Design, Synthesis and Evaluation of Bitopic Arylpiperazine-phthalimides as Selective Dopamine D3 Receptor Agonists

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1. Docking

The SYBYL-X 2.0 program was used for ligand sketching, hydrogen addition, and minimization with the Trips force field and Gasteriger-Huckel charges.\textsuperscript{1} The dopaminergic receptors, D\textsubscript{3}R (PDB code: 3PBL) and D\textsubscript{2}R (PDB code: 6C38), were refined using the Biopolymer module implemented in SYBYL-X 2.0. After the removal of all the ligands and water molecules, the N- and C-termini were treated with charges. Subsequently, hydrogens were added and staged minimizations were performed using AMBER7_FF99 force field and Gasteriger-Marsili charges. The most selective D\textsubscript{3}R ligand among the identified compounds, compound 9i, was docked into the refined D\textsubscript{3}R and D\textsubscript{2}R with the program LeDock (www.lephar.com).\textsuperscript{2} Briefly, a rectangular box was generated in the LeDockGUI, a free graphics user interface of VMD, based on the binding pockets of eticlopride in D\textsubscript{3}R and risperidone in D\textsubscript{2}R. The compound 9i was docked into the dopaminergic receptors using LeDock program and for each ligands, 30 poses were generated and clustered by a RMSD cutoff of 1 Å. The docking results were analyzed by VMD frame by frame. The final reasonable binding conformations were accomplished by docking energy and poses. Docking poses were further minimized with the CHARMM force field.\textsuperscript{3}

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2. Chemistry

2.1 General synthesis of N-phenylpiperazines

To a solution of bis(2-chloroethyl)amine hydrochloride in 1ml diethylene glycol monomethyl ether was added varying substitution anilines, the mixture was allowed to reflux at 130°C for 15 hours. After cooling, 1 mL MeOH was added to reaction mixture followed by addition of 100 mL Et₂O. The resulting precipitate was filtered and washed with Et₂O to give corresponding piperazine hydrochloride salts. The salts were directly used in next step without further purification.

1-(4-Chlorophenyl)piperazine (8a)

Compound 8a was obtained as a yellowish solid. Yield: 50%. ¹H NMR (300 MHz, DMSO-d₆) δ 9.55 (s, 2H), 7.34–7.18 (m, 2H), 7.07–6.83 (m, 2H), 3.46–3.29 (m, 4H), 3.17 (s, 4H).

1-(3-Chlorophenyl)piperazine (8b)
Compound 8b was obtained as a yellowish solid. Yield: 65.3%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.21 (t, \(J = 8.1\) Hz, 1H), 6.98–6.87 (m, 2H), 6.84–6.72 (m, 1H), 3.56–3.44 (m, 4H), 3.40 (d, \(J = 2.0\) Hz, 4H).

1-(2-Chlorophenyl)piperazine (8c)

Compound 8c was obtained as a colorless solid. Yield: 70%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 9.90 (s, 2H), 7.39 (ddd, \(J = 7.76, 1.53, 0.54\) Hz, 1H), 7.29–7.21 (m, 1H), 7.12–7.00 (m, 2H), 3.42 (d, \(J = 10.83\) Hz, 8H).

1-(2,3-Dichlorophenyl)piperazine (8d)

Compound 8d was obtained as a yellowish solid. Yield: 63.1%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.21–7.04 (m, 2H), 6.95 (dd, \(J = 5.7, 3.9\) Hz, 1H), 3.04 (ddd, \(J = 12.7, 5.0, 1.8\) Hz, 8H).

1-(2,4-Dichlorophenyl)piperazine (8e)

Compound 8e was obtained as a yellowish solid. Yield: 76.5%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.37 (d, \(J = 2.4\) Hz, 1H), 7.20 (dd, \(J = 8.6, 2.4\) Hz, 1H), 6.96 (d, \(J = 8.6\) Hz, 1H), 3.13–3.05 (m, 4H), 3.02 (d, \(J = 5.4\) Hz, 4H).

1-(3-(Trifluoromethyl)phenyl)piperazine (8f)

Compound 8f was obtained as a yellowish solid. Yield: 61.9%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.41 (t, \(J = 7.9\) Hz, 1H), 7.21 (d, \(J = 7.7\) Hz, 1H), 7.16–7.04 (m, 2H), 3.56 (dd, \(J = 6.4, 3.8\) Hz, 4H), 3.42 (dd, \(J = 6.4, 3.8\) Hz, 4H).

1-(4-Fluorophenyl)piperazine (8g)

Compound 8g was obtained as yellowish solid. Yield: 75%. \(^1H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.32 – 7.25 (m, 2H), 7.13 – 7.04 (m, 2H), 3.61 – 3.54 (m, 4H), 3.51 (dd, \(J = 6.46, 3.47\) Hz, 4H).

1-(2-Fluorophenyl)piperazine (8h)

Compound 8h was obtained as yellowish solid. Yield: 87.5%. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.11–6.88 (m, 4H), 3.06 (s, 8H).

1-(2,3-Difluorophenyl)piperazine (8i)
Compound \textit{8i} was obtained as yellowish solid. Yield: 97.9\%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.12–6.99 (m, 1H), 6.97–6.85 (m, 1H), 6.80 (t, $J = 7.75$ Hz, 1H), 3.39 (s, 8H).

\textbf{1-(2,4-Difluorophenyl)piperazine (8j)}

Compound \textit{8j} was prepared as yellowish solid. Rf= 0.091 (CH$_2$Cl$_2$/MeOH=10:1). Yield: 52.8\%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.95 (s, 2H), 6.94 (td, $J = 9.53$, 9.21, 5.65 Hz, 1H), 6.90–6.74 (m, 2H), 3.39 (d, $J = 7.82$ Hz, 8H).

\textbf{1-(2,6-Difluorophenyl)piperazine (8k)}

Compound \textit{8k} was afforded as yellowish solid. Rf= 0.121 (CH$_2$Cl$_2$/MeOH=10:1). Yield: 84.3\%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.95 (s, 2H), 7.02–6.89 (m, 1H), 6.84 (tdd, $J = 8.71$, 5.91, 3.20 Hz, 1H), 3.39 (d, $J = 8.1$ Hz, 8H).

\textbf{1-(4-Chloro-2-fluorophenyl)piperazine (8l)}

Compound \textit{8l} was obtained as a colorless solid. Yield: 72.7\%. Rf= 0.061 (CH$_2$Cl$_2$/MeOH=10:1). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.95 (s, 2H), 7.17–7.00 (m, 2H), 6.88 (dd, $J = 12.85$, 4.93 Hz, 1H), 3.50–3.32 (m, 8H).

\textbf{1-(2-Fluoro-5-(trifluoromethyl)phenyl)piperazine (8m)}

Compound \textit{8m} was furnished as colorless solid. Yield: 39.1\%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.31 (dd, $J = 9.22$, 3.41 Hz, 1H), 7.18 (ddd, $J = 15.41$, 8.20, 5.25 Hz, 2H), 3.46 (d, $J = 2.54$ Hz, 8H).

\textbf{3.2 General procedure of N-alkylation}

$N$-(4-Bromobutyl)phthalimide was added to a suspension of 4-phenylpiperazine, K$_2$CO$_3$ and NaI in acetonitrile. The reaction mixture was allowed to reflux overnight and monitored by TLC (CH$_2$Cl$_2$/MeOH, 10:1). Upon completion of reaction, K$_2$CO$_3$ was removed by filtration and washed by acetone. The resulting filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting with EtOAc/hexane (1:4$\rightarrow$4:1) to furnish target compounds.

\textbf{2-(4-(4-Chlorophenyl)piperazin-1-yl)butyl}isoindoline-1,3-dione (9a)
Compound 9a was obtained as pale-yellow solid. Rf = 0.234 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 73.8%. Mp: 138–141°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.85 (dd, $J$ = 5.37, 3.10 Hz, 2H), 7.74 (dd, $J$ = 5.48, 3.02 Hz, 2H), 7.27 – 7.10 (m, 2H), 6.90 – 6.76 (m, 2H), 3.73 (t, $J$ = 6.98 Hz, 2H), 3.26 – 3.12 (m, 4H), 2.69 (s, 4H), 2.59 – 2.43 (m, 2H), 1.73 (dd, $J$ = 14.17, 7.09 Hz, 2H), 1.68 – 1.56 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 172.62, 153.47, 138.07, 135.84, 132.86, 128.90, 127.18, 121.43, 61.71, 56.73, 52.65, 41.52, 30.37, 27.40.

2-(4-(4-(3-Chlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9b)

Compound 9b was obtained as light yellow solid. Rf = 0.25 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 75%. Mp: 111–112°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.45, 3.04 Hz, 2H), 7.71 (dd, $J$ = 5.46, 3.04 Hz, 2H), 7.15 (t, $J$ = 8.12 Hz, 1H), 6.86 (t, $J$ = 2.12 Hz, 1H), 6.78 (ddd, $J$ = 8.90, 4.92, 1.77 Hz, 2H), 3.73 (t, $J$ = 7.10 Hz, 2H), 3.24 – 3.08 (m, 4H), 2.63 – 2.50 (m, 4H), 2.48 – 2.35 (m, 2H), 1.73 (dd, $J$ = 14.87, 7.44 Hz, 2H), 1.66 – 1.48 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.42, 149.29, 133.88, 132.11, 130.58, 128.72, 127.54, 123.57, 123.16, 120.33, 58.02, 53.35, 51.18, 37.85, 26.60, 24.23.

2-(4-(4-(2-Chlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9c)

Compound 9c was furnished as light yellow solid. Rf = 0.242 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 73.1%. Mp: 141–143°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.45, 3.05 Hz, 2H), 7.71 (dd, $J$ = 5.45, 3.04 Hz, 2H), 7.15 (t, $J$ = 8.12 Hz, 1H), 6.86 (t, $J$ = 2.12 Hz, 1H), 6.78 (ddd, $J$ = 8.90, 4.92, 1.77 Hz, 2H), 3.73 (t, $J$ = 7.10 Hz, 2H), 3.24 – 3.08 (m, 4H), 2.63 – 2.50 (m, 4H), 2.48 – 2.35 (m, 2H), 1.73 (dd, $J$ = 14.87, 7.44 Hz, 2H), 1.66 – 1.48 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.45, 152.33, 134.90, 133.93, 132.11, 129.99, 123.20, 119.15, 115.65, 113.79, 57.96, 53.03, 48.61, 37.83, 26.58, 24.19.

2-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9d)

Compound 9d was prepared as colorless solid. Rf = 0.136 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 77.7%. Mp: 121–123°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.45, 3.04 Hz, 2H), 7.71 (dd, $J$ = 5.45, 3.05 Hz, 2H), 7.20 – 7.09 (m, 2H), 6.94 (dd, $J$ = 6.39, 3.20 Hz, 1H), 3.73 (t, $J$ = 7.10 Hz, 2H), 3.05 (s, 4H), 2.62 (s, 4H), 2.49 – 2.39 (m, 2H), 1.74 (dt, $J$ = 14.86, 7.47 Hz, 2H), 1.64 – 1.51 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.45, 151.30, 133.98, 133.91, 132.13, 127.42, 124.50, 123.19, 118.58, 57.97, 53.28, 51.30, 37.86, 26.60, 24.22.
2-(4-(4-(2,4-Dichlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9e)

Compound 9e was obtained as colorless solid. Yield: 76.5%. Mp: 106–107°C. 

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.44, 3.04 Hz, 2H), 7.71 (dd, $J$ = 5.44, 3.05 Hz, 2H), 7.34 (d, $J$ = 2.43 Hz, 1H), 7.17 (dd, $J$ = 8.61, 2.45 Hz, 1H), 6.95 (d, $J$ = 8.64 Hz, 1H), 3.73 (t, $J$ = 7.11 Hz, 2H), 3.02 (s, 4H), 2.61 (s, 4H), 2.50 – 2.37 (m, 2H), 1.73 (dd, $J$ = 14.84, 7.44 Hz, 2H), 1.65 – 1.49 (m, 2H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.45, 148.12, 133.92, 132.14, 130.27, 129.42, 128.08, 127.59, 123.19, 121.12, 57.97, 53.25, 51.20, 37.87, 26.60, 24.22.

2-(4-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9f)

Compound 9f was obtained as colorless solid. Rf= 0.219 (n-hexane/EtOAc = 1:1). Yield: 86.9%. Mp: 96–98°C. 

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.47, 3.02 Hz, 2H), 7.71 (dd, $J$ = 5.45, 3.05 Hz, 2H), 7.33 (t, $J$ = 7.95 Hz, 1H), 7.10 (s, 1H), 7.08 – 7.00 (m, 2H), 4.12 (q, $J$ = 7.15 Hz, 1H), 3.73 (t, $J$ = 7.11 Hz, 2H), 3.31 – 3.13 (m, 4H), 2.70 – 2.50 (m, 4H), 2.49 – 2.38 (m, 2H), 1.75 (dt, $J$ = 14.90, 7.47 Hz, 2H), 1.68 – 1.48 (m, 2H), 1.26 (t, $J$ = 7.14 Hz, 1H). 

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.44, 151.39, 133.92, 132.14, 131.37 (q, $J_{CF}$=31.67 Hz), 129.50, 124.35 (dd, $J_{CF}$=271.92 Hz), 123.19, 118.58, 115.68 (q, $J_{CF}$=3.73 Hz), 112.07 (q, $J_{CF}$=3.88 Hz), 60.39, 57.92, 53.01, 48.61, 37.83, 26.56, 24.17, 21.04, 14.20.

2-(4-(4-(4-Fluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9g)

Compound 9g was prepared as colorless crystal. Rf= 0.125 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 86.9%. Mp: 116–118°C. 

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.47, 3.02 Hz, 2H), 7.71 (dd, $J$ = 5.46, 3.04 Hz, 2H), 7.02 – 6.90 (m, 2H), 6.91 – 6.79 (m, 2H), 3.73 (t, $J$ = 7.10 Hz, 2H), 3.16 – 3.03 (m, 4H), 2.68 – 2.53 (m, 4H), 2.49 – 2.34 (m, 2H), 1.74 (dt, $J$ = 14.86, 7.46 Hz, 2H), 1.65 – 1.47 (m, 2H). 

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.48, 158.31, 155.94, 148.00 (d, $J_{CF}$=2.22 Hz), 133.94, 132.13, 123.21, 117.73 (d, $J_{CF}$=7.59 Hz), 115.48 (d, $J_{CF}$=22.14 Hz), 58.00, 53.24, 50.15, 37.86, 26.61, 24.22.

2-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9h)

Compound 9h was obtained as pale-yellow crystals. Rf= 0.182 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 90.3%. Mp: 120–122°C. 

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J$ = 5.46, 3.04 Hz, 2H), 7.71 (dd, $J$ = 5.46, 3.05 Hz, 2H), 7.09 – 6.97 (m, 2H), 6.97 – 6.87 (m, 2H), 3.73 (t, $J$ = 7.10 Hz, 2H),
3.20 – 3.02 (m, 4H), 2.62 (d, \( J = 4.25 \text{ Hz}, 4 \text{H} \)), 2.50 – 2.35 (m, 2H), 1.73 (dd, \( J = 14.81, 7.41 \text{ Hz}, 2 \text{H} \)), 1.66 – 1.48 (m, 2H). \(^{13}\text{C} \text{ NMR (101 MHz, CDCl}_3 \delta 168.42, 155.69 (d, \( J_{CF}=245.86 \text{ Hz} \)), 140.15 (d, \( J_{CF}=8.50 \text{ Hz} \)), 133.89, 132.11, 124.41 (d, \( J_{CF}=3.55 \text{ Hz} \)), 123.16, 122.33 (d, \( J_{CF}=7.97 \text{ Hz} \)), 118.87 (d, \( J_{CF}=3.03 \text{ Hz} \)), 116.04 (d, \( J_{CF}=20.82 \text{ Hz} \)), 58.02, 53.26, 50.52, 50.49, 37.84, 26.58, 24.19.

\( \text{2-(4-(4-(2,3-Difluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9i)} \)

Compound 9i was afforded as colorless solid. Rf= 0.265 (CH\(_2\)Cl\(_2\)/EtOAc=1:1). Yield: 87.7%. Mp: 101–103°C. \(^1\text{H} \text{ NMR (400 MHz, CDCl}_3 \delta 7.83 (td, \( J = 5.27, 5.27, 2.09 \text{ Hz}, 2 \text{H} \)), 7.72 (td, \( J = 5.44, 5.24, 2.09 \text{ Hz}, 2 \text{H} \)), 6.95 – 6.84 (m, 1H), 6.85 – 6.73 (m, 2H), 3.72 (dd, \( J = 13.32, 6.23 \text{ Hz}, 2 \text{H} \)), 3.19 – 3.03 (m, 4H), 2.71 – 2.54 (m, 4H), 2.48 – 2.39 (m, 2H), 1.74 (dt, \( J = 14.91, 7.41 \text{ Hz}, 2 \text{H} \)), 1.65 – 1.49 (m, 2H). \(^{13}\text{C} \text{ NMR (101 MHz, CDCl}_3 \delta 168.45, 151.51 (dd, \( J = 245.74, 11.96 \text{ Hz} \)), 143.96 (dd, \( J = 247.0, 13.73 \text{ Hz} \)), 141.98 (d, \( J = 5.62 \text{ Hz} \)), 133.93, 132.11, 123.55 (d, \( J = 8.53, 4.89 \text{ Hz} \)), 123.19, 113.67, 109.87 (d, \( J = 17.69 \text{ Hz} \)), 57.98, 53.16, 50.45, 50.42, 37.84, 26.58, 24.18.

\( \text{2-(4-(4-(2,4-Difluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9j)} \)

Compound 9j was afforded as colorless solid. Rf= 0.156 (CH\(_2\)Cl\(_2\)/EtOAc=1:1). Yield: 66.0%. Mp: 101–103°C. \(^1\text{H} \text{ NMR (400 MHz, CDCl}_3 \delta 7.84 (dd, \( J = 5.44, 3.05 \text{ Hz}, 2 \text{H} \)), 7.72 (dd, \( J = 5.44, 3.05 \text{ Hz}, 2 \text{H} \)), 6.95 – 6.84 (m, 1H), 6.85 – 6.73 (m, 2H), 3.13 – 2.93 (m, 4H), 2.61 (s, 4H), 2.50 – 2.37 (m, 2H), 1.83 – 1.66 (m, 2H), 1.66 – 1.47 (m, 2H). \(^{13}\text{C} \text{ NMR (101 MHz, CDCl}_3 \delta 168.43, 151.51 (dd, \( J = 245.74, 11.96 \text{ Hz} \)), 143.96 (dd, \( J = 247.0, 13.73 \text{ Hz} \)), 141.98 (d, \( J = 5.62 \text{ Hz} \)), 133.93, 132.11, 123.55 (d, \( J = 8.53, 4.89 \text{ Hz} \)), 123.19, 113.67, 109.87 (d, \( J = 17.69 \text{ Hz} \)), 57.98, 53.16, 50.45, 50.42, 37.84, 26.58, 24.18.

\( \text{2-(4-(4-(2,6-Difluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9k)} \)

Compound 9k was afforded as colorless solid. Rf= 0.265 (CH\(_2\)Cl\(_2\)/EtOAc=1:1). Yield: 60.2%. Mp: 102–103°C. \(^1\text{H} \text{ NMR (300 MHz, CDCl}_3 \delta 7.90 – 7.78 (m, 2H), 7.76 – 7.66 (m, 2H), 6.89 (td, \( J = 9.64, 9.27, 5.80 \text{ Hz}, 1 \text{H} \)), 6.84 – 6.71 (m, 2H), 3.73 (t, \( J = 7.04 \text{ Hz}, 2 \text{H} \)), 3.14 – 2.94 (m, 4H), 2.72 – 2.52 (m, 4H), 2.50 – 2.35 (m, 2H), 1.75 (dt, \( J = 14.30, 6.91 \text{ Hz}, 2 \text{H} \)), 1.58 (ddd, \( J = 14.90, 8.16, 4.43 \text{ Hz}, 2 \text{H} \)).
2-(4-(4-(4-Chloro-2-fluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9l)

Compound 9l was obtained as light yellow solid. Rf= 0.219 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 75.2%. Mp: 89–91°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.83 (dd, $J$ = 5.45, 3.05 Hz, 2H), 7.71 (dd, $J$ = 5.45, 3.04 Hz, 2H), 7.10 – 6.93 (m, 2H), 6.84 (t, $J$ = 8.75 Hz, 1H), 3.73 (t, $J$ = 7.09 Hz, 2H), 3.16 – 2.97 (m, 4H), 2.60 (d, $J$ = 3.98 Hz, 4H), 2.49 – 2.37 (m, 2H), 1.73 (dd, $J$ = 14.91, 7.43 Hz, 2H), 1.64 – 1.49 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.38, $\delta$ 155.25 (d, $J$ = 249.82 Hz), 139.01 (d, $J$ = 8.60 Hz), 133.89, 132.09, 126.56 (d, $J$ = 10.03 Hz), 124.45 (d, $J$ = 3.52 Hz), 123.15, 119.50 (d, $J$ = 3.85 Hz), 116.74 (d, $J$ = 24.30 Hz), 57.94, 53.11, 50.46, 50.42, 37.82, 26.56, 24.16.

2-(4-(4-(2-Fluoro-5-(trifluoromethyl)phenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (9m)

Compound 9m was prepared as colorless solid. Rf= 0.617 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 87.5%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.92 – 7.79 (m, 2H), 7.76 – 7.66 (m, 2H), 7.23 – 7.01 (m, 3H), 3.73 (t, $J$ = 7.05 Hz, 2H), 3.24 – 3.02 (m, 4H), 2.72 – 2.54 (m, 4H), 2.51 – 2.33 (m, 2H), 1.73 (dd, $J$ = 14.34, 7.33 Hz, 2H), 1.58 (dd, $J$ = 14.85, 8.70 Hz, 2H).

2-(4-(4-(2-Methoxyphenyl)piperidin-1-yl)butyl)isoindoline-1,3-dione (11a)

Compound 11a was prepared as colorless solid. Rf= 0.219 (CH$_2$Cl$_2$/EtOAc=1:1). Yield: 75.2%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.83 (dd, $J$ = 5.47, 3.02 Hz, 2H), 7.70 (dd, $J$ = 5.44, 3.05 Hz, 2H), 7.23 – 7.11 (m, 2H), 6.91 (td, $J$ = 7.47, 1.02 Hz, 1H), 6.84 (dd, $J$ = 8.17, 0.90 Hz, 1H), 3.81 (s, 3H), 3.72 (t, $J$ = 7.08 Hz, 2H), 3.02 (d, $J$ = 11.48 Hz, 2H), $\delta$ 2.99 – 2.89 (m, 1H), 2.45 – 2.34 (m, 2H), 2.06 (td, $J$ = 11.54, 11.43, 2.84 Hz, 2H), 1.84 – 1.64 (m, 6H), 1.59 (ddd, $J$ = 12.17, 8.24, 4.33 Hz, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.42, 156.81, 134.48, 133.87, 132.15, 126.77, 126.56, 123.16, 120.62, 110.26, 58.59, 55.31, 54.62, 37.90, 35.08, 32.11, 26.78, 24.46.

2-(4-(4-(5-chloro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)butyl)isoindoline-1,3-dione (11b)

Compound 11b was obtained as light colorless solid. Rf= 0.441 (CH$_2$Cl$_2$/MeOH=10:1). Yield: 75.2%. Mp: 198–203°C. $^1$H NMR (400 MHz, CDCl$_3$/CD$_3$OD = 1:1) $\delta$ 7.85 (ddd, $J$ = 9.21, 3.87,
2.42 Hz, 2H), 7.82 – 7.74 (m, 2H), 7.29 (d, J = 8.45 Hz, 1H), 7.13 – 6.89 (m, 2H), 4.34 (tt, J = 12.34, 4.24 Hz, 1H), 3.75 (t, J = 6.87 Hz, 2H), 3.14 (d, J = 11.7 Hz, 2H), 2.46 (ddd, J = 16.12, 13.97, 5.69 Hz, 4H), 2.23 (t, J = 11.13 Hz, 2H), 1.89 – 1.69 (m, 4H), 1.70 – 1.53 (m, 2H).

3. Biological Assays

The binding properties of compounds on D3R and D2R based on competition-binding experiment with radioligand as described. Briefly, HEK 293 cells were transfected with D2R or D3R plasmids and cultured by minimal essential medium (MEM) without and within fetal bovine serum for 4 hours and 24 hours, respectively. Cells were seeded in 24-well plates and after overnight were treated with [3H]-sulpiride (2.2 nM for D2R and 4.4 nM for D3R) and synthesized compounds for 150 minutes at 4 ºC. Upon completion of binding, cells were rinsed with ice-cold MEM and lysed in 1% sodium dodecyl sulfate. Finally, the remaining radioligand was detected by a liquid scintillation counter. The resulting dose-response curves were obtained by fitting nonlinear regression using Graphpad Prism, and IC50 values were calculated from the fitting curves. Ki values were converted from IC50 values according to the equation of the Cheng-Prusoff.

4. Functional evaluation

Cellular cAMP levels were measured using an indirect reporter gene method5,6. The reporter system that consisted of a plasmid containing the firefly luciferase gene was used under the control of multiple cAMP responsive elements and a pRL-TK control vector. Cells expressing D3R (about 1.9 pmol/mg protein) were transfected with reporter genes, and were seeded in 24-well plates. Cells were treated with 2 μM forskolin and quinpirole (10^{-12}–10^{-8} M) for 4h. Relative luciferase expression was measured using a dual luciferase assay kit (Promega, Madison, WI, USA).

Reference

1. St. Louis, M., Sybyl-x, Tripos International, USA.

