

Supporting Information

Synthesis and *in vitro* pharmacological evaluation of indolyl carboxylic amide analogues as D₃ dopamine receptor selective ligands

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Experimental section

General: Synthetic intermediates were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Lancaster Synthesis (Windham, MA) and used as received unless otherwise stated. Tetrahydrofuran (THF) was freshly distilled from sodium hydride prior to use. All other reagents and solvents were purchased as reagent grade and used without further purification.

All air-sensitive reactions were carried out using oven-dried glassware under an inert nitrogen atmosphere unless otherwise stated. Four amines, 9a, 9b, 9c and 9d in Scheme 1 were prepared as previously reported.²⁸ Standard handling techniques for air sensitive materials were employed throughout this study. When the reactions involved extraction with dichloromethane (CH₂Cl₂), ethyl acetate, or ethyl ether (Et₂O), the organic solutions were dried over anhydrous Na₂SO₄ and concentrated on a rotary evaporator under reduced pressure. Yields were not optimized. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. ¹H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer with CDCl₃ as solvent and tetramethylsilane (TMS) as the internal standard. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of residual solvents are observed at 7.26 (CDCl₃) or 2.50/3.30 (DMSO-d₆). The following abbreviations were used to describe peak patterns wherever appropriate: b = broad, s = singlet, d = doublet, t = triplet, m = multiplet. Analytical thin layer chromatography (TLC) was carried out on Analtech GHLF silica gel glass plates, and visualization was aided by UV. Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. High resolution mass spectrometry were conducted by the Washington University Mass Spectrometry Resource. The purity of the target compounds was determined by elemental analysis and by HPLC methods. All the compounds reported in this article have a purity ≥ 95%.

1. chemistry

1.1. Ethyl 5-(2-(2-hydroxyethoxy)ethoxy)-1H-indole-2-carboxylate (11b)

To a solution of ethyl 5-hydroxy-1*H*-indole-2-carboxylate (10, 0.71 g, 3.46 mmol) and 2-(2-chloroethoxy)ethanol (1.27g, 10.4 mmol) in *N,N*-dimethylformamide (DMF) (30 mL), potassium carbonate (1.42 g, 10.4 mmol) was added with stirring. The mixture was heated at 100 °C by oil-bath overnight. After cooling down to room temperature, the mixture was partitioned between dichloromethane (50 mL) and water (300 mL). The organic layer was collected. Additional dichloromethane (50 mL) was used to extract the product from aqueous solution. The organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. After filtration, concentration, the product was purified through a silica gel column using ethyl acetate/hexane (1:1, v/v) as mobile phase to afford compound **11b** (1.02 g, 46%), a pale yellow solid. The ¹H NMR (CDCl₃) is δ 1.40 (t, *J* = 7.2 Hz, 3H), 2.25 (s, 1H), 3.69 (m, *J* = 4.8 Hz, 2H), 3.74 – 3.82 (m, 2H), 3.90 (t, *J* = 6.9 Hz, 2H), 4.17 (t, *J* = 4.8 Hz, 2H), 4.30 - 4.42 (m, 2H), 7.02 (dd, *J* = 3.0, 6.0 Hz, 1H), 7.09 (d, *J* = 1.0 Hz, 1H), 7.12 – 7.16 (m, 1H), 7.31 (d, *J* = 4.5 Hz, 1H), 8.88 (s, 1H).

1.2. Ethyl 5-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-1*H*-indole-2-carboxylate (**11c**)

A similar procedure for making compound **11b** was followed to afford **11c** (366 mg, 16%) as colorless grease. The ¹H NMR (CDCl₃) is δ 1.40 (t, *J* = 4.2 Hz, 3H), 3.60 – 3.66 (m, 2H), 3.68 – 3.78 (m, 6H), 3.89 (t, *J* = 5.1 Hz, 2H), 4.17 (t, *J* = 3.9 Hz, 2H), 4.35 – 4.45 (m, 2H), 7.03 (dd, *J* = 3.0, 5.4 Hz, 1H), 7.08 (d, *J* = 1.2 Hz, 1H), 7.14 – 7.15 (m, 1H), 7.30 (d, *J* = 4.5 Hz, 1H), 8.88 (s, 1H).

1.3. Ethyl 5-(2-fluoroethoxy)-1*H*-indole-2-carboxylate (**12a**)

To a mixture of ethyl 5-hydroxy-1*H*-indole-2-carboxylate (10, 0.6 g, 2.93 mmol) in acetone (30 mL), potassium carbonate (1.22 g, 8.80 mmol), 1-bromo-2-fluoroethane (1.11 g, 8.8 mmol) was added with stirring. After the mixture was refluxed overnight, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), and then washed with saline (3 × 20 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified via flash column chromatography using ethyl acetate/hexane (20/100, v/v) as mobile phase to afford **12a** (534 mg, 73%), a white solid. The ¹H NMR (CDCl₃) is δ 1.41 (t, *J* = 6.9 Hz, 3H), 4.21 (t, *J* =

4.2 Hz, 1H), 4.30 (t, $J = 4.2$ Hz, 1H), 4.35 – 4.48 (m, 2H), 4.71 (t, $J = 4.2$ Hz, 1H), 4.86 (t, $J = 4.2$ Hz, 1H), 7.05 (d, $J = 4.5$ Hz, 1H), 7.09 (d, $J = 1.2$ Hz, 1H), 7.14 (d, $J = 0.3$ Hz, 1H), 7.33 (d, $J = 4.5$ Hz, 1H), 8.89 (s, 1H).

1.4. Ethyl 5-(2-(2-fluoroethoxy)ethoxy)-1H-indole-2-carboxylate (12b)

To a solution of 11b (300 mg, 1.02 mmol) in dichloromethane (15 mL) cooled with ice-water bath was added diethylaminosulfur trifluoride (DAST) (247 mg, 1.53 mmol) slowly. The reaction mixture was stirred at ambient temperature overnight. After the solvent was evaporated under reduced pressure, the residue was dissolved in ethyl acetate (80 mL) and washed with water (40 mL), saturated sodium carbonate aqueous solution (40 mL), saturated sodium chloride aqueous solution (40 mL) separately. The organic layers were dried over anhydrous sodium sulfate and concentrated. The crude product was purified via flash column chromatography using ethyl acetate/hexane (40/60, v/v) to afford **12b** (85 mg, 28%), a white solid. The ^1H NMR (CDCl_3) is δ 1.40 (t, $J = 4.2$ Hz, 3H), 3.76 – 3.82 (m, 1H), 3.86 – 3.96 (m, 3H), 4.19 (t, $J = 4.8$ Hz, 2H), 4.34 – 4.50 (q, 2H), 4.53 (t, $J = 3.6$ Hz, 1H), 4.69 (t, $J = 3.9$ Hz, 1H), 7.03 (d, $J = 4.5$ Hz, 1H), 7.09 (s, 1H), 7.11 – 7.15 (m, 1H), 7.31 (d, $J = 4.5$ Hz, 1H), 8.77 (s, 1H).

1.5. Ethyl 5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-1H-indole-2-carboxylate (12c)

A similar procedure for making 12b was followed to afford **12c** (223 mg, 60%) as colorless grease. The ^1H NMR (CDCl_3) is 1.40 (t, $J = 6.9$ Hz, 3H), 3.68 – 3.84 (m, 6H), 3.89 (t, $J = 4.8$ Hz, 2H), 4.07 – 4.20 (m, 2H), 4.35 – 4.44 (m, 2H), 4.48 (t, $J = 4.2$ Hz, 1H), 4.64 (t, $J = 4.2$ Hz, 1H), 7.02 (dd, $J = 3.0, 6.0$ Hz, 1H), 7.08 (d, $J = 1.5$ Hz, 1H), 7.11 – 7.21 (m, 1H), 7.30 (d, $J = 4.2$ Hz, 1H), 8.84 (s, 1H).

1.6. 5-(2-Fluoroethoxy)-1H-indole-2-carboxylic acid (13a)

To a solution of compound 12a (520 mg, 2.08 mmol) in methanol (30 mL) and water (5 mL) was added sodium hydroxide (160 mg, 4 mmol). The mixture was stirred at ambient temperature overnight and then refluxed for additional 6 hrs. Methanol was evaporated under reduced pressure and the residue was neutralized using 2N HCl aqueous solution. The crude product was extracted with dichloromethane (3 x 15 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated. After

removing the solvent, compound **13a** (337 mg, 73%) was afforded as a white solid. The ^1H NMR (DMSO- d_6) is δ 4.13 – 4.22 (m, 1H), 4.22 - 4.32 (m, 1H), 4.63 – 4.72 (m, 1H), 4.79 – 4.88 (m, 1H), 6.90 – 7.01 (m, 1H), 7.01 – 7.13 (m, 2H), 7.32 – 7.46 (m, 1H), 10.43 (s, 1H).

1.7. 5-(2-(2-Fluoroethoxy)ethoxy)-1H-indole-2-carboxylic acid (**13b**)

A similar procedure for making **13a** was followed to afford **13b** (94 mg, 95%) as pale yellow solid. The ^1H NMR (DMSO- d_6) is δ 3.67 (t, $J = 4.2$ Hz, 1H), 3.73 – 3.83 (m, 3H), 4.05 (t, $J = 4.5$ Hz, 2H), 4.41 (t, $J = 4.2$ Hz, 1H), 4.57 (t, $J = 4.2$ Hz, 1H), 6.82 (dd, $J = 2.4, 9.0$ Hz, 1H), 6.90 (d, $J = 0.6$ Hz, 1H), 6.96 (d, $J = 0.9$ Hz, 1H), 7.28 (d, $J = 4.5$ Hz, 1H), 7.84 (s, 1H), 11.25 (s, 1H).

1.8. 5-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)-1H-indole-2-carboxylic acid (**13c**)

A similar procedure for making **13a** was followed to afford **13c** (204 mg, 66%), a pale yellow solid. The ^1H NMR (DMSO- d_6) is δ 3.55 – 3.64 (m, 5H), 3.87 (t, $J = 3.9$ Hz, 1H), 3.73 (t, $J = 9.0$ Hz, 2H), 4.04 (t, $J = 7.8$ Hz, 2H), 4.39 (t, $J = 4.5$ Hz, 1H), 4.55 (t, $J = 4.2$ Hz, 1H), 6.84 (dd, $J = 3.3, 6.0$ Hz, 1H), 6.91 (d, $J = 0.6$ Hz, 1H), 7.02 (d, $J = 0.9$ Hz, 1H), 7.28 (d, $J = 4.5$ Hz, 1H), 8.17 (s, 1H), 11.52 (s, 1H).

1.9. 5-Hydroxy-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (**14a**)

General procedure for making amides: Procedure A 5-Hydroxyindole-2-carboxylic acid (350 mg, 1.98 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (494 mg, 2.4 mmol), HOBt (384 mg, 2.4 mmol) and compound **9a** (522 mg, 2 mmol) were dissolved in dichloromethane (30 mL) and stirred at ambient temperature overnight under nitrogen atmosphere. The mixture was washed with saturated sodium carbonate aqueous solution and dried over anhydrous sodium sulfate. After concentration, the product was purified by silica gel column chromatography (methanol/dichloromethane, 15/85. v/v) to afford **14a** (208 mg, 25%) as colorless grease. MP (oxalate salt): 162 °C (decomposed). The ^1H NMR (CDCl_3) is δ 1.65 – 1.75 (m, 2H), 1.75 – 2.00 (m, 2H), 2.52 (t, $J = 8.1$ Hz, 2H), 2.73 (s, 4H), 3.14 (s, 4H), 3.51 (d, $J = 5.7$ Hz, 2H), 3.86 (s, 3H), 6.56 (s, 1H), 6.65 (d, $J = 0.6$ Hz, 1H), 6.83 – 7.06 (m, 6H), 7.28 (s, 1H), 9.08 (s, 1H). HRMS (ESI) calcd for monoisotopic $\text{C}_{25}\text{H}_{31}\text{N}_4\text{O}_3$ m/z 423.2391 $[\text{M}+\text{H}]^+$, found 423.2406.

1.10. 5-(2-fluoroethoxy)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (14b)

Procedure A was followed to afford **14b** (178 mg, 73%), a slightly yellow solid. MP (oxalate salt): 176.9-177.7 °C. The ¹H NMR (CDCl₃) is δ 1.20 – 1.60 (m, 2H), 1.60 – 1.95 (m, 2H), 2.50 (t, *J* = 6.6 Hz, 2H), 2.70 (d, *J* = 0.3 Hz, 4H), 3.12 (s, 4H), 3.52 (d, *J* = 3.0 Hz, 2H), 3.86 (s, 3H), 4.19 (t, *J* = 4.2 Hz, 1H), 4.28 (t, *J* = 4.2 Hz, 1H), 4.69 (t, *J* = 4.2 Hz, 1H), 4.85 (t, *J* = 4.2 Hz, 1H), 6.61 (s, 1H), 6.76 (d, *J* = 0.6 Hz, 1H), 6.82 – 7.09 (m, 6H), 7.34 (d, *J* = 4.5 Hz, 1H), 9.09 (s, 1H). Elemental Analysis results C₂₆H₃₃FN₄O₃·0.5 H₂C₂O₄·0.5H₂O (C, H, N). Calcd: C: 62.05, H: 6.75, N: 10.72, Found: C: 62.36, H: 6.71, N: 10.64.

1.11. 5-(2-(2-Fluoroethoxy)ethoxy)-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-1H-indole-2-carboxamide (14c)

Procedure A was followed to afford **14c** (140 mg, 78%) as colorless grease. MP (oxalate salt): 180.1-181.3 °C. The ¹H NMR (CDCl₃) is δ 1.58 – 1.78 (m 2H), 1.78 – 1.98 (m, 2H), 2.42 – 2.58 (m, 2H), 2.71 (s, 4H), 3.13 (s, 4H), 3.52 (d, *J* = 3.0 Hz, 2H), 3.79 (t, *J* = 2.4 Hz, 1H), 3.83 – 4.00 (m, 6H), 4.17 (t, *J* = 5.4 Hz, 2H), 4.53 (t, *J* = 3.0 Hz, 1H), 4.68 (t, *J* = 3.0 Hz, 1H), 6.56 – 6.66 (s, br, 1H), 6.77 (s, 1H), 6.82 – 7.28 (m, 6H), 7.32 (d, *J* = 3.0 Hz, 1H), 9.11 (s, 1H). Elemental Analysis results: C₂₈H₃₇FN₄O₄·H₂C₂O₄·1.2H₂O (C, H, N). Calcd: C: 60.13, H: 7.03, N: 9.67, Found: C: 60.37, H: 6.89, N: 9.29.

1.12. 5-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)-N-(4-(4-(2-methoxy-phenyl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (14d)

Procedure A was followed to afford **14d** (228 mg, 98%) as colorless grease. MP (oxalate salt): 148.1-149.0 °C. The ¹H NMR (CDCl₃) is δ 1.62 – 1.72 (m, 2H), 2.03 (s, 2H), 2.50 (t, *J* = 4.5 Hz, 2H), 2.69 (s, 4H), 3.12 (s, 4H), 3.47 – 3.59 (m, 2H), 3.69 – 3.8 (m, 6H), 3.86 (s, 3H), 3.89 (t, *J* = 3.6 Hz, 2H), 4.16 (t, *J* = 4.8 Hz, 2H), 4.48 (t, *J* = 3.9 Hz, 1H), 4.64 (t, *J* = 4.2 Hz, 1H), 6.60 (s, br, 1H), 6.76 (d, *J* = 0.9 Hz, 1H), 6.83 – 7.04 (m, 5H), 7.04 (d, *J* = 1.2 Hz, 1H), 7.31 (d, *J* = 4.2 Hz, 1H), 9.17 (s, 1H). Elemental

Analysis results: $C_{30}H_{41}FN_4O_5 \cdot 0.5H_2C_2O_4 \cdot H_2O$ (C, H, N). Calcd: C: 60.08, H: 7.16, N: 9.04, Found: C: 60.36, H: 6.91, N: 9.02.

1.13. (E)-N-(4-(4-(2-(2-Fluoroethoxy)phenyl)piperazin-1-yl)but-2-en-1-yl)-1H-indole-2-carboxamide (15a)

Procedure A was followed to afford **15a** (120 mg, 79%) as colorless grease. MP (oxalate salt): 200.7-202.0 °C. The 1H NMR ($CDCl_3$) is δ 2.66 (s, 4H), 3.00 – 3.21 (m, 6H), 4.02 – 4.18 (m, 2H), 4.21 (t, J = 4.2 Hz, 1H), 4.31 (t, J = 4.20 Hz, 1H), 4.70 (t, J = 3.9 Hz, 1H), 4.86 (t, J = 4.2 Hz, 1H), 5.72 – 5.94 (m, 2H), 6.24 (s, 1H), 6.82 – 6.90 (m, 2H), 6.92 – 7.02 (m, 3H), 7.15 (t, J = 8.1 Hz, 1H), 7.30 (t, J = 8.1 Hz, 1H), 7.43 (dd, J = 0.4, 7.5 Hz, 1H), 7.66 (dd, J = 0.9, 8.4 Hz, 1H), 9.16 (s, 1H). Elemental Analysis results: $C_{25}H_{29}FN_4O_2 \cdot 0.5H_2C_2O_4$ (C, H, N). Calcd: C: 64.85, H: 6.28, N: 11.63, Found: C: 64.46, H: 6.27, N: 11.41.

1.14. (E)-N-(4-(4-(2-(2-Fluoroethoxy)phenyl)piperazin-1-yl)but-2-en-1-yl)-5-hydroxy-1H-indole-2-carboxamide (15b)

Procedure A was followed to afford **15b** (107 mg, 34%), a white solid. MP (oxalate salt): 200 °C (decomposed). The 1H NMR ($CDCl_3$) is δ 2.70 (s, 4H), 3.02 – 3.24 (m, 6H), 4.04 – 4.18 (m, 2H), 4.20 (t, J = 4.2 Hz, 1H), 4.30 (t, J = 5.1 Hz, 1H), 4.70 (t, J = 5.1 Hz, 1H), 4.86 (t, J = 5.1 Hz, 1H), 5.70 - 5.84 (m, 2H), 6.21 (s, 1H), 6.60 (d, J = 1.2 Hz, 1H), 6.72 – 6.88 (m, 2H), 6.90 – 7.00 (m, 4H), 7.25 (d, J = 7.5 Hz, 1H), 9.07 (s, 1H). Elemental Analysis results: $C_{25}H_{29}FN_4O_3 \cdot 0.5 H_2C_2O_4$ (C, H, N). Calcd: C: 62.76, H: 6.08, N: 11.26, Found: C: 62.49, H: 6.42, N: 11.32.

1.15. (E)-N-(4-(4-(2-(2-Fluoroethoxy)phenyl)piperazin-1-yl)but-2-en-1-yl)-5-methoxy-1H-indole-2-carboxamide (15c)

Procedure A was followed to afford **15c** (178 mg, 60%), a white solid. MP (oxalate salt): 145.7-147.3 °C. The 1H NMR ($CDCl_3$) is δ 2.67 (s, 4H), 3.05 – 3.25 (m, 6H), 3.85 (s, 3H), 4.04 – 4.16 (m, 2H), 4.21 (t, J = 4.2 Hz, 1H), 4.30 (t, J = 4.2 Hz, 1H), 4.70 (t, J = 4.2 Hz, 1H), 4.86 (t, J = 3.9 Hz, 1H), 5.70 - 5.90 (m, 2H), 6.21 (s, br., 1H), 6.76 (d, J = 2.4 Hz, 1H), 6.82 – 6.90 (m, 1H), 6.92 – 7.03 (m, 4H),

7.05 (d, $J = 2.4$ Hz, 1H), 7.30 (d, $J = 9$ Hz, 1H), 9.08 (s, 1H). Elemental Analysis results: $C_{26}H_{31}FN_4O_3 \cdot 0.5 H_2C_2O_4 \cdot H_2O$ (C, H, N). Calcd: C: 61.24, H: 6.47, N: 10.58, Found: C: 61.10, H: 6.51, N: 10.38.

1.16. (E)-5-Methoxy-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)but-2-en-1-yl)-1H-indole-2-carboxamide (15d)

Procedure A was followed to afford **15d** (209 mg, 99%), a white solid. MP (oxalate salt): 170.9–172.2 °C. The 1H NMR ($CDCl_3$) is δ 2.68 (s, 4H), 3.02 – 3.22 (m, 6H), 3.85 (s, 3H), 3.86 (s, 3H), 4.04 – 4.18 (m, 2H), 5.70 – 5.90 (m, 2H), 6.16 – 6.24 (s, br., 1H), 6.75 (d, $J = 2.4$ Hz, 1H), 6.84 – 7.03 (m, 5H), 7.05 (d, $J = 2.4$ Hz, 1H), 7.35 (d, $J = 9$ Hz, 1H), 9.07 (s, 1H). HRMS (ESI) calcd for monoisotopic $C_{25}H_{31}N_4O_3$ m/z 435.2396 $[M+H]^+$, found 435.2391.

1.17. (E)-5-(2-fluoroethoxy)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)but-2-en-1-yl)-1H-indole-2-carboxamide (16a)

Procedure A was followed to afford **16a** (186 mg, 74%), a pale yellow solid. MP (oxalate salt): 176.3 °C (decomposed). The 1H NMR ($CDCl_3$) is δ 2.68 (s, 4H), 3.02 – 3.20 (m, 6H), 3.86 (s, 3H), 4.12 (t, $J = 5.1$ Hz, 2H), 4.22 (t, $J = 4.2$ Hz, 1H), 4.30 (t, $J = 4.2$ Hz, 1H), 4.70 (t, $J = 4.2$ Hz, 1H), 4.86 (t, $J = 4.5$ Hz, 1H), 5.76 – 5.86 (m, 2H), 6.21 (s, 1H), 6.75 (d, $J = 0.6$ Hz, 1H), 6.83 – 7.04 (m, 5H), 7.08 (d, $J = 1.2$ Hz, 1H), 7.34 (d, $J = 4.5$ Hz, 1H), 9.09 (s, 1H). Elemental Analysis results: $C_{26}H_{31}FN_4O_3 \cdot 0.5 H_2C_2O_4 \cdot 0.5H_2O$ (C, H, N). Calcd: C: 62.29, H: 6.39, N: 10.76, Found: C: 62.46, H: 6.38, N: 10.40.

1.18. (E)-5-(2-(2-Fluoroethoxy)ethoxy)-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)but-2-en-1-yl)-1H-indole-2-carboxamide (16b)

Procedure A was followed to afford **16b** (230 mg, 70%), as pale brown grease. MP (oxalate salt): 166.5–168.3 °C. The 1H NMR ($CDCl_3$) is δ 2.69 (s, 4H), 3.00 – 3.40 (m, 6H), 3.79 (t, $J = 4.2$ Hz, 1H), 3.86 (s, 3H), 3.87 – 3.96 (m, 3H), 4.12 (t, $J = 4.5$ Hz, 2H), 4.18 (t, $J = 4.8$ Hz, 2H), 4.53 (t, $J = 4.2$ Hz, 1H), 4.68 (t, $J = 4.2$ Hz, 1H), 5.72 – 5.90 (m, 2H), 6.24 (t, $J = 3.3$ Hz, 1H), 6.75 (d, $J = 0.6$ Hz, 1H), 6.70 – 7.05 (m, 5H), 7.07 (d, $J = 0.9$ Hz, 1H), 7.20 (d, $J = 4.5$ Hz, 1H), 9.23 (s, 1H). Elemental Analysis

results: $C_{28}H_{35}FN_4O_4 \cdot 1.5H_2C_2O_4 \cdot H_2O$ (C, H, N). Calcd: C: 56.10, H: 6.07, N: 8.44, Found: C: 56.18, H: 6.06, N: 8.39.

1.19. (E)-5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)but-2-en-1-yl)-1H-indole-2-carboxamide (16c)

Procedure A was followed to afford **16c** (186 mg, 95%), a pale brown solid. MP (oxalate salt): 161.1–162.5 °C. The 1H NMR ($CDCl_3$) is δ 2.66 (s, 4H), 2.92 – 3.20 (m, 6H), 3.60 – 3.92 (m, 11H), 4.00 – 4.21 (m, 4H), 4.49 (t, $J = 4.2$ Hz, 1H), 4.64 (t, $J = 4.2$ Hz, 1H), 5.64 – 5.84 (m, 2H), 6.18 (s, br., 1H), 6.73 (d, $J = 4.5$ Hz, 1H), 6.78 – 7.09 (m, 5H), 7.09 – 7.35 (m, 2H), 9.06 (s, 1H). Elemental Analysis results: $C_{30}H_{39}FN_4O_5 \cdot H_2C_2O_4 \cdot 0.25H_2O$ (C, H, N). Calcd: C: 59.20, H: 6.44, N: 8.63, Found: C: 59.17, H: 6.37, N: 8.67.

1.20. N-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-1H-indole-3-carboxamide(17a)

Procedure A was followed to afford **17a** (98 mg, 21%), a pale brown solid. MP (oxalate salt): 205.3 °C (decomposed). The 1H NMR ($CDCl_3$) is δ 1.71 (s, 4H), 2.40 – 2.58 (m, 2H), 2.67 (s, 4H), 3.12 (s, 4H), 3.50 (d, $J = 3.0$ Hz, 2H), 4.20 (t, $J = 4.2$ Hz, 1H), 4.30 (t, $J = 4.2$ Hz, 1H), 4.69 (t, $J = 3.9$ Hz, 1H), 4.85 (t, $J = 4.2$ Hz, 1H), 6.72 (s, 1H), 6.81 – 7.01 (m, 3H), 7.05 (s, 1H), 7.14 – 7.24 (m, 1H), 7.40 – 7.50 (m, 1H), 7.62 (s, 1H), 7.81 (d, $J = 1.5$ Hz, 1H), 8.05 (t, $J = 4.8$ Hz, 1H), 10.76 (s, 1H). HRMS (ESI) calcd for monoisotopic $C_{25}H_{31}FN_4O_2$ m/z 439.2504 $[M+H]^+$, found 439.2489.

1.21. N-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-6-methoxy-1H-indole-3-carboxamide (17b)

Procedure A was followed to afford **17b** (206 mg, 59%), as colorless grease. MP (oxalate salt): 210.9–212.1 °C. The 1H NMR ($CDCl_3$) is δ 1.6 (s, 2H), 1.87 (s, 2H), 2.47 (t, $J = 6.6$ Hz, 2H), 2.65 (s, 4H), 3.10 (s, 4H), 3.45 – 3.60 (m, 2H), 3.86 (s, 3H), 4.19 (t, $J = 3.9$ Hz, 1H), 4.29 (t, $J = 3.9$ Hz, 1H), 4.68 (t, $J = 3.9$ Hz, 1H), 4.84 (t, $J = 4.2$ Hz, 1H), 6.29 (s, 1H), 6.70 – 7.00 (m, 5H), 7.27 (d, $J = 4.5$ Hz, 1H), 7.52 – 7.62 (m, 2H), 9.00 (s, 1H). Elemental Analysis results: $C_{26}H_{33}FN_4O_3 \cdot 0.5H_2C_2O_4 \cdot 0.5H_2O$ (C, H, N). Calcd: C: 62.05, H: 6.75, N: 10.54, Found: C: 62.02, H: 6.57, N: 10.65.

1.22. (E)-5-Methoxy-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)but-2-en-1-yl)-1H-indole-3-carboxamide (17c)

Procedure A was followed to afford **17c** (197mg, 65%), as pale yellow grease. MP (oxalate salt): 186.3-187.9 °C. The ¹H NMR (CDCl₃) is δ 2.69 (s, 4H), 3.02 – 3.20 (m, 6H), 3.85 (s, 3H), 3.87 (s, 3H), 4.09 – 4.18 (m, 2H), 5.79 – 5.84 (m, 2H), 6.01 (s, 1H), 6.83 – 7.04 (m, 5H), 7.28 (dd, *J* = 0.3, 4.5 Hz, 1H), 7.57 (d, *J* = 1.2 Hz, 1H), 7.64 (d, *J* = 1.5 Hz, 1H), 8.69 (s, 1H). Elemental Analysis results: C₂₅H₃₀N₄O₃·0.5H₂C₂O₄·1.25H₂O (C, H, N). Calcd: C: 62.20, H: 6.73, N: 11.16, Found: C: 62.54, H: 6.54, N: 10.77.

1.23. (E)-N-(4-(4-(2-(2-fluoroethoxy)phenyl)piperazin-1-yl)but-2-en-1-yl)-5-methoxy-1H-indole-3-carboxamide (17d)

Procedure A was followed to afford **17d** (95 mg, 89%) as white solid. MP (oxalate salt): 213.3-214.4 °C. The ¹H NMR (CDCl₃) is δ 2.67 (s, 4H), 3.02 – 3.22 (m, 6H), 3.88 (s, 3H), 4.10 – 4.16 (m, 2H), 4.20 (t, *J* = 4.8 Hz, 1H), 4.30 (t, *J* = 3.9 Hz, 1H), 4.69 (t, *J* = 4.2 Hz, 1H), 4.85 (t, *J* = 3.9 Hz, 1H), 5.80 – 5.85 (m, 2H), 5.95 (s, 1H), 6.81 – 7.01 (m, 5H), 7.29 (d, *J* = 6.6 Hz, 1H), 7.58 (d, *J* = 1.2 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 8.51 (s, 1H). Elemental Analysis results: C₂₆H₃₁FN₄O₃·0.5H₂C₂O₄·0.2H₂O (C, H, N). Calcd: C: 62.95, H: 6.34, N: 10.88, Found: C: 62.95, H: 6.32, N: 10.84.

1.24. (E)-N-(4-(4-(2-(2-Fluoroethoxy)phenyl)piperazin-1-yl)but-2-en-1-yl)-6-methoxy-1H-indole-3-carboxamide (17e)

Procedure A was followed to afford **17e** (165 mg, 80%), a white solid. MP (oxalate salt): 187 °C (decomposed). The ¹H NMR (CDCl₃) is δ 2.67 (s, 4H), 3.02 – 3.42 (m, 6H), 3.84 (s, 3H), 4.10 – 4.16 (m, 2H), 4.20 (t, *J* = 3.9 Hz, 1H), 4.29 (t, *J* = 4.2 Hz, 1H), 4.69 (t, *J* = 3.9 Hz, 1H), 4.85 (t, *J* = 4.2 Hz, 1H), 5.78 – 5.88 (m, 2H), 6.00 (s, 1H), 6.81 - 7.00 (m, 6H), 7.64 (d, *J* = 1.2 Hz, 1H), 7.86 (d, *J* = 4.5 Hz, 1H), 8.52 (s, 1H). Elemental Analysis results: C₂₆H₃₁FN₄O₃·0.5 H₂C₂O₄·H₂O (C, H, N). Calcd: C: 61.24, H: 6.47, N: 10.58, Found: C: 61.27, H: 6.49, N: 10.42.

2. *In Vitro* Binding Studies

2.1. Dopamine receptor binding assays

The binding properties of membrane-associated receptors were characterized by a filtration binding assay.³⁴ For human D₂, D₃, and D₄ dopamine receptors expressed in HEK 293 cells, 50 μ L of membrane homogenates were suspended in 50 mM Tris-HCl/150 mM NaCl/10 mM EDTA buffer, pH = 7.5 and incubated with 50 μ L of [¹²⁵I]IABN³⁴ at 37°C for 60 min, using 20 μ M (+)-butaclamol to define the non-specific binding. The radioligand concentration was equal to approximately 0.5 times the K_d value and the concentration of the competitive inhibitor ranged over 5 orders of magnitude for competition experiments. For each competition curve, two concentrations of inhibitor per decade were used and triplicates were performed. Binding was terminated by the addition of the cold wash buffer (10mM Tris-HCl/150mM NaCl, pH = 7.5) and filtration over a glass-fiber filter (Schleicher and Schuell No. 32). A Packard Cobra gamma counter was used to measure the radioactivity. The equilibrium dissociation constant and maximum number of binding sites was generated using unweighted non-linear regression analysis of data modeled according to the equation describing mass action binding. The concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC₅₀ value) was determined by using nonlinear regression analysis to analyze the data of competitive inhibition experiments. Competition curves were modeled for a single site and the IC₅₀ values were converted to equilibrium dissociation constants (K_i values) using the Cheng and Prusoff³⁵ correction. Mean K_i values \pm S.E.M. are reported for at least three independent experiments.

2.2. Sigma Receptor Binding Assays

Before determining the σ_1 and σ_2 receptor binding assays, the compounds were dissolved in either DMF, DMSO, or ethanol and then diluted in 50 mM Tris-HCl buffer containing 150 mM NaCl and 100 mM EDTA at pH = 7.4. The procedures for isolating the membrane homogenates and performing the σ_1 and σ_2 receptor binding assays have been described previously.^{26, 36}

Briefly, the σ_1 receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (~300 μg protein) and ~5 nM (+)-[^3H]-pentazocine (34.9 Ci/mmol, Perkin Elmer, Boston, MA). The total incubation time was 90 min at room temperature. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol. After 90 min, the reaction was terminated by the adding 150 μL of ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) using a 96 channel transfer pipette (Fisher Scientific, Pittsburgh, PA). The samples were harvested and filtered rapidly through a 96-well fiber glass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris-HCl buffer at pH = 8.0 for 1 h. Each filter was washed 3 times with 200 μL of ice-cold wash buffer, and the filter counted in a Wallac 1450 MicroBeta liquid scintillation counter (Perkin Elmer, Boston, MA).

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg protein) and ~5 nM [^3H]-DTG (58.1 Ci/mmol, Perkin Elmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_\square sites. The incubation time was 2 h at room temperature. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol. All other procedures were identical to those described above for the σ_1 receptor binding assay.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration that inhibits 50% of the specific binding of the radioligand (IC_{50} value). Competitive curves were best fit to a one-site fit and gave pseudo-Hill coefficients of 0.6–1.0. K_i values were calculated using Cheng and Prusoff method³⁵ and were presented as the mean \pm S.E.M. For these calculations, we used a K_d value of 7.89 nM for (+)-[^3H]-pentazocine and guinea pig brain and a K_d value of 30.73 nM for [^3H]-DTG and rat liver.²⁶

2.3. Whole cell adenylyl cyclase assay

The accumulation of ^3H -cyclic AMP in HEK cells was measured by a modification of the Shimizu et al method.³⁷ Transfected HEK cells were treated with serum-free media containing 2,8-[^3H]adenine (ICN Pharmaceutical Inc., Costa Mesa, CA) and cells were incubated at 37°C for 75 min. Cells and

drugs diluted in serum-free media containing 0.1 mM 3-isobutyl-1-methylxanthine (Sigma) were mixed to give a final volume of 500 μ L and cells were incubated for 20 min at 37°C. The reaction was stopped by addition of 500 μ L of 10% trichloroacetic acid and 1 mM cyclic AMP. After centrifugation, the supernatants were fractionated using Dowex AG1-X8 and neutral alumina to separate the [³H]ATP and the [³H]cyclic AMP. Individual samples were corrected for column recovery by monitoring the recovery of the cyclic AMP using spectrophotometric analysis at OD 259 nm.^{34, 37}