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Supporting Information

Design, Synthesis and Evaluation of Potent and Selective Ligands for the Dopamine 3 Receptor (D_3) with a Novel *in vivo* Behavioral Profile

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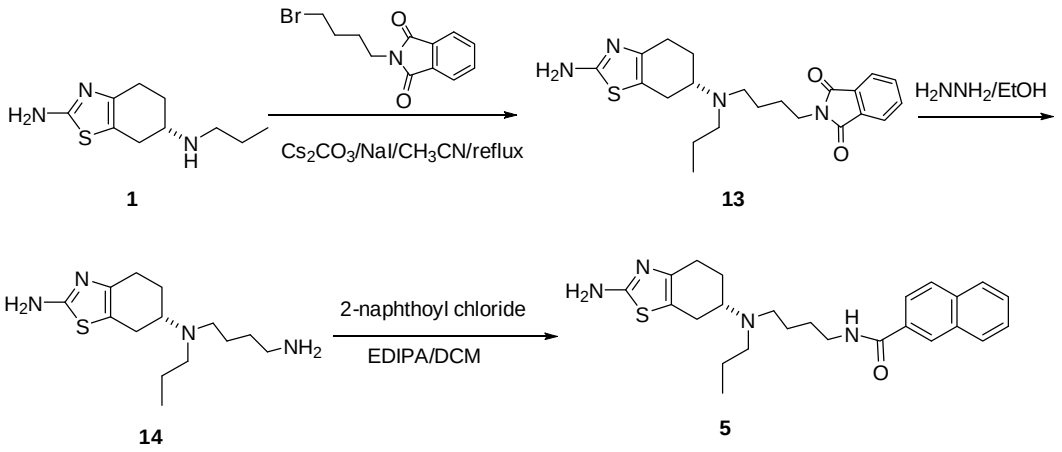
I. Chemistry

Solvents and reagents were obtained commercially and were used without further purification. Reactions were monitored by TLC carried out on 250 μm E. Merck silica gel plates (60F-254) using UV light as visualizing agent. E. Merck silica gel (60, particle size 15-40 μm) was used for flash column chromatography. NMR spectra were recorded on a Bruker Avance300 spectrometer (300 MHz). Chemical shifts (δ) are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. High resolution electrospray ionization mass spectra (MS) were run on a Micromass AutoSpec Ultima mass spectrometer. Elemental analysis (EA) was performed by the Department of Chemistry, University of Michigan (Ann Arbor) using a Perkin-Elmer 2400 Series II Analyzer. HPLC analysis was performed on a Waters 2795 using a Waters SunFire C18 (150 mm×4.6 mm) column, mobile phase flow 1.0 mL/min, gradient water (with 0.1 % TFA)/acetonitrile (with 0.1 % TFA) 0~50%, and UV detection at 254 nm.

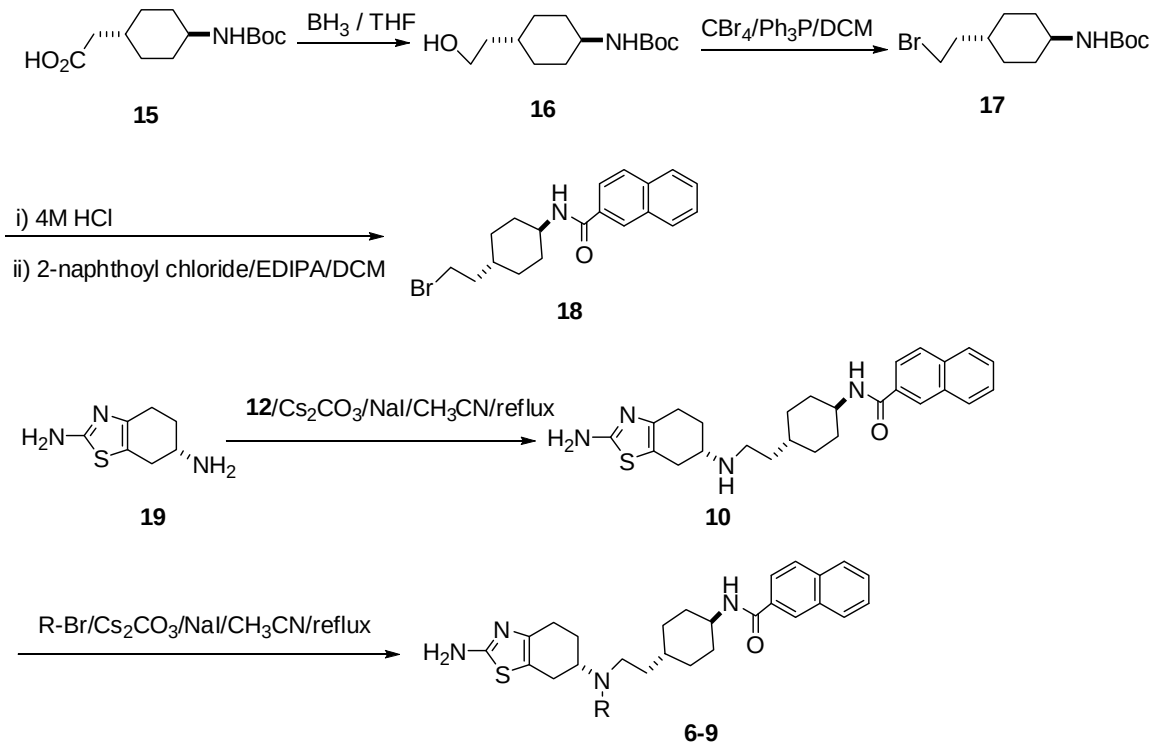
Synthesis

The synthesis of compound **5** is outlined in Scheme Scheme 1. Briefly, commercially available pramipexole **1**, purchased from APAC Pharmaceutical Co., USA, was treated with *N*-(4-bromobutyl)-phthalimide in the presence of Cs_2CO_3 in acetonitrile to afford amine **13**. Compound **13** was treated with hydrazine to remove the phthalimide protective group to give the amine **14**, followed by reaction with 2-naphthoyl chloride to furnish **5**.



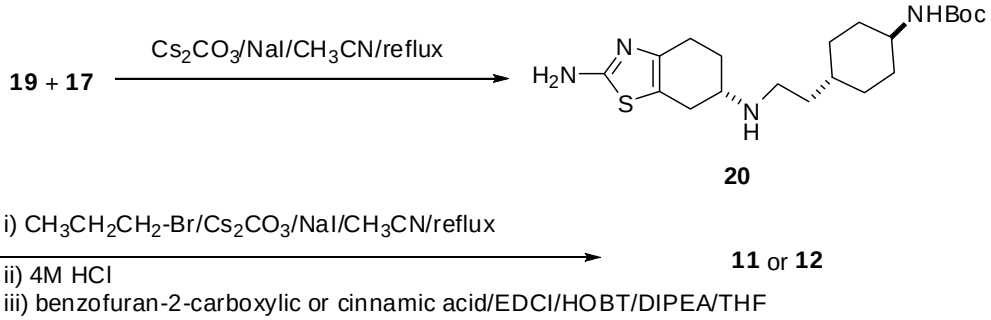
Scheme 1. Synthetic route for compound **5.**

The syntheses of compounds **6**-**10** is shown in Scheme 2. The carboxylic acid group in compound **15** was reduced to alcohol **16** by borane. Compound **16** was treated with tetrabromomethane and triphenylphosphione in dichloromethane to give bromide 17. Deprotection of Boc protective group, followed by 2-naphthyl chloride treatment afforded bromide **18**. (6S)-4,5,6,7-tetrahydro-1,3-benzothiazol-2,6-diamine (**19**) was reacted with the bromide **18** in the presence of Cs_2CO_3 and NaI in acetonitrile to give compound **10**, which was also used as the key intermediate for the synthesis of compounds **6**-**9**. Compound **10** was treated with different bromides and the products purified by chromatography to afford the desired compounds **6**-**9**.



Scheme 2. Synthesis of compounds **6-10.**

The synthesis of compounds **11** and **12** is outlined in Scheme 3. Briefly, (6*S*)-4,5,6,7-tetrahydro-1,3-benzothiazol-2,6-diamine (**19**) was reacted with the bromide **17** in the presence of Cs_2CO_3 and NaI in acetonitrile to give the key intermediate **20**. Compound **15** was treated with n-propyl bromide, deprotected by 4M HCl, and condensed with benzofuran-2-carboxylic acid or cinnamic acid to give designed compound **11** or **12**.



Scheme 3. Synthesis of compounds **11** and **12**.

(S)-2-(4-((2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)butyl)isoindoline-1,3-dione (13). *N*-(4-bromobutyl)-phthalimide (2.25 g, 7.98 mmol), cesium carbonate (2.6 g**,** 7.98 mmol), and sodium iodide (1.80 g, 12 mmol) were added to a solution of pramipexole **1** (1.53 g, 7.25 mmol) in acetonitrile (50 mL). After refluxing for 3 hr, the mixture was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 5:95) gave **13** as a colorless oil (2.7 g, 90.4 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88-8.00 (m, 2H), 7.78-7.60 (m, 2H), 4.90 (s, 2H), 3.70 (t, *J*=7.2 Hz, 2H), 3.10-2.95 (m, 1H), 2.78-2.30 (m, 8H), 2.05-1.90 (m, 1H), 1.77-1.60 (m, 3H), 1.56-1.40 (m, 4H), 0.87 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ168.89, 165.83, 145.53, 134.28, 132.53, 123.58, 117.96, 57.59, 52.97, 50.41, 38.34, 27.01, 26.81, 26.71, 26.05, 25.33, 22.64, 12.24.

(*S*)-N^6-(4-aminobutyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (14) Hydrazine hydrate (5 mL) was added to a solution of **13** (3.4 g, 8.25 mmol) in ethanol (30 mL) and the mixture was refluxed for 2 hr. The mixture was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of solvent gave **14** as a colorless oil (2.32 g, 99 %), which was used for the next step without further purification.

(*S*)-*N*-(4-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)butyl)-2-naphthamide (5) 2-Naphthoyl chloride (0.92 g, 4.8 mmol) and DIPEA (0.62 g, 4.8

mmol) were added to a solution of **14** (1.13 g, 4.0 mmol) in anhydrous dichloromethane. After stirring at room temperature for 2 hr, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/EtOAc, 1:9) to give **5** as a colorless solid (1.40 g, 80 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.96-7.75 (m, 4H), 7.60-7.45 (m, 2H), 6.72 (t, *J*=5.7 Hz, 1H), 4.95 (s, 2H), 3.52 (q, *J*=6.2 Hz, 2H), 3.10-2.95 (m, 1H), 2.73-2.35 (m, 8H), 2.00-1.88 (m, 1H), 1.80-1.37 (m, 7H), 0.89 (t, *J*=7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) 168.1, 166.00, 145.46, 135.04, 133.01, 132.50, 129.26, 128.79, 128.14, 127.94, 127.67, 127.12, 124.06, 117.72, 57.73, 53.11, 50.64, 40.57, 27.96, 26.99, 26.13, 25.21, 22.57, 12.31. Free base was converted into its HCl salt. Anal. Calcd for $C_{25}H_{32}N_4OS{\cdot}2HCl{\cdot}2H_2O$: C, 55.05; H, 7.02; N, 10.27. Found: C, 54.62; H, 7.10; N, 10.12.

***Trans-tert*-butyl-4-(2-hydroxyethyl)cyclohexylcarbamate (16)** 1.0 M BH_3 (15 mL, 15 mmol) in THF was added to a solution of **15** (2.57 g, 10 mmol) in anhydrous THF (30 mL). The reaction mixture was stirred at 0 ℃ for 1 hr. The reaction was quenched with water and extracted with ethyl acetate. Solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (Hexane/EtOAc, 1:1) to give **16** as a colorless oil (2.40 g, 98 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.39 (s, broad, 1H), 3.69 (t, *J*=6.6 Hz, 2H), 3.45-3.25 (m, 1H), 2.05-1.94 (m, 2H), 1.85-1.70 (m, 2H), 1.55-1.23 (m, 4H), 1.45 (s, 9H), 1.20-0.95 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.67, 79.47, 61.17, 50.22, 40.03, 33.81, 33.76, 32.24, 28.83.

***Trans-tert*-butyl-4-(2-bromoethyl)cyclohexylcarbamate (17)** Triphenylphosphine (3.9 g, 14.8 mmol) and carbon tetrabromide (4.9 g, 14.8 mmol) were added to a solution of **16** (3.0 g, 12.3 mmol) in dichloromethane (40 mL). The solution was stirred at 0 ℃ for 1 hr and room temperature for 1 hr. Solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (Hexane/EtOAc, 6:1) to give **17** as a colorless solid (3.7 g, 98 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.5 (s, broad), 3.44 (t, *J*=7.0 Hz, 2H), 3.47-3.30 (m, 1H), 2.10-1.92 (m, 2H), 1.82-1.70 (m, 4H), 1.45 (s, 9H), 1.53-1.35 (m, 1H), 1.25-0.90 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ155.63, 79.49, 50.13, 39.92, 35.59, 33.80, 33.57, 32.17, 31.54, 31.29, 29.08, 28.83, 28.55.

***Trans-N*-(4-(2-bromoethyl)cyclohexyl)-2-naphthamide (18)** 4.0 M HCl solution in dioxane (4.0 mL, 16.0 mmol) was added to a solution of **17** (1.0 g, 3.3 mmol) in dioxane (15 mL) and the reaction mixture was stirred at room temperature for 2 hr. Solvent was evaporated *in vacuo* and the residue was used directly for the next step without further purification. 2-Naphthoyl chloride (750 mg, 3.93 mmol) and triethylamine (707 mg, 7.0 mmol) were added to a solution of the above residue in dichloromethane (20 mL). After stirring at room temperature for 2 hr, the mixture was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 3:1) to give **18** as a colorless solid (0.92 g, 77 % for two ateps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (s, 1H), 7.98-7.80 (m, 4H), 7.65-7.50 (m, 2H), 6.15 (d, *J*=8.1 Hz, 1H), 4.10-3.95 (m, 1H), 3.52 (t, *J*=7.0 Hz, 2H), 2.30-2.15 (m, 2H), 1.97-1.80 (m, 4H), 1.65-1.48 (m, 1H), 1.43-1.10 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.83, 134.65, 132.61, 132.12, 128.86, 128.39, 127.73, 127.55, 127.22, 126.72, 123.63, 49.08, 39.46, 35.19, 32.89, 31.79, 31.07.

***Trans*-*N*-(4-(2-((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-ylamino)ethyl)cyclohexyl)-2-naphthamide (10)** Bromide **18** (1.18 g, 3.28 mmol), cesium carbonate (1.59 g**,** 4.90 mmol), and sodium iodide (0.737 g, 4.90 mmol) were added to a solution of **19** (0.554 g, 3.28 mmol) in acetonitrile (30 mL). After refluxing for 48 hr, the mixture was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 1:6) gave **10** as a colorless solid (0.90 g, 61 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (s, 1H), 7.95-7.84 (m, 4H), 7.58-7.28 (m, 2H), 6.12 (d, *J*=8.7 Hz, 1H), 4.81 (s, 2H), 4.02-3.99 (m, 1H), 3.04-2.80 (m, 2H), 2.76-2.35 (m, 5H), 2.30-2.00 (m, 3H), 1.95-1.60 (m, 3H), 1.55-1.10 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.76, 165.32, 145.11, 134.64, 132.63, 132.19, 128.86, 128.40, 127.74, 127.53, 127.17, 126.72, 123.59, 116.54, 54.09, 49.22, 45.05, 37.51, 35.01, 33.19, 31.88, 30.14, 29.51, 24.97; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 449.2375; found 449.2369; purity HPLC 100.0 %, t_R = 29.903 min.

***Trans*-*N*-(4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)cyclohexyl)-2-naphthamide (6)** *n*-Propyl bromide (38 mg, 0.28 mmol), cesium carbonate (108 mg**,** 0.33 mmol), and sodium iodide (50 mg, 0.33 mmol) were added to a solution of **10** (100 mg, 0.22 mmol) in acetonitrile (15 mL). After refluxing for 48 hr, the mixture was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 1:6)

gave **6** as a colorless oil (35 mg, 32 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (s, 1H), 7.89-7.80 (m, 4H), 7.56-7.40 (m, 2H), 6.21 (d, *J*=8.1 Hz, 1H), 4.93 (s, 2H), 4.10-3.90 (m, 1H), 3.15-2.95 (m, 1H), 2.80-2.30 (m, 8H), 2.20-1.90 (m, 3H), 1.89-1.61 (m, 3H), 1.55-1.05 (m, 9H), 0.90 (t, *J*=7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.76, 165.55, 145.10, 134.61, 132.60, 132.19, 128.84, 128.35, 127.71, 127.50, 127.18, 126.68, 123.63, 117.33, 57.44, 52.62, 49.25, 48.52, 35.98, 35.17, 33.20, 31.95, 26.59, 25.78, 24.87, 22.16, 11.88; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 491.2845; found 491.2845; purity HPLC 100.0%, t_R = 31.816 min.

***Trans*-*N*-(4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(butyl)amino)ethyl)cyclohexyl)-2-naphthamide (7).** **7** was prepared under similar conditions as described for **6** (28 %).^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.92-7.81 (m, 4H), 7.57-7.40 (m, 2H), 6.26 (s, broad, 1H), 5.01 (s, 2H), 4.05-3.90 (m, 1H), 3.40-2.40 (m, 8H), 2.30-2.10 (m, 3H), 2.00-1.50 (m, 7H), 1.49-1.05 (m, 8H), 0.95 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.18, 135.04, 133.01, 132.49, 129.28, 128.77, 128.13, 127.95, 127.65, 127.11, 124.07, 50.83, 49.49, 49.13, 35.63, 33.38, 32.16, 25.05, 20.94, 14.31; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 505.3001; found 505.2995; purity HPLC 99.7 %, t_R = 34.009 min.

***Trans*-*N*-(4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(isopentyl)amino)ethyl)cyclohexyl)-2-naphthamide (8).** **8** was prepared under similar conditions as described for **6** (25 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (s, 1H),

7.93-7.80 (m, 4H), 7.56-7.48 (m, 2H), 6.15 (d, *J*=8.1 Hz, 1H), 4.87 (s, 2H), 4.10-3.90 (m, 1H), 3.50-2.50 (m, 8H), 2.30-2.10 (m, 3H), 2.00-1.10 (m, 14 H), 0.94 (d, *J*=6.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.78, 144.81, 134.65, 132.61, 132.06, 128.88, 128.39, 127.74, 127.56, 127.24, 126.72, 123.64; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 519.3158; found 519.3150; purity HPLC 98.3 %, t_R = 35.819 min.

***Trans*-*N*-(-4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(2-cyclohexylethyl)amino)ethyl)cyclohexyl)-2-naphthamide (9).** **9** was prepared under similar conditions as described for **6** (18 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (s, 1H), 7.90-7.70 (m, 4H), 7.55-7.35 (m, 2H), 6.36 (s, broad, 1H), 5.17 (s, 2H), 4.10-3.90 (m, 1H), 3.50-2.45 (m, 8H), 2.35-2.05 (m, 3H), 2.00-0.80 (m, 24 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.81, 166.24, 144.63, 134.63, 132.60, 132.03, 128.89, 128.34, 127.71, 127.54, 127.31, 126.68, 123.72, 58.81, 49.06, 48.79, 48.63, 35.98, 35.38, 35.16, 34.80, 33.22, 32.88, 31.72, 29.69, 26.55, 26.37, 26.14, 24.63; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 559.3471; found 559.3465; purity HPLC 100.0 %, t_R = 40.403 min.

***Trans*-*tert*-butyl-4-(2-((S)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-ylamino)ethyl)cyclohexylcarbamate (20).** Bromide **17** (1.1 g, 3.6 mmol), cesium carbonate (1.47 g**,** 4.5 mmol), and sodium iodide (0.68 g, 4.5 mmol) were added to a solution of **19** (0.63 g, 3.0 mmol) in acetonitrile (40 mL). After refluxing for 48 hr, the mixture was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 1:6) gave **20** as a colorless oil

(0.8 g, 61 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.93 (s, 2H), 4.02 (d, *J*=7.4 Hz, 1H), 3.50-3.30 (m, 1H), 3.10-2.95 (m, 1H), 2.80-2.35 (m, 8H), 2.05-1.90 (m, 3H), 1.85-1.60 (m, 3H), 1.55-1.00 (m, 9H), 1.44 (s, 9H), 0.87 (t, J=7.2 Hz, 3H).

***Trans*-*N*-(4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)cyclohexyl)benzofuran-2-carboxamide (11).** 4.0 M HCl solution in dioxane (1.0 mL, 4.0 mmol) was added to a solution of **20** (87 mg, 0.2 mmol) in dioxane (10 mL) and the reaction mixture was stirred at room temperature for 2 hr. Solvent was evaporated *in vacuo* and the residue was used directly for the next step without further purification. To a solution of the residue in THF (20 mL), were added benzofuran-2-carboxylic acid (32 mg, 0.2 mmol), EDCI (38 mg, 0.2 mmol), HOBT (27 mg, 0.2 mmol), and DIPEA (103 mg, 0.8 mmol). The reaction mixture was stirred at room temperature for 2 hr. Solvent was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 1:6) gave **11** as a colorless oil (60 mg, 63 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.68 (d, *J*=7.6 Hz, 1H), 7.52-7.28 (m, 4H), 6.49 (d, *J*=8.2 Hz, 1H), 4.82 (s, broad, 2H), 3.98-3.85 (m, 1H), 3.50-2.25 (m, 8H), 2.20-1.10 (m, 16H), 0.94 (t, *J*=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.50, 155.06, 149.22, 128.06, 127.22, 124.08, 123.11, 112.08, 110.71, 52.80, 48.68, 35.63, 33.04, 31.74, 19.26, 11.97, 11.77; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 481.2637; found 481.2637; purity HPLC 99.2 %, t_R = 29.761 min.

***Trans*-*N*-(4-(2-(((S)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)cyclohexyl)cinnamamide (12).** 4.0 M HCl solution in dioxane (1.0 mL, 4.0 mmol) was added to a solution of **20** (87 mg, 0.2 mmol) in dioxane (10 mL) and the reaction mixture was stirred at room temperature for 2 hr. Solvent was evaporated *in vacuo* and the residue was used directly for the next step without further purification. To a solution of the residue in THF (20 mL), were added cinnamic acid (29 mg, 0.2 mmol), EDCI (38 mg, 0.2 mmol), HOBT (27 mg, 0.2 mmol), and DIPEA (103 mg, 0.8 mmol). The reaction mixture was stirred at room temperature for 2 hr. Solvent was evaporated *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, and dried over anhydrous Na_2SO_4. Flash column chromatography (MeOH/EtOAc, 1:6) gave **12** as a colorless oil (75 mg, 80 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, *J*=15.6 Hz, 1H, 7.52-7.35 (m, 5H), 6.39 (d, *J*=15.6 Hz, 1H), 5.60 (s, broad, 1H), 4.90 (s, 2H), 3.95-3.80 (m, 1H), 3.30-2.30 (m, 8H), 2.20-1.95 (m, 3H), 1.93-1.02 (m, 13H), 0.92 (t, *J*=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.87, 165.05, 144.85, 140.66, 134.92, 129.54, 128.77, 127.73, 121.10, 69.73, 57.86, 52.54, 48.71, 35.18, 33.06, 31.80, 30.84, 26.34, 25.36, 24.76, 18.88, 11.79; HRMS-Electrospray (*m/z*): $[M+H]^+$ calcd 467.2845; found 467.2830; purity HPLC 95.4 %, t_R = 29.227 min.

Table S1. Purity of target compounds determined by HPLC, including impurity tracings information.

Target compounds			Impurity tracings	
#	Retention time (min)	Purity (%)	Retention time (min)	%
6	31.816	100.0	-	-
7	34.009	99.7	33.453	0.3
8	35.819	98.3	23.632	1.7
9	40.403	100.0	-	-
10	29.903	100.0	-	-
11	29.761	99.2	23.715	0.8
12	29.227	95.4	28.895	4.6

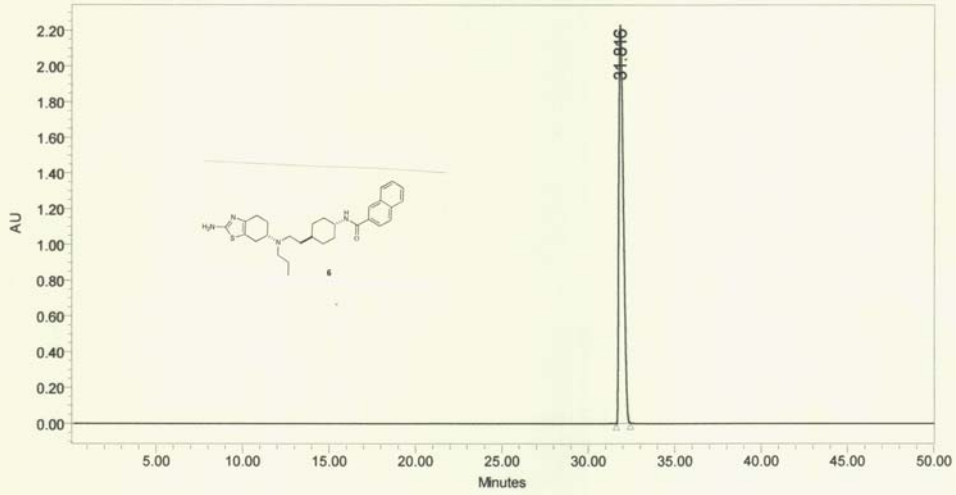
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-998	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/10/08 9:31:08 PM
Vial:	1:A,4	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/11/08 10:43:18 AM
Injection Volume:	20.00 ul	Processing Method:	Default
Run Time:	50.0 Minutes	Channel Name:	WWn Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	31.816	11130704	100.00

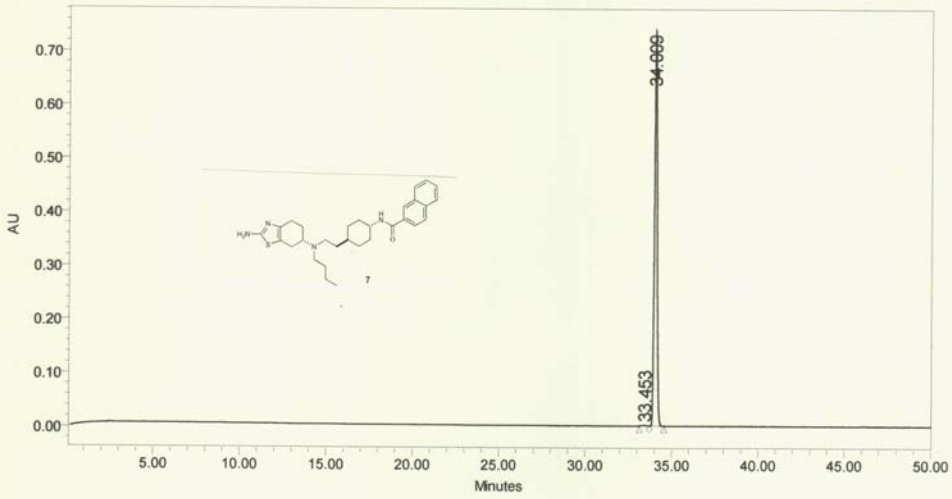
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1016	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/11/08 1:33:11 AM
Vial:	1:A,7	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/11/08 11:06:05 AM
Injection Volume:	20.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvln Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	33.453	24557	0.30
2	34.009	3033314	99.70

Report Method: Analytical HPLC

Printed 4:50:22 PM 8/8/08

Page: 1 of 1

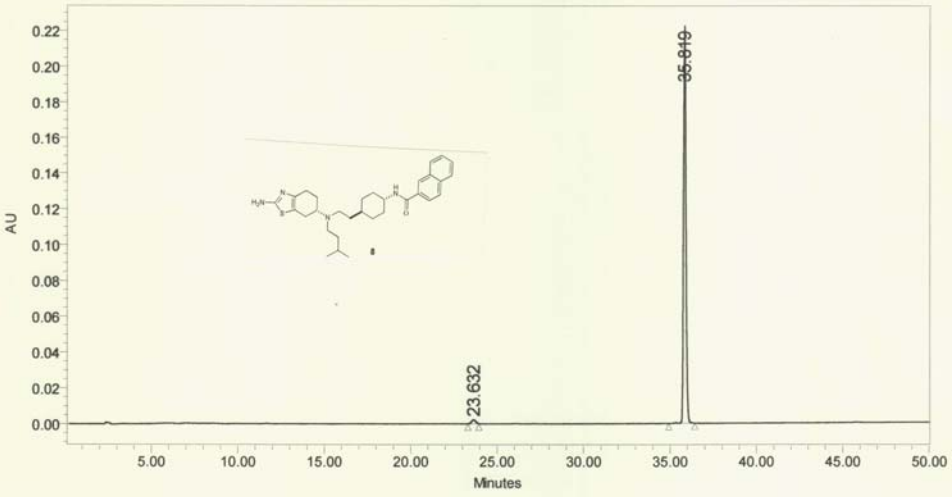
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1022	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/12/08 5:41:16 PM
Vial:	1:A,2	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/12/08 10:14:35 PM
Injection Volume:	25.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvln Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	23.632	37997	1.75
2	35.819	2133227	98.25

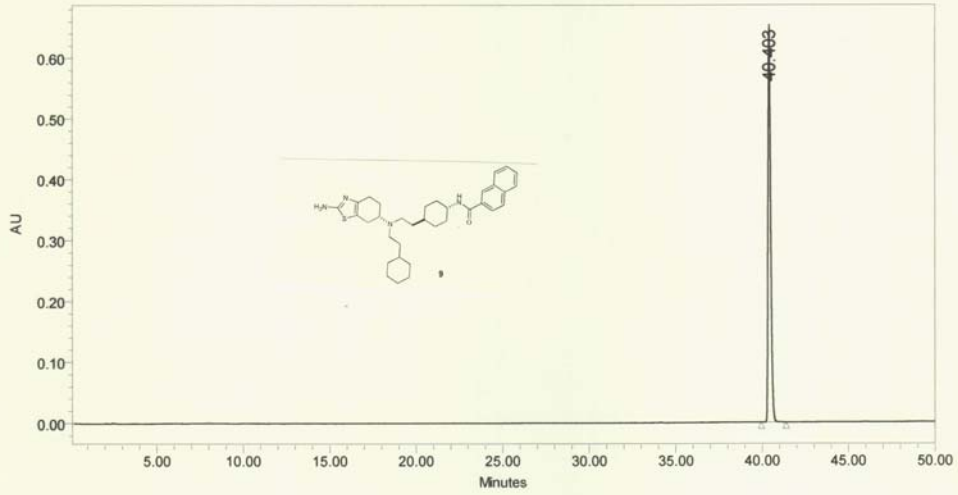
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1024	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/11/08 2:53:51 AM
Vial:	1:A,8	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/11/08 11:13:58 AM
Injection Volume:	20.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvln Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	40.403	7113017	100.00

Report Method: Analytical HPLC

Printed 4:50:44 PM 8/8/08

Page: 1 of 1

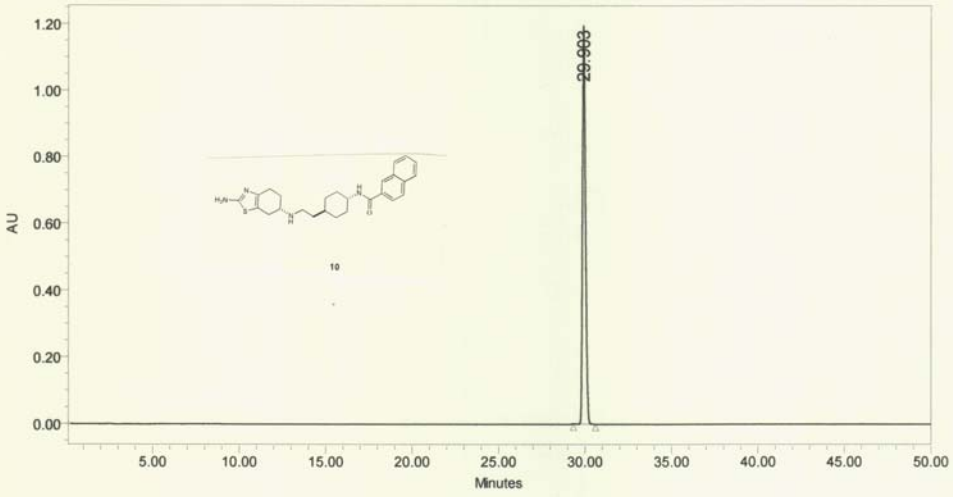
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1014	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/11/08 12:12:31 AM
Vial:	1:A,6	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/11/08 11:03:49 AM
Injection Volume:	20.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvln Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	29.903	14394815	100.00

Report Method: Analytical HPLC

Printed 4:49:55 PM 8/8/08

Page: 1 of 1

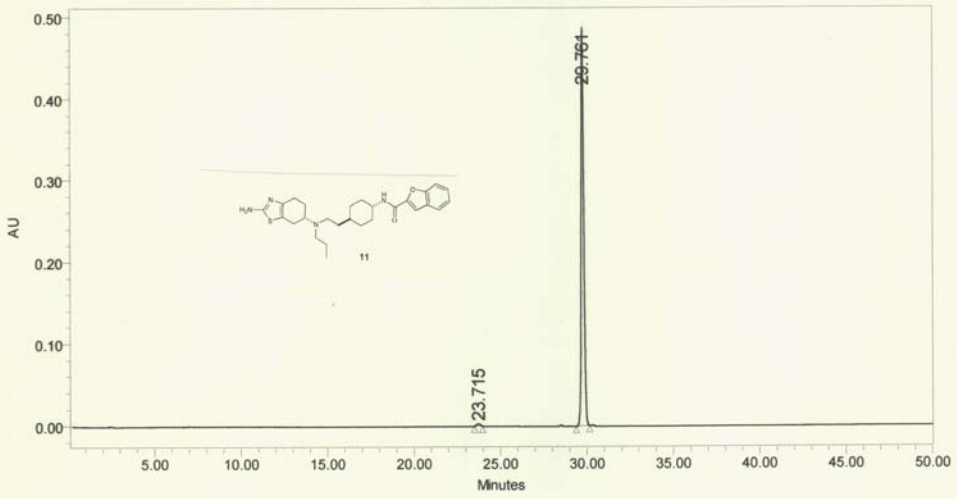
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1034	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/12/08 7:01:56 PM
Vial:	1:A,3	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/12/08 10:20:06 PM
Injection Volume:	25.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvln Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	23.715	40122	0.80
2	29.761	4991442	99.20

Report Method: Analytical HPLC

Printed 4:59:27 PM 8/8/08

Page: 1 of 1

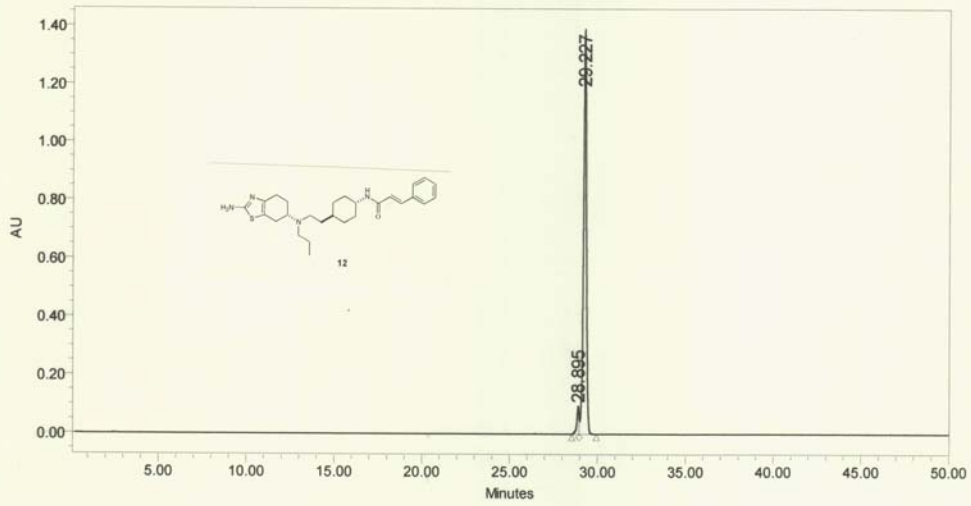
Individual Sample Report

Reported by User: System

Project Name: Jan2006

SAMPLE INFORMATION

Sample Name:	CJ-1037	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/10/08 10:51:48 PM
Vial:	1:A,5	Acq. Method Set:	0to50%B in 50min_Delay 30min
Injection #:	1	Date Processed:	6/11/08 2:05:04 PM
Injection Volume:	20.00 ul	Processing Method:	Analytical HPLC
Run Time:	50.0 Minutes	Channel Name:	Wvn Ch1
Sample Set Name:	HK	Proc. Chnl. Descr.:	PDA 254.0 nm



	RT	Area	% Area
1	28.895	787692	4.60
2	29.227	16343369	95.40

Report Method: Analytical HPLC

Printed 4:56:49 PM 8/8/08

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II. Computational modeling methods

The human dopamine subtype 3 (D_3) receptor was homology-modeled using the crystal structure of human β2 Adrenergic (β2AD) receptor (PDB entry: 2RH1) at 2.4 Å resolution as the template.[1] The sequence alignment used was based on sequence analysis of 493 members of the amine sub-family of GPCR proteins.[2] Initial 3D models of the human D_3 were generated using the program Modeller (version 9v2).[3] The initial D_3 receptor models from Modeller were then inserted into a 2-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) membrane[4] in a TIP3 water environment. Molecular dynamic (MD) simulations were performed to further refine the modeled structures of the D_3 receptor using Gromacs (version 3.3.1) with united atom representation for the receptor.[5]

For docking, all the binding poses of our compounds with the D_3 receptor were predicted using the GOLD program (version 3.1).[6,7] The center of the binding site for the D_3 receptor was set at centre of the Asp110 with a radius of 13 Å, large enough to cover the binding pocket. For each genetic algorithm (GA) run, a maximum number of 200 000 operations were performed on a population of 5 islands of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. The docking simulations were terminated after 10 runs for each compound. GoldScore implemented in Gold was used as the fitness function to evaluate the docked conformations. The 10 conformations ranked highest by each fitness function were saved for analysis of the predicted docking modes. For the docking poses reported in Figure 1, these were the highest ranked conformations from the docking simulations.

III. In Vitro dopamine receptor binding assays

Determination of D_3 dopamine receptor affinity and selectivity were performed in membranes prepared from the brains of adult, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN). All compounds were dissolved in 100% EtOH at a concentration of 5 mM.

[^{3}H]PD 128907 binding assays. [^{3}H]PD 128907 binding assays for D_3 receptors dopamine receptors were performed as previously described in detail.[8,9] Rat ventral striatum (nucleus accumbens and olfactory tubercles) was prepared in assay buffer (50 mM Tris, 1 mM EDTA; pH 7.4 at 23° C) to yield a final concentration of 10 mg original wet weight (o.w.w.)/ml. Membranes were incubated with [^{3}H]PD 128907 (0.3 nM; 116 Ci/mmol; Amersham, Arlington Heights, IL) and various concentrations of competing compounds (10^{-10} to 10^{-4} M). Nonspecific binding was defined by 1 μM spiperone. Assay tubes were incubated at 23° C for 3 hrs. The reaction was terminated by rapid vacuum filtration. Data were analyzed using SigmaPlot 8.0.2. using the K_D value for [^{3}H]PD 128907 of 0.3 nM.[8] K_i values are expressed at the mean ± SEM of 3-6 independent determinations.

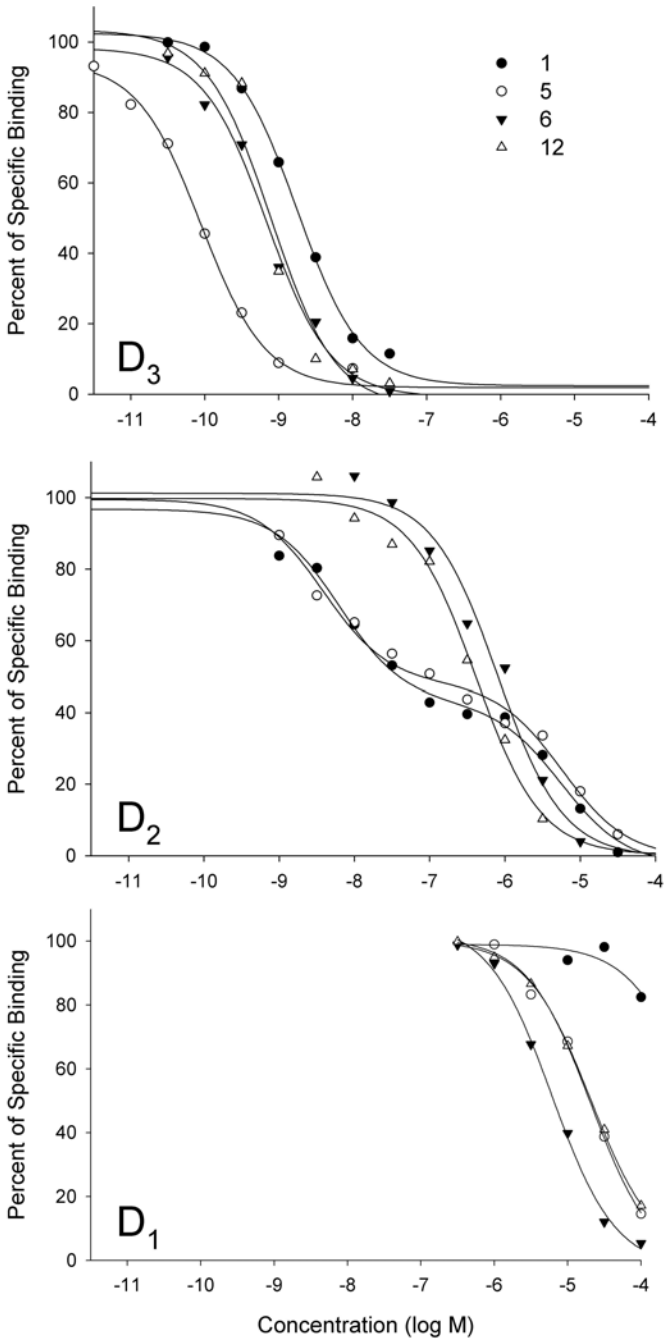
[^{3}H]Spiperone binding assays. [^{3}H]spiperone binding assays for D_2-like receptors were performed as previously described in detail[9,10] and as described for [^{3}H]PD 128907 except for the following. Assays were performed using membranes prepared from rat caudate-putamen and the final membrane homogenate concentration was 1.5 mg o.w.w./ml. The assay buffer was 50 mM Tris-HCl, 5 mM KCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$, pH 7.4 at 23°C; the concentration of [^{3}H]spiperone (24 Ci/mmol; Amersham) was

0.2 nM; and the incubation time was 90 min at 23° C. Nonspecific binding was defined in the presence of 1 μM (+)-butaclamol. K_i values were determined using the K_D value for [^{3}H]spiperone of 0.1 nM.[10]

[^{3}H]SCH 23390 binding assays. [^{3}H] SCH 23390 binding assays for D_1-like dopamine receptors were performed as previously described in detail[9] and as described for [^{3}H]spiperone binding except the concentration of [^{3}H]SCH 23390 (73 Ci/mmol; Amersham) was 0.3 nM. K_i values were determined using the K_D value for [^{3}H]SCH 23390 of 0.3 nM.[9]

The binding curves for compounds **1**, **5**, **6** and **12** are shown in Figure S1.

Figure S1. Competitive binding curves of compounds 1, 5, 6 and 12 in our in vitro binding assays.


Percent of Specific Binding
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
-4
1
5
6
12
D3
D2
D1
Concentration (log M)

III. *In vivo* Yawning and hypothermia assays in rats

Rats were purchased from Harlan (Indianapolis, IN) and housed three to a cage for yawning studies, and one to a cage for hypothermia studies. Rats used in the hypothermia studies had a radio-telemetric probe (E-4000 E-Mitter, Mini-Mitter, Bend, OR, USA) implanted into their peritoneal cavity, and were allowed 7 days to recover prior to experimentation as previously described.[11] Yawning studies were performed as previously described,[11,12] with yawning defined as a prolonged (~1 sec.), wide opening of the mouth followed by a rapid closure. The capacity of pramipexole, and other ligands investigated to induce yawning and hypothermia was assessed using a single dosing procedure (one dose per rat), with yawns recorded for a period of 60 min, and hypothermia for a period of 120 min after s.c. (1ml/kg) administration of compounds. The capacity of CJ-1037 to alter the induction of yawning and hypothermia by pramipexole was assessed using a multiple dosing procedure in which rats were first treated with CJ-1037 (0.0, 10.0, or 32.0 mg/kg) followed by five successive doses of pramipexole each separated by 30 min. Yawning was recorded for 30 min after each injection. Determination of changes in core body temperature for compounds alone were determined by comparing body temperature 30 and 60 min after each single dose of compounds to that obtained 1 min prior to injection of that compound, while the effects of CJ-1037 on pramipexole-induced hypothermia were determined by comparing the differences in core body temperature 30 min after each dose of CJ-1037 alone, or in combination with pramipexole to the body temperature obtained 1 min prior to the injection of CJ-1037. Yawns and changes in core body temperature are presented as the mean ± standard error of the mean (SEM) with 8 (yawning) or 6 (hypothermia) rats per

group. A one-way, repeated-measures ANOVA with post-hoc Dunnett's tests was used to determine significant changes in yawning or body temperature compared to vehicle treated animals, while significant effects of CJ-1037 on pramipexole-induced yawning and hypothermia were determined by two-way, repeated-measures ANOVA with post-hoc Bonferroni tests(GraphPad Prism; GraphPad Software Inc., San Diego, CA).

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