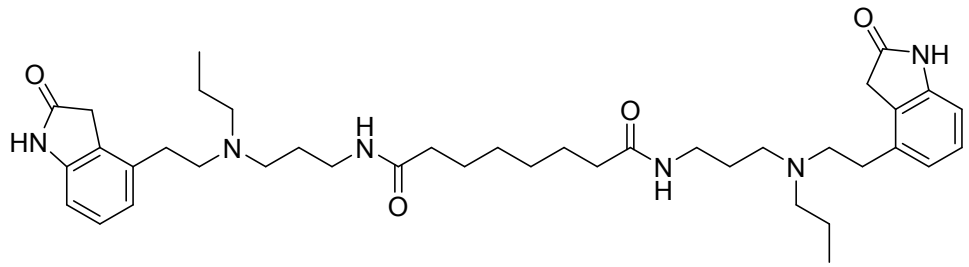
$N^1$,$N^4$-*Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)succinamide (**15a**)* ($C_{36}H_{52}N_6O_4$)




The title compound **15a** (2 mg, 4%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.60 (br s, 2H), 7.16 (t, *J* = 7.8 Hz, 2H), 6.95 (br t, *J* = 5.2 Hz, 2H), 6.85 (d, *J* = 7.3 Hz, 2H), 6.76 (d, *J* = 7.6 Hz, 2H), 3.51 (s, 4H), 3.25 (q, *J* = 6.4 Hz, 4H), 2.74 (m, 8H), 2.61 (t, *J* = 6.6 Hz, 4H), 2.52 (m, 4H), 2.42 (s, 4H), 1.66 (m, 4H), 1.52 (m, 4H), 0.92 (t, *J* = 7.4 Hz, 6H). LCMS-2: *m/z* (ESI 20 V) 631.8 ($MH^-$, 100); (basic) $t_R$ 0.96 min, 97%; (acidic) $t_R$ 0.61 min, 97%. HRMS ($C_{36}H_{52}N_6O_4$): Calcd. 633.4128 $[M+H]^+$, Found 633.4148.

$N^1$,$N^8$-*Bis(3-((2-(2-oxoindolin-4yl)ethyl)(propyl)amino)propyl)octanediamide* ***(15b)***

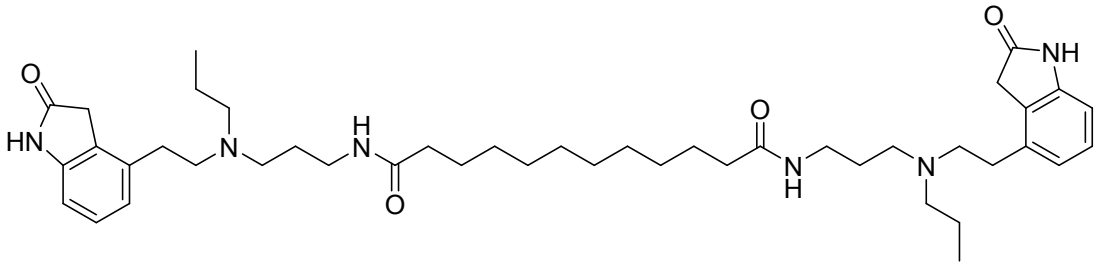
($C_{40}H_{60}N_6O_4$)



Reaction was based on 54.5 μmol of dicarboxylic acid. The title compound **15b** (6 mg, 16%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.37 (br s, 2H), 7.14 (t, *J* = 7.8 Hz, 2H), 6.84 (d, *J* = 7.8 Hz, 2H), 6.83 – 6.78 (br t, 2H), 6.76 (d, *J* = 7.7 Hz, 2H), 3.47 (s, 4H), 3.27 (q, *J* = 5.6 Hz, 4H), 2.68 (m, 8H), 2.56 (t, *J* = 6.2 Hz, 4H), 2.45 (m, 4H), 2.03 (t, *J* = 7.5 Hz, 4H), 1.62 (m, 4H), 1.55 (m, 4H), 1.48 (m, 4H), 1.28 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 6H). LCMS-2: *m/z* (ESI 20 V) 687.9 ($MH^-$, 100); (basic) $t_R$ 1.00 min, >99%; (acidic) $t_R$ 0.62 min, 97%. HRMS ($C_{40}H_{60}N_6O_4$): Calcd. 689.4754 $[M+H]^+$, Found 689.4772.

$N^1$,$N^{12}$-*Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)dodecanediamide* ***(15c)***

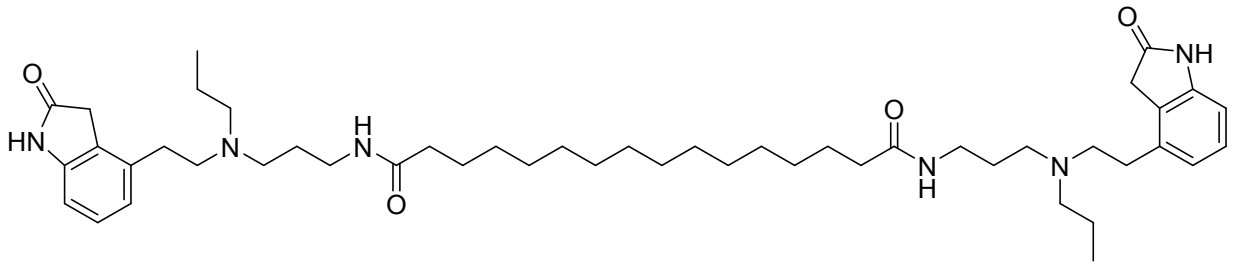
($C_{44}H_{68}N_6O_4$)



The title compound **15c** (11 mg, 17%) was obtained as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.11 (br s, 2H), 7.17 (t, *J* = 7.8 Hz, 2H), 6.90 – 6.81 (m, 4H), 6.78 (d, *J* = 7.7 Hz, 2H), 3.48 (s, 4H), 3.32 (q, *J* = 5.9 Hz, 4H), 2.72 (m, 8H), 2.60 (t, *J* = 6.1 Hz, 4H), 2.47 (m, 4H), 2.05 (m, 4H), 1.66 (m, 4H), 1.60 – 1.45 (m, 8H), 1.35 – 1.15 (m, 12H), 0.93 (t, *J* = 7.4 Hz, 6H). LCMS-2: *m/z* (ESI 20 V) 744.0 ($MH^-$, 100); (basic) $t_R$ 1.22 min, 97%; (acidic) $t_R$ 0.79 min, 97%. HRMS ($C_{44}H_{68}N_6O_4$): Calcd. 745.5380 $[M+H]^+$, Found 745.5357.

$N^1$,$N^{16}$-*Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)hexadecanediamide* (***15d***)

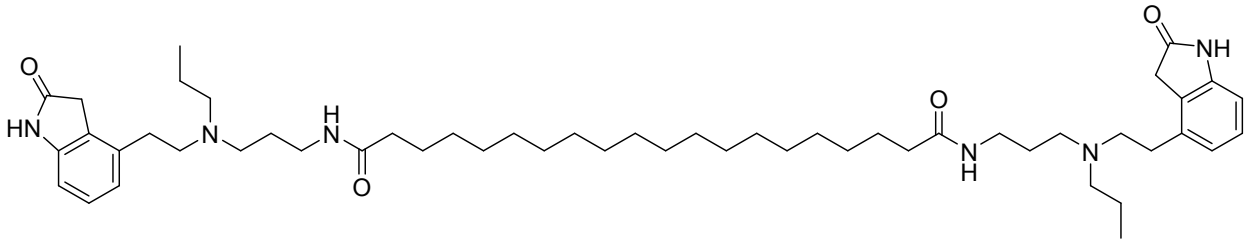
($C_{48}H_{76}N_6O_4$)



The title compound **15d** (16 mg, 22%) was obtained as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.12 (br s, 2H), 7.16 (t, *J* = 7.8 Hz, 2H), 6.89 – 6.82 (m, 4H), 6.77 (d, *J* = 7.7 Hz, 2H), 3.48 (s, 4H), 3.33 (q, *J* = 5.8 Hz, 4H), 2.70 (m, 8H), 2.60 (t, *J* = 6.2 Hz, 4H), 2.47 (m, 4H), 2.10 (m, 4H), 1.66 (m, 4H), 1.60 (m, 4H), 1.50 (m, 4H), 1.35 – 1.15 (m, 20H), 0.92 (t, *J* = 7.4 Hz, 6H). LCMS-2: *m/z* (ESI 20 V) 800.0 ($MH^-$, 100); (basic) $t_R$ 1.39 min, 96%; (acidic) $t_R$ 0.91 min, 98%. HRMS ($C_{48}H_{76}N_6O_4$): Calcd. 801.6006 $[M+H]^+$, Found 801.6021.

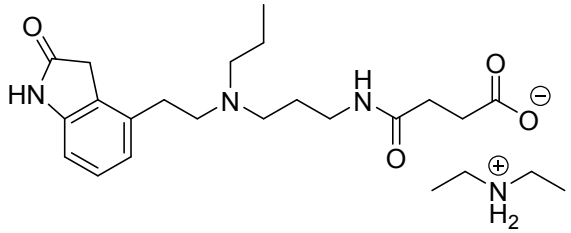
$N^1$,$N^{20}$-*Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)icosanediamide* (***15e***)

($C_{52}H_{84}N_6O_4$)



The title compound **15e** (25 mg, 31%) was obtained as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (br s, 2H), 7.18 (t, *J* = 7.8 Hz, 2H), 6.87 (d, *J* = 7.3 Hz, 2H), 6.82 – 6.77 (br t, *J* = 7.8 Hz, 2H), 6.76 (t, *J* = 7.7 Hz, 2H), 3.50 (s, 4H), 3.34 (q, *J* = 5.9 Hz, 4H), 2.71 (m, 8H), 2.61 (t, *J* = 6.2 Hz, 4H), 2.48 (m, 4H), 2.11 (m, 4H), 1.68 (m, 4H), 1.58 (m, 4H), 1.52 (m, 4H), 1.35 – 1.20 (m, 28H), 0.94 (t, *J* = 7.4 Hz, 6H). LCMS-2: *m/z* (ESI 20 V) 856.0 ($MH^-$, 100); (basic) $t_R$ 1.65 min, 95%; (acidic) $t_R$ 1.06 min, 90%. HRMS ($C_{52}H_{84}N_6O_4$): Calcd. 857.6632 $[M+H]^+$, Found 857.6640.

*Diethylammonium 4-oxo-4-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)-butanoate (**16**)* ($C_{24}H_{40}N_4O_4$)

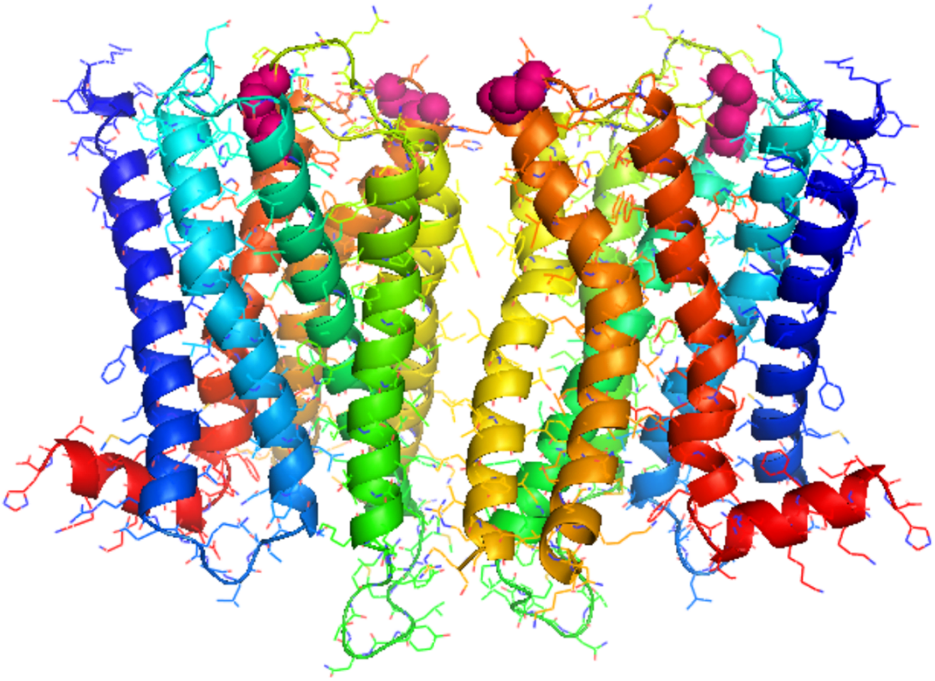


The title compound **16** (2 mg, 4%) was obtained as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.42 (br s, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.01 (m, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 3.56 (s, 2H), 3.24 (q, *J* = 5.7 Hz, 2H), 2.84 (q, *J* = 7.2 Hz, 4H), 2.81 – 2.72 (m, 4H), 2.66 (t, *J* = 6.8 Hz, 2H), 2.59 (m, 4H), 2.41 (m, 2H), 1.64 (m, 2H), 1.55 (m, 2H), 1.25 (t, *J* = 7.2 Hz, 6H), 0.94 (t, *J* = 7.3 Hz, 3H). LCMS-2: *m/z* (ESI 20 V) 376.3 ($MH^+$, 100); (basic) $t_R$ 0.69 min, 86%; (acidic) $t_R$ 0.62 min, 85%. HRMS ($C_{20}H_{29}N_3O_4$): Calcd. 376.2236 $[M+H]^+$, Found 376.2240.

Molecular Modeling

**Homology modeling of dopamine $D_2$ receptor in active conformation:** The homology model of the dopamine $D_2$ receptor in active conformation was constructed with Modeller9.11,[3] using the $\beta_2$ adrenergic receptor in a complex with the $G_s$ protein crystal structure (PDB ID: 3SN6)[4] as a template. Sequence alignment involving sequences of human $D_1$-$D_5$ receptors, was done with MOE Molecular Environment[5] module for GPCRs and corrected manually to satisfy conserved residues in the transmembrane helices as well as to consider appropriate position of a disulfide bridge linking TM3 and extracellular loop 2, e2. The model has a sequence identity of 35.56% and sequence similarity of 56.30% with the template. In particular e2 loop has a sequence identity of 9.10% and sequence similarity of 27.30% with the e2 loop of the template. Sequence alignment was visualized with Chimera.[6] A population of 200 models was generated. N-end, C-end and intracellular loop 3 (i3) were not modeled due to the lack of suitable templates. The other loops were refined with MD-slow refinement method of Modeller9.11. The best model was selected on the basis of the lowest values of Modeller9.11 objective function. The software programs PyMol 0.99[7] and SPDBV[8] were also used for results visualization.

**Modeling of dopamine $D_2$ receptor homodimer in different conformational states:** The preliminary dopamine $D_2$ homodimer model was manually built with PyMol v. 0.99.[7] There are several experimentally justified interfaces to build dopamine $D_2$ receptor dimer model. Independent studies by Guo *et al.* and Lee *et al.* provided evidence that transmembrane domain IV is involved in the dimerization of the dopamine $D_2$ receptors.[9-12] In addition, Guo *et al*.[11] also determined that TM1 may be involved in the oligomerization of the dopamine $D_2$ receptor. Consequently, there are at least three possible interfaces to be considered: TM4TM5 in both receptors, TM1-TM2 in both receptors and TM4-TM5 in one protomer and TM1-TM2 in the other protomer. Most probably, in the higher-order oligomers all these interfaces occur as it was proposed for rhodopsin[13] and the $D_2$ receptor.[12] In this study, a symmetric homodimer with a TM3TM4TM5 interface from both individual protomers was built. This particular interface was chosen since it is the commonly used approach to build GPCR dimers[14] and the same interface has been used for the dopamine $D_2$ homodimer[12,15] and $D_2$- adenosine $A_{2A}$ heterodimermodels.[16] This model was used as input for protein-protein docking with Rosetta[17] with the local refinement option in order to ensure only membrane-realistic results. 200 models were generated and the best one was selected on the basis of Rosetta interface score.



Dopamine $D_2$ homodimer model in active conformation using a TM3TM4TM5 interface from both individual protomers. Transmembrane helices in both protomers colored in a spectrum-like manner, from dark blue (TM1) to red (TM7). Disulphide bonds shown as magenta spheres.

**Docking of ropinirole derivatives:** The size of orthosteric site extending to the allosteric site is 147 Å$^3$ (pocket identified by SiteID and surface measured by MOLCAD, both included in Sybyl-X, v. 2.1[18]). The size of a pocket available for bivalent ligands is twice that size, i.e. 294 Å$^3$ extended by the region between protomers which cannot be easily measured as it is not a well-defined pocket. The size of the bivalent ligand pocket may be approximated by the molecular surface and volume of the biggest ligand **15e** in its docked conformation, which are 1667.20 Å$^2$ and 945.40 Å$^3$, respectively, as measured by VegaZZ v. 3.0.1[19]. Ligands were prepared with LigPrep module of Schrödinger suite of software.[20] Protonation states at physiological pH was assessed with Epik[21] modules of Schrödinger suite of software. All compounds were protonated at one or two (when available) protonable nitrogen atoms. In case of compound **16** ionization of carboxyl group was considered. Compounds **15d** and **15e** were additionally subjected to conformational analysis with the genetic algorithm of Sybyl-X[18] in order to find suitable starting conformations. Ropinirole and compounds **4**, **7**, **9**, **10**, **12**, **15a** and **16** were docked with Glide with XP settings to the model of receptor monomer, indicating Asp3.49 as the binding site. In case of compound **12** extensive conformational search was enabled. Bivalent ligands **15b**, **15c**, **15d** and **15e** were manually placed in the $D_2$ homodimer model using PyMol v. 0.99.[7] These 4 ligand-receptor complexes were minimized with MOE Molecular Environment (with implicit membrane simulation and fixed receptor backbone) and then re-docked with Glide using refinement option. Docking protocol was validated for docking of monovalent ligands by docking a set of high affinity agonists as well as true decoys (i.e. compounds with experimentally confirmed lack of $D_2$ receptor activity) as found in CHEMBL database. Docking protocol was not validated in this way for bivalent ligands due to lack of true decoys. However, the methodology used for docking of bivalent ligands was successfully applied to $D_2$ receptor bivalent ligands available in literature which may be treated as partial validation. The stability of ligand-receptor complexes was assessed in molecular dynamic simulations with Desmond v. 3.0.3.1.[22] Ligand-receptor complexes were inserted into POPC membrane and solvated with water. Ions were added to neutralize protein charges and to the concentration of 0.15 M NaCl. The complexes embedded in membrane were first minimized and then subjected to 1 ns MD in NVT ensemble, followed by 20 ns MD in NPT ensemble.

References


1 D. Wu, A. Chen, C. S. Johnson, Jr., *J. Magn. Reson., Ser. A* **1995,** *115*, 260.

2 J. D. Hayler, S. L. B. Howie, R. G. Giles, A. Negus, P. W. Oxley, T. C. Walsgrove, S. E. Walsh, R. E. Dagger, J. M. Fortunak, A. Mastrocola, *J. Heterocyclic Chem.* **1995,** *32*, 875.

3 N. Eswar, M. A. Marti-Renom, B. Webb, M. S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, A. Sali. Comparative Protein Structure Modeling With MODELLER. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., Supplement 15, 5.6.1-5.6.30, **2006**.

4 S. G. Rasmussen, B. T. DeVree, Y. Zou, A. C. Kruse, K. Y. Chung, T. S. Kobilka, F. S. Thian, P. S. Chae, E. Pardon, D. Calinski, J. M. Mathiesen, S. T. Shah, J. A. Lyons, M. Caffrey, S. H. Gellman, J. Steyaert, G. Skiniotis, W. I. Weis, R. K. Sunahara, B. K. Kobilka, *Nature* **2011,** *477*, 549-55.

5 Molecular Operating Environment (MOE) software, http://www.chemcomp.com/ software.htm.

6 E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605.

7 The PyMOL Molecular Graphics System, Version 0.99, Schrödinger, LLC.

8 N. Guex, M. C. Peitsch, *Electrophoresis* **1997**, *18*, 2714.

9 W. Guo, L. Shi, J. A. Javitch, *J. Biol. Chem*. **2003**, *278*, 4385.

10 W. Guo, L. Shi, M. Filizola, H. Weinstein, J. A. Javitch, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17495.

11 W. Guo, E. Urizar, M. Kralikova, J. C. Mobarec, L. Shi, M. Filizola, J. A. Javitch, *EMBO J*., **2008**, *27*, 2293.

12 S. P. Lee, B. F. O'Dowd, R. D. Rajaram, T. Nguyen, S. R. George, *Biochemistry* **200**3, *42*, 11023.

13 B. Jastrzebska, D. Fotiadis, G. F. Jang, R. E. Stenkamp, A. Engel, K. Palczewski, *J. Biol. Chem.* **2006**, *281*, 11917.

14 J. Selent, A. A. Kaczor, *Curr. Med. Chem.* **2011**, *18*, 4588.

15 Y. Liang, D. Fotiadis, S. Filipek, D. A. Saperstein, K. Palczewski, A. Engel, *J. Biol. Chem.* **2003**, *278*, 21655.

16 A. Soriano, R. Ventura, A. Molero, R. Hoen, V. Casadó, A. Cortés, F. Fanelli F. Albericio, C. Lluís, R. Franco, M. Royo , *J. Med. Chem.* **2009**, *52*, 5590.

17 O. Schueler-Furman, C. Wang, D. Baker, *Proteins* **2005**, *60*, 187.

18 Sybyl-X suite, www.certara.com

19 A. Pedretti L. Villa, G. Vistoli. *J. Comput. Aided. Mol. Des.* **2004**, *18*, 167.

20 LigPrep, Version 2.4: Schrödinger, LLC, New York, **2010**.

21 Epik, Version 2.1: Schrödinger, LLC, New York, **2010**.

22 K. J. Bowers, E. Chow, H. Xu, R. O. Dror, M. P. Eastwood, B. A. Gregersen, J. L. Klepeis, I. Kolossváry, M. A. Moraes, F. D. Sacerdoti, J. K. Salmon, Y. Shan, D. E. Shaw, Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters, Proceedings of the ACM/IEEE Conference on Supercomputing (SC06), Tampa, Florida, November 11–17, **2006**.