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 ($\delta$) 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 x 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 homogenized 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 transferred 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).

**$[^{35}S]$GTP$\gamma$S 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 $[^{35}S]$GTP$\gamma$S 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 $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ 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 ($B_{\text{max}}$, fmol mg$^{-1}$) values could be extracted. The initial rate of $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding was calculated as $k.B_{\text{max}}$ 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, hD₂ agonist).

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

**tert-Butyl (3-bromopropyl)carbamate** (C\(_8\)H\(_{16}\)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\(_2\)Cl\(_2\) (30 mL). Et\(_3\)N (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\(_2\)Cl\(_2\) (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)(I)** (C\(_{16}\)H\(_{24}\)N\(_2\)O)

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\(_2\)Cl\(_2\) (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\(_2\)Cl\(_2\) (2 × 50 mL). The combined organic layers were washed with water, dried with anhydrous Na\(_2\)SO\(_4\), 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_{2}O)

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 NaHCO₃ (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 (CH₂Cl₂ → CH₂Cl₂: 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. K₂CO₃ 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. ³¹H NMR (400 MHz, d₆-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).

¹³C NMR (101 MHz, d₆-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 (CH₂), 46.3 (CH₂), 34.6 (CH₂), 29.0 (CH₂), 18.9 (CH₂), 11.0 (CH₃). LCMS-1: m/z (ESI 20 V) 219.2 (MH⁺, 100). HRMS (C_{13}H_{18}N_{2}O): 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\textsubscript{10}H\textsubscript{10}ClNO)

Obtained byproduct when using method A to synthesize compound 4. \(^1\)H NMR (400 MHz, \textit{d}_6-DMSO) \(\delta\) 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, \textit{d}_6-DMSO) \(\delta\) 176.2 (C), 143.5 (C), 134.1 (C), 127.5 (CH), 124.97 (C), 121.9 (CH), 107.5 (CH), 44.2 (CH\textsubscript{2}), 35.5 (CH\textsubscript{2}), 34.5 (CH\textsubscript{2}). LCMS-1: \(m/z\) (ESI 20 V) 196.1 (MH\(^+\), 100).

2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (6)\(^2\) (C\textsubscript{17}H\textsubscript{17}NO\textsubscript{4}S)

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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2} was added and the layers were separated. The aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers were washed with water, then dried with anhydrous Na\textsubscript{2}SO\textsubscript{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\textsubscript{2}Cl\textsubscript{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\textsubscript{3}) \(\delta\) 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). $^1$H 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-H$^+$, 100).

$^1$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). $^1$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).

4-(2-(Propylamino)ethyl)indolin-2-one (C$_{13}$H$_{18}$N$_2$O)

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$_2$Cl$_2$ (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$_2$Cl$_2$ (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). $^1$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).
4-(2-(Propylamino)ethyl)indolin-2-one (70.3 mg, 322 µmol) was suspended in acetone (5 mL). K$_2$CO$_3$ (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$_2$Cl$_2$ and washed with 1 M aq. K$_2$CO$_3$ solution and water. The organic layer was dried with anhydrous Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (CH$_2$Cl$_2$ 100% → CH$_2$Cl$_2$: 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$_2$O$_3$): 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_{3}O_{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_{2}CO_{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_{2}Cl_{2} → CH_{2}Cl_{2}: MeOH 9:1) gave the title compound 9 (522 mg, 38%) as a purple oil which was immediately stored under N\textsubscript{2} to minimize decomposition. \textsuperscript{1}H NMR (400 MHz, \textit{d}_{6}-DMSO) \(\delta\) 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). \textsuperscript{13}C NMR (101 MHz, \textit{d}_{6}-DMSO) \(\delta\) 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\textsubscript{2}), 53.5 (CH\textsubscript{2}), 50.8 (CH\textsubscript{2}), 38.3 (CH\textsubscript{2}), 34.5 (CH\textsubscript{2}), 29.7 (CH\textsubscript{2}), 28.2 (CH\textsubscript{3}), 26.9 (CH\textsubscript{2}), 19.9 (CH\textsubscript{2}), 11.7 (CH\textsubscript{3}). LCMS-1: \textit{m/z} (ESI 20 V) 376.5 (MH\textsuperscript{+}, 100). HRMS (C_{21}H_{33}N_{3}O_{3}): Calcd. 376.2595 [M+H]\textsuperscript{+}, 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_{4}O_{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$_3$) $\delta$ 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_{4}O_{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_{3}O)

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$_2$Cl$_2$ and 2 M aq. NaOH solution was added until pH=13. The water layer was extracted with CH$_2$Cl$_2$. 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$_3$) $\delta$ 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$_3$): $\delta$ 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$_2$), 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$_3$O): Calcd. 276.2070 [M+H]$^+$, Found 276.2080. HPLC: $t_R$ 4.52 min, 95% (214 nm), 98% (254 nm).

N-(3-((2-(2-Oxindolin-4-yl)ethyl)(propyl)amino)propyl)decanamide (12) (C$_{26}$H$_{43}$N$_3$O$_2$)

Decanoyl chloride (58 $\mu$L, 280 $\mu$mol) and DIPEA (30.8 $\mu$L, 335 $\mu$mol) were added to a solution of 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (11) (77 mg, 280 $\mu$mol) in CH$_2$Cl$_2$ (5 mL). The reaction mixture was stirred at room temperature for 15 min before another portion of decanoyl chloride (58 $\mu$L, 280 $\mu$mol) 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$_2$Cl$_2$. 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$_3$) $\delta$ 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$_3$) $\delta$ 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 (CH₂), 52.1 (CH₂), 38.0 (CH₂), 36.8 (CH₂), 35.0 (CH₂), 31.9 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.0 (CH₂), 25.4 (CH₂), 25.2 (CH₂), 22.7 (CH₂), 18.6 (CH₂), 14.1 (CH₃), 11.6 (CH₃). LCMS-2: m/z (ESI 20 V) 430.7 (MH⁺, 100); (basic) tᵣ 1.31 min, >99%; (acidic) tᵣ 0.97 min, 95%. HRMS (C₂₆H₄₃N₃O₂): 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 CH₂Cl₂ (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 CH₂Cl₂ (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 CH₂Cl₂ and 1 M aq. NaOH solution. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂. 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.

N¹,N⁴-Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)succinamide (15a) (C₃₆H₅₂N₆O₄)

The title compound 15a (2 mg, 4%) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃) 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) tᵣ 0.96 min, 97%; (acidic) tᵣ 0.61 min, 97%. HRMS (C₃₆H₅₂N₆O₄): Calcd. 633.4128 [M+H]⁺, Found 633.4148.
N¹,N⁸-Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)octanediamide (15b)
(C₄₀H₆₀N₆O₄)

Reaction was based on 54.5 μmol of dicarboxylic acid. The title compound 15b (6 mg, 16%) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃) 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) tᵣ 1.00 min, >99%; (acidic) tᵣ 0.62 min, 97%. HRMS (C₄₀H₆₀N₆O₄): Calcd. 689.4754 [M+H]⁺, Found 689.4772.

N¹,N¹²-Bis(3-((2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)dodecanediamide (15c)
(C₄₄H₆₈N₆O₄)

The title compound 15c (11 mg, 17%) was obtained as a white solid. ¹H NMR (400 MHz, CDCl₃) 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) tᵣ 1.22 min, 97%; (acidic) tᵣ 0.79 min, 97%. HRMS (C₄₄H₆₈N₆O₄): Calcd. 745.5380 [M+H]⁺, Found 745.5357.
N₁,N₁₆-Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)hexadecanediame (15d)
(C₄₈H₇₆N₆O₄)

The title compound 15d (16 mg, 22%) was obtained as a white solid. ¹H NMR (400 MHz, CDCl₃) 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) tᵣ 1.39 min, 96%; (acidic) tᵣ 0.91 min, 98%. HRMS (C₄₈H₇₆N₆O₄): Calcd. 801.6006 [M+H]⁺, Found 801.6021.

N₁,N₂₀-Bis(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)icosanediame (15e)
(C₅₂H₈₄N₆O₄)

The title compound 15e (25 mg, 31%) was obtained as a white solid. ¹H NMR (400 MHz, CDCl₃) 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) tᵣ 1.65 min, 95%; (acidic) tᵣ 1.06 min, 90%. HRMS (C₅₂H₈₄N₆O₄): 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\textsubscript{24}H\textsubscript{40}N\textsubscript{4}O\textsubscript{4})

The title compound 16 (2 mg, 4%) was obtained as a white solid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 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\textsuperscript{+}, 100); (basic) \(t_R\) 0.69 min, 86%; (acidic) \(t_R\) 0.62 min, 85%. HRMS (C\textsubscript{20}H\textsubscript{29}N\textsubscript{3}O\textsubscript{4}): Calcd. 376.2236 [M+H]\textsuperscript{+}, Found 376.2240.

**Molecular Modeling**

**Homology modeling of dopamine D\textsubscript{2} receptor in active conformation:** The homology model of the dopamine D\textsubscript{2} receptor in active conformation was constructed with Modeller9.11\textsuperscript{3} using the \(\beta\textsubscript{2}\) adrenergic receptor in a complex with the G\textsubscript{s} protein crystal structure (PDB ID: 3SN6)\textsuperscript{4} as a template. Sequence alignment involving sequences of human D\textsubscript{1}-D\textsubscript{5} receptors, was done with MOE Molecular Environment\textsuperscript{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.\textsuperscript{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\textsuperscript{7} and SPDBV\textsuperscript{8} were also used for results visualization.
Modeling of dopamine D₂ receptor homodimer in different conformational states: The preliminary dopamine D₂ homodimer model was manually built with PyMol v. 0.99. There are several experimentally justified interfaces to build dopamine D₂ 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 D₂ receptors. In addition, Guo et al. also determined that TM1 may be involved in the oligomerization of the dopamine D₂ receptor. Consequently, there are at least three possible interfaces to be considered: TM4-TM5 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 and the D₂ receptor. In this study, a symmetric homodimer with a TM3-TM4-TM5 interface from both individual protomers was built. This particular interface was chosen since it is the commonly used approach to build GPCR dimers and the same interface has been used for the dopamine D₂ homodimer and D₂- adenosine A₂A heterodimer models. This model was used as input for protein-protein docking with Rosetta 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 D₂ homodimer model in active conformation using a TM3-TM4-TM5 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 Å³ (pocket identified by SiteID and surface measured by MOLCAD, both included in Sybyl-X, v. 2.1\cite{18}). The size of a pocket available for bivalent ligands is twice that size, i.e. 294 Å³ 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 Å² and 945.40 Å³, respectively, as measured by VegaZZ v. 3.0.1\cite{19}. Ligands were prepared with LigPrep module of Schrödinger suite of software\cite{20}. Protonation states at physiological pH was assessed with Epik\cite{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\cite{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 D₂ homodimer model using PyMol v. 0.99\cite{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 D₂ 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 D₂ 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\cite{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.


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


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