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

Yongkai Cao[a,b,c,1], Ningning Sun[b,1], Jiumei Zhang[c,1], Zhiguo Liu[d], Yi-zhe Tang[c], Zhengzhi Wu[c,*], Kyeong-Man Kim[b,*] and Seung Hoon Cheon[b,*]

a Integrated Chinese and Western Medicine Postdoctoral Research Station, Jinan University, Guangzhou 510632, China

b College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Republic of Korea

c The First Affiliated Hospital of Shenzhen University, Shenzhen 518035,China

d Chemical Biology Research at School of Pharmaceutical sciences, Wenzhou Medical University, Wenzhou 325035, China

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.[1] The dopaminergic receptors, D_3R (PDB code: 3PBL) and D_2R (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_3R ligand among the identified compounds, compound **9i**, was docked into the refined D_2R and D_3R with the program LeDock (www.lephar.com).[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_3R and risperidone in D_2R. 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.[3]

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[1] These authors contribute equally to this work.

* Corresponding authors: Tel.: +82625302929, fax: +82625302911, e-mail: shcheon@jnu.ac.kr (S.H. Cheon); Tel.: +82625302936, fax: +82625302949, e-mail: kmkim@jnu.ac.kr (K.M. Kim); Tel. and fax: +8675525622938, E-mail: szwzz001@163.com (Z. Wu).

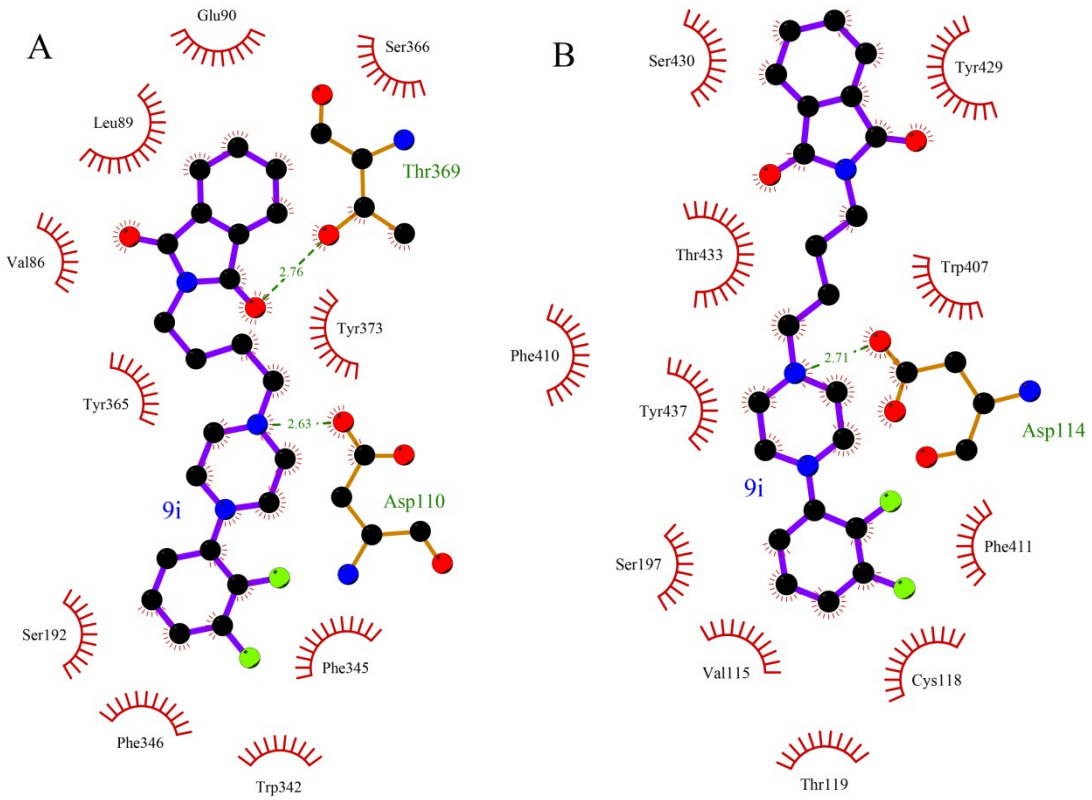

Figure S1. 2D diagram of interactions between **9i** and D_3R (A) and D_2R (B)

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 Et_2O. The resulting precipitate was filtered and washed with Et_2O to give corresponding piperazine hydrochloride salts.[4] 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%. ^{1}H NMR (300 MHz, DMSO-d_6) 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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%. ^{1}H NMR (500 MHz, cd_3od) δ 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%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11–6.88 (m, 4H), 3.06 (s, 8H).

1-(2,3-Difluorophenyl)piperazine (8i)

Compound **8i** was obtained as yellowish solid. Yield: 97.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12–6.99 (m, 1H), 6.97–6.85 (m, 1H), 6.80 (t, *J* = 7.75 Hz, 1H), 3.39 (s, 8H).

1-(2,4-Difluorophenyl)piperazine (8j)

Compound **8j** was prepared as yellowish solid. Rf= 0.091 (CH_2Cl_2/MeOH=10:1). Yield: 52.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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).

1-(2,6-Difluorophenyl)piperazine (8k)

Compound **8k** was afforded as yellowish solid. Rf= 0.121 (CH_2Cl_2/MeOH=10:1). Yield: 84.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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).

1-(4-Chloro-2-fluorophenyl)piperazine (8l)

Compound **8l** was obtained as a colorless solid. Yield: 72.7%. Rf= 0.061 (CH_2Cl_2/MeOH=10:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 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).

1-(2-Fluoro-5-(trifluoromethyl)phenyl)piperazine (8m)

Compound **8m** was furnished as colorless solid. Yield: 39.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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).

3.2 General procedure of *N*-alkylation

N-(4-Bromobutyl)phthalimide was added to a suspension of 4-phenylpiperazine, K_2CO_3 and NaI in acetonitrile. The reaction mixture was allowed to reflux overnight and monitored by TLC (CH_2Cl_2/MeOH, 10:1). Upon completion of reaction, K_2CO_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→1:3) to furnish target compounds.

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

Compound **9a** was obtained as pale-yellow solid. Rf= 0.234 (CH_2Cl_2/EtOAc=1:1). Yield: 73.8%. Mp: 138–141°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 75%. Mp: 111–112°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.45, 3.04 Hz, 2H), 7.71 (dd, *J* = 5.46, 3.04 Hz, 2H), 7.34 (dd, *J* = 7.89, 1.50 Hz, 1H), 7.25 – 7.16 (m, 1H), 7.03 (dd, *J* = 8.06, 1.46 Hz, 1H), 6.95 (td, *J* = 7.78, 1.51 Hz, 1H), 3.81 – 3.67 (m, 2H), 3.06 (s, 4H), 2.63 (s, 4H), 2.51 – 2.38 (m, 2H), 1.74 (dt, *J* = 14.85, 7.51 Hz, 2H), 1.66 – 1.50 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 73.1%. Mp: 141–143°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.43, 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, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 77.7%. Mp: 121–123°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 86.9%. Mp: 116–118°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.45, 3.04 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, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 90.3%. Mp: 120–122°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.46, 3.03 Hz, 2H), 7.71 (dd, *J* = 5.44, 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 Hz, 4H), 2.50 – 2.35 (m, 2H), 1.73 (dd, *J* = 14.81, 7.41 Hz, 2H), 1.66 – 1.48 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.42, 155.69 (d, J_{CF}=245.86 Hz), 140.15 (d, J_{CF}=8.50 Hz), 133.89, 132.11, 124.41 (d, J_{CF}=3.55 Hz), 123.16, 122.33 (d, J_{CF}=7.97 Hz), 118.87 (d, J_{CF}=3.03 Hz), 116.04 (d, J_{CF}=20.82 Hz), 58.02, 53.26, 50.52, 50.49, 37.84, 26.58, 24.19.

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_2Cl_2/EtOAc=1:1). Yield: 87.7%. Mp: 101–103°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (td, *J* = 5.27, 5.27, 2.09 Hz, 2H), 7.72 (td, *J* = 5.44, 5.24, 2.09 Hz, 2H), 6.95 (tdd, *J* = 8.17, 5.88, 2.00 Hz, 1H), 6.82 – 6.71 (m, 1H), 6.72 – 6.62 (m, 1H), 3.72 (dd, *J* = 13.32, 6.23 Hz, 2H), 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 Hz, 2H), 1.65 – 1.49 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.45, 151.51 (dd, *J* = 245.74, 11.96 Hz), 143.96 (dd, *J* = 247.0, 13.73 Hz), 141.98 (d, *J* = 5.62 Hz), 133.93, 132.11, 123.55 (dd, *J* = 8.53, 4.89 Hz), 123.19, 113.67, 109.87 (d, *J* = 17.69 Hz), 57.98, 53.16, 50.45, 50.42, 37.84, 26.58, 24.18.

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_2Cl_2/EtOAc=1:1). Yield: 66.0%. Mp: 101–103°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.44, 3.05 Hz, 2H), 7.72 (dd, *J* = 5.44, 3.05 Hz, 2H), 6.95 – 6.84 (m, 1H), 6.85 – 6.73 (m, 2H), 3.79 – 3.67 (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}C NMR (101 MHz, $CDCl_3$) δ 168.43, 157.81 (dd, J_{CF}=11.78, 242.94 Hz), 155.57 (dd, J_{CF}=11.23, 249.15 Hz), 136.75 (dd, *J* = 8.95, 3.35 Hz), 133.91, 132.11, 123.17, 119.35 (dd, *J* = 9.25, 4.25 Hz), 110.62 (dd, *J* = 21.32, 3.72 Hz), 104.62 (dd, *J* = 25.81, 0.89 Hz), 57.98, 53.23, 50.88 (d, *J* = 2.55 Hz), 37.83, 26.58, 24.17.

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_2Cl_2/EtOAc=1:1). Yield: 60.2%. Mp: 102–103°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.78 (m, 2H), 7.76 – 7.66 (m, 2H), 6.89 (td, *J* = 9.64, 9.27, 5.80 Hz, 1H), 6.84 – 6.71 (m, 2H), 3.73 (t, *J* = 7.04 Hz, 2H), 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 Hz, 2H), 1.58 (ddd, *J* = 14.90, 8.16, 4.43 Hz, 2H).

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_2Cl_2/EtOAc=1:1). Yield: 75.2%. Mp: 89–91°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 168.38, δ 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_2Cl_2/EtOAc=1:1). Yield: 87.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 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_2Cl_2/EtOAc=1:1). Yield: 75.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 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), δ 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, $CDCl_3$) δ 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-1*H*-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_2Cl_2/MeOH=10:1). Yield: 75.2%. Mp: 198–203°C. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD = 1:1) δ 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 D_3R and D_2R based on competition-binding experiment with radioligand as described. Briefly, HEK 293 cells were transfected with D_2R or D_3R 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 [^{3}H]-sulpiride (2.2 nM for D_2R and 4.4 nM for D_3R) 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 IC_{50} values were calculated from the fitting curves. Ki values were converted from IC_{50} values according to the equation of the Cheng-Prusoff.

4. Functional evaluation

Cellular cAMP levels were measured using an indirect reporter gene method[5,6]. 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 D_3R (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).

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