Received Date : 17-Feb-2016
Revised Date : 20-Jun-2016
Accepted Date : 13-Aug-2016
Article type : Research Article

Synthesis and biological evaluation of novel flavanone derivatives as potential antipsychotic agents

Hong-shun Gu[1], Xi Chen[1], Jian-wei Zhang[2], Lan Zhang[1,*], Lin Li[1,*]

[1] *Department of Pharmacology, Xuanwu Hospital of Capital Medical University; Beijing Institute for Brain Disorders; Beijing Engineering Research Center for Nerve System Drugs; Key Laboratory for Neurodegenerative Diseases of Ministry of Education, Beijing 100053, China.*

[2]*School of Chemical Biology and Pharmaceutical Sciences, Capital Medical University, Beijing 100069, China.*

[*]*Corresponding author:*

Professor Lin Li, Department of Pharmacology, Xuanwu Hospital of Capital Medical University, 45 Chang-chun Street, Beijing 100053, China.

Tel.: +86-10-83198886; Fax: +86-10-63042809;

E-mail address: linlixw@126.com

Professor Lan Zhang, Department of Pharmacology, Xuanwu Hospital of Capital Medical University, 45 Chang-chun Street, Beijing 100053, China.

Tel.: +86-10-63132779;

E-mail: lanizhg@hotmail.com

Abstract: In the present study, a series of novel flavanone derivatives were designed and synthesized, and the antipsychotic activities of the target compounds were evaluated *in vitro* and *in vivo*. The results showed that synthesized compounds **7a–7g** decreased the activity of dopamine D_2 receptors in HEK293 cells co-transfected with D_2 receptor/G protein α16a, with IC_{50} values of 0.051–0.35 μM. Compounds **7a–7g** inhibited the over-production of nitric oxide stimulated by lipopolysaccharide/interferon-γ in BV-2 microglial cells. In mice, intragastric administration of **7d**, **7e**, and **7g** reversed the increase in locomotor activity induced by MK-801 (an antagonist of NMDA receptors), and decreased the hyperactivity of climbing behavior induced by apomorphine (a dopamine receptor agonist). These results suggest that some of the novel flavanone derivatives have potential antipsychotic effects, and may be useful in the treatment of schizophrenia.

Key words: flavanone derivatives; schizophrenia; dopamine D_2 receptor; apomorphine; MK-801; neuroinflammation

Schizophrenia is a chronic and complex neuropsychiatric illness, afflicting approximately 1% of the population worldwide (1). Typical antipsychotics antagonize dopamine receptors and mainly ameliorate the positive symptoms of schizophrenia, but they fail to manage the negative symptoms and cognitive impairment. Moreover, serious side effects such as extrapyramidal symptoms and hyperprolactinemia limit the use of these drugs (2). Atypical antipsychotics (e.g., clozapine, ziprasidone, risperidone, quetiapine, and olanzapine) potently antagonize the serotonin 2A receptor (5-HT_{2A} receptor) along with a dopamine D_2 receptor blockade. A major advantage of atypical antipsychotics is their effectiveness in suppressing negative and cognitive symptoms. However, it has been proven that atypical antipsychotics cause numerous side effects, such as substantial weight gain and the time from the start of QRS waves to end point of T wave (QT) interval prolongation.

Recently, some studies have found that people with schizophrenia have increased blood concentrations of inflammatory cytokines (3). There exist several randomized clinical trials of the non-steroidal anti-inflammatory drugs (such as celecoxib (4) and aspirin (5,6) as adjuncts to antipsychotics, and one trial found that adjunctive treatment with the antioxidant N-acetylcysteine significantly reduced psychopathology in schizophrenic patients (7). The evidence from first-degree relatives of patients with schizophrenia supports the idea that increased inflammation is associated with schizophrenia (8–10).

Flavanones are a series of naturally occurring compounds present in natural medicine and plant-based foods. They possess a broad range of pharmacological properties including radical-scavenging, anti-inflammatory, anti-cancer, and anti-mutagenic properties (11). An increasing number of reports have shown that flavanones (such as hesperidin (12),

naringenin (13, 14), nobiletin (15), and tangeretin (16)) inhibit inflammation caused by activated microglia. However, the low content of these flavanones in natural products limits further research and development, and it is not easy to obtain them by separation and purification. On the other hand, chemical synthesis may reduce the waste of resources. Meanwhile, it has been reported that some synthesized antipsychotic agents containing an arylpiperazine (piperidine) group have antagonistic effects on dopamine receptors (17–22). Therefore, in the present study, we designed and synthesized a series of new compounds with the arylpiperazine (piperidine) group linked to flavanones. We then investigated the preliminary antipsychotic effects of the target compounds on the dopamine D_2 receptor and on neuroinflammation *in vitro*, and on hyperactive behavior in mouse models of schizophrenia (induced by MK-801 and by apomorphine) *in vivo*.

Materials and methods

Chemical synthesis

General information

Optical rotations were measured on a JASCO P-2000 automatic digital polarimeter (Japan). Ultraviolet (UV) spectra were obtained on a JASCO V650 spectrophotometer (Japan). Infrared (IR) spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (USA). 1D Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz on a Bruker Avance spectrometer (Germany) in $CDCl_3$ solution. Chemical shifts were given in δ values (ppm) using tetramethylsilane (TMS) as the internal standard. Electrospray ionization mass spectrometry (ESIMS) data were measured on a Waters Micromass Quattro Micro API

LC/MS/MS mass spectrometer (USA). High-resolution ESIMS (HRESIMS) data were recorded on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer (USA). Analytical thin layer chromatography (TLC) was performed on silica gel GF254 (Qingdao Marine Chemical Inc., China). Column chromatographic purification was carried out using silica gel. Reagents were all of analytical grade or of chemical purity.

General synthesis for novel flavanone derivatives is shown in Fig. 1.

Procedure for synthesis of 1-[2-Hydroxy-4,6-bis(methoxymethoxy)phenyl] ethanone (2)

A flame-dried flask was charged with acetone (30 mL), anhydrous potassium carbonate (8.28 g, 60 mmol), and ketone **1** (5.04 g, 30 mmol). The mixture was stirred at room temperature (RT) for 5 min, and MOMCl (5.93 g, 75 mmol) was added dropwise. The mixture was maintained at RT for 1.5 h, and then filtered to remove the anhydrous potassium carbonate. The organic layer was concentrated under reduced pressure to give thick yellow oil. Column chromatography of the crude product on silica gel using 10% EtOAc in petroleum ether yielded **2** (4.33 g, 56%) as a colorless oil.

Procedure for synthesis of 1-(4,6-bis(methoxymethoxy)-2-hydroxy-phenyl)-3-(4-methoxy-phenyl)-propenone 4 (a–c)

A mixture of 10 mmol of sodium hydroxide, 15 mmol of **2**, and 25 mmol of corresponding substituted benzaldehyde in 50 mL of methanol was refluxed for 3 h, acidified (2 N HCl), and extracted with ethyl acetate (3×25 mL). The combined organic layers were evaporated under reduced pressure, and the crude products **3** (**a–c**) were then directly used for the next

step.

Sodium acetate (5.0 g, 0.4 mmol) was added to a stirring solution of crude **3** (**a–c**) in methanol (50 mL). The solution was refluxed for 14 h, and then evaporated under reduced pressure. The crude product was washed with distilled water (50 mL) and extracted with ethyl acetate (3×25 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated. The crude materials were purified on a silica gel column using 25% EtOAc in petroleum ether to yield **4** (**a**–**c**) as yellow oils.

5,7-Bis(methoxymethoxy)-2-(4-methoxyphenyl)chroman-4-one (4a):

Yield: 72%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 7.43 (d, 2H, J=8.7 Hz), 6.99 (d, 2H, J=8.7 Hz), 6.36 (d, 1H, J=2.1 Hz), 6.33 (d, 1H, J=2.1 Hz), 5.50 (dd, 1H, J=13.2, 2.7 Hz), 5.23 (s, 2H), 5.22 (s, 2H), 3.77 (s, 3H), 3.41 (s, 3H), 3.78 (s, 3H), 3.11 (dd, 1H, J=16.5, 13.2 Hz), and 2.61 (dd, 1H, J=16.5, 3.0 Hz). ESIMS *m/z* 375.2 $[M+H]^+$.

5,7-Bis(methoxymethoxy)-2-(4-methylphenyl)chroman-4-one (4b):

Yield: 75%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 7.37 (d, 2H, J=7.8 Hz), 7.26 (d, 2H, J=7.8 Hz), 6.45 (d, 1H, J=2.4 Hz), 6.41 (d, 1H, J=2.4 Hz), 5.40 (dd, 1H, J=13.2, 2.7 Hz), 5.32 (s, 2H), 5.20 (s, 2H), 3.55 (s, 3H), 3.49 (s, 3H), 3.04 (dd, 1H, J=16.5, 13.2 Hz), 2.72 (dd, 1H, J=16.51, 2.7 Hz), and 2.39 (s, 3H). ESIMS *m/z* 359.1 $[M+H]^+$.

5,7-Bis(methoxymethoxy)-2-(4-fluorophenyl)chroman-4-one (4c):

Yield: 81%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 7.60 (d, 2H, J=8.7 Hz), 7.29 (d, 2H, J=8.7 Hz), 6.37 (d, 1H, J=2.1 Hz), 6.35 (d, 1H, J=2.1 Hz), 5.61 (dd, 1H, J=13.2, 3.0 Hz), 5.23 (s, 2H), 5.22 (s, 2H), 3.42 (s, 3H), 3.79 (s, 3H), 3.12 (dd, 1H, J=16.2, 13.2 Hz), and 2.60 (dd, 1H, J=16.2, 3.0 Hz). ESIMS *m/z* 363.1 $[M+H]^+$.

Procedure for the preparation of 5 (a–c)

A mixture of 10 mmol of **4 (a–c)** and 1 mL of concentrated HCl in 15 mL of MeOH was refluxed for 0.5 h. After the solvent was evaporated under vacuum, the residue was dissolved in ice-cold water, and the precipitate was filtered off, washed with water, and recrystallized from absolute ethanol to give **5** (**a**–**c**).

5,7-Dihydroxy-2-(4-methoxyphenyl)chroman-4-one (5a):

Yield: 82%. $[\alpha]_D^{20}$ −17.4 (*c* 0.1, MeOH). ^{1}H NMR δ ppm (300 MHz, DMSO-d_6): 12.14 (br s, 1H), 10.78 (br s, 1H), 7.44 (d, 2H, *J*＝8.7 Hz), 6.97 (d, 2H, *J*＝8.7 Hz), 5.90 (d, 1H, *J*＝2.1 Hz), 5.89 (d, 1H, *J*＝1,8 Hz), 5.53 (dd, 1H, *J*＝12.6, 2.7 Hz), 3.77 (s, 3H), 3.28 (dd, 1H, *J*＝14.1, 12.6 Hz), and 2.72 (dd, 1H, *J*＝14.1, 3.0 Hz). ESIMS *m/z* 287.1 $[M+H]^+$.

5,7-Dihydroxy-2-(4-methylphenyl)chroman-4-one (5b):

Yield: 82%. $[\alpha]_D^{20}$ −13.5 (*c* 0.1, MeOH). ^{1}H NMR δ ppm (300 MHz, DMSO-d_6): 12.13 (br s, 1H), 10.81 (br s, 1H), 7.40 (d, 2H, *J*＝7.8 Hz), 7.23 (d, 2H, *J*＝7.8 Hz), 5.91 (d, 1H, *J*＝2.4 Hz), 5.90 (d, 1H, *J*＝2.4 Hz), 5.54 (dd, 1H, *J*＝12.3, 2.4 Hz), 3.23 (dd, 1H, *J*＝17.1, 12.4 Hz), 2.72 (dd, 1H, *J*＝17.1, 3.0 Hz), and 3.32 (s, 3H). ESIMS *m/z* 271.1 $[M+H]^+$.

5,7-Dihydroxy-2-(4-fluorophenyl)chroman-4-one (5c):

Yield: 86%. $[\alpha]_D^{20}$ −3.1 (*c* 0.1, MeOH). ^{1}H NMR δ ppm (300 MHz, DMSO-d_6): 12.12 (br s, 1H), 10.85 (br s, 1H), 7.58 (d, 2H, *J*＝8.4 Hz), 7.27 (d, 2H, *J*＝8.7 Hz), 5.93 (d, 1H, *J*＝2.1 Hz), 5.91 (d, 1H, *J*＝2.1 Hz), 5.60 (dd, 1H, *J*＝12.6, 2.7 Hz), 3.25 (dd, 1H, *J*＝17.1, 12.6 Hz), and 2.77 (dd, 1H, *J*＝17.1, 3.0 Hz). ESIMS *m/z* 275.0 $[M+H]^+$.

Procedure for the preparation of 6 (a–c)

1,4-Dibromobutane (10 mmol) was added to a solution of **5** (**a**–**c**) (5 mmol) and potassium carbonate in acetone (30 mL), and the mixture was refluxed for 3 h. After cooling to room temperature, the mixture was filtered, solvent was evaporated, and residue was purified by column chromatography (petroleum ether/EtOAc 15:1) to yield **6** (**a**–**c**) as yellow oils.

7-(4-Bromobutoxy)-5-hydroxy-2-(4-methoxyphenyl)chroman-4-one (6a):

Yield: 52%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (s, 1H), 7.40 (d, 2H, J=8.7 Hz), 6.97 (d, 2H, J=8.7 Hz), 6.07 (d, 1H, J=2.4 Hz), 6.04 (d, 1H, J=2.4 Hz), 5.38 (dd, 1H, J =12.9, 3.0 Hz), 4.02 (t, 2H, J=6.0 Hz), 3.85 (s, 3H), 3.49 (t, 2H, J=6.0 Hz), 3.11 (dd, 1H, J=17.1, 12.9 Hz), 2.80 (dd, 1H, J=17.1, 3.0 Hz), 2.08–2.03 (m, 2H), and 2.01–1.93 (m, 2H). ESIMS *m/z* 423.1 $[M + H]^+$.

7-(4-Bromobutoxy)-5-hydroxy-2-(4-methylphenyl)chroman-4-one (6b):

Yield: 45%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 12.05 (s, 1H), 7.35 (d, 2H, J=8.1 Hz), 7.25 (d, 2H, J=8.1 Hz), 6.07 (d, 1H, J=2.4 Hz), 6.04 (d, 1H, J=2.4 Hz), 5.40 (dd, 1H, J =12.9, 3.0 Hz), 4.04 (t, 2H, J=6.0 Hz), 3.47 (t, 2H, J=6.0 Hz), 3.11 (dd, 1H, J=17.1, 12.9 Hz), 2.81 (dd, 1H, J=17.1, 3.0 Hz), 2.38 (s, 3H), 2.09–2.03 (m, 2H), and 2.02–1.93 (m, 2H). ESIMS *m/z* 405.2 $[M + H]^+$.

7-(4-Bromobutoxy)-5-hydroxy-2-(4-fluorophenyl)chroman-4-one (6c):

Yield: 47%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (br s, 1H), 7.75 (d, 2H, J=8.7 Hz), 7.46 (d, 2H, J=8.7 Hz), 6.08 (d, 1H, J=2.1 Hz), 6.05 (d, 1H, J=2.4 Hz), 5.51 (dd, 1H, J =12.6, 2.7 Hz), 4.03 (t, 2H, J=6.0 Hz), 3.45 (t, 2H, J=6.0 Hz), 2.85 (dd, 1H, J=17.1, 3.0 Hz), 2.08–2.01 (m, 2H), and 2.00–1.90 (m, 2H). ESIMS *m/z* 409.1 $[M + H]^+$.

Procedure for the preparation of 7 (a–c)

For the preparation of **7** (**a–c**), 1-(2-methoxyphenyl)piperazine (2.5 mmol) and a catalytic amount of KI were added to a suspension of **6** (**a**–**c**) (2 mmol) and K_2CO_3 (1.22 mmol) in acetonitrile (15 mL). The resulting mixture was refluxed for 1 h. After filtration, the resulting filtrate was evaporated to dryness under reduced pressure. The residue was evaporated under reduced pressure, and the crude product was purified by means of chromatography (petroleum ether/EtOAc 2:1) to yield **7** (**a**–**c**).

5-hydroxy-2-(4-methoxyphenyl)-7-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butoxy)chroman-4-one (7a):

Compounds **7a** was obtained as yellow sticky residue, with yield 26%. The molecular formula was established as $C_{31}H_{36}N_2O_6$ by HR-Q-TOFMS at *m/z* 533.2712 $[M + H]^+$ (calcd 533.2646) and the 1D NMR spectroscopic data, representing fourteen indices of hydrogen deficiency. The ^{1}H NMR spectrum exhibited one hydroxy protons [δ_H 12.04 (br s, 1H)], six aromatic protons, 7.40 (d, 2H, J=8.7 Hz), 7.04–6.86 (m, 2H), 6.08 (d, 1H, J= 2.1 Hz), 6.05 (br s, 1H, J=1.8 Hz), 5.38 (dd, 1H, J=12.9, 2.7 Hz), 3.85 (s, 3H), 3.16–3.06 (m, 1H), 2.80 (dd, 1H, J=17.1, 3.0 Hz) indicated that 7a with the same skeletal structure of flavanones as that observed for **5a**. The proton signals at 4.03 (t, 2H, J=6.0 Hz), 2.49 (t, 2H, J=7.2 Hz), 1.87–1.82 (m, 2H), and 1.75–1.68 (m, 2H) suggesting that **7a** possessing the butoxy fragment as that observed for **6a**, furthermore, the other proton signals at 3.16–3.06 (m, 4H), 2.68 (m, 4H) suggesting the presence of 4-(4-(2-methoxyphenyl)piperazin-1-yl) fragment. $[\alpha]_D^{20}$ −5.3 (*c* 0.1, MeOH). UV (MeOH):

λ_{max} (log ε) 208.5 (4.73), and 287.5 (4.36) nm. IR (KBr): ν_{max} 2939, 2833, 1641, 1574, 1514, 1503, 1448, 1302, 1242, 1163, and 829 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (br s, 1H), 7.40 (d, 2H, J=8.7 Hz), 7.04–6.86 (m, 6H), 6.08 (d, 1H, J=2.1 Hz), 6.05 (br s, 1H, J=1.8 Hz), 5.38 (dd, 1H, J=12.9, 2.7 Hz), 4.03 (t, 2H, J=6.0 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 3.16–3.06 (m, 5H), 2.80 (dd, 1H, J=17.1, 3.0 Hz), 2.68 (m, 4H), 2.49 (t, 2H, J=7.2 Hz), 1.87–1.82 (m, 2H), and 1.75–1.68 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 195.9, 167.5, 164.1, 162.9, 160.1, 152.3, 141.4, 130.5, 127.7, 122.9, 121.0, 118.2, 114.2, 111.3, 103.1, 95.6, 94.6, 79.0, 68.3, 58.1, 55.4, 53.4, 50.6, 43.2, 27.0, and 23.2. ESIMS *m/z* 532.9 $[M + H]^+$; HR-Q-TOFMS: 533.2712 $[M + H]^+$ (calcd for $C_{31}H_{36}N_2O_6$, 533.2646).

5-hydroxy-2-(4-methylphenyl)-7-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butoxy)chroman-4-one (7b):

Yellow sticky residue, yield: 20%. $[\alpha]_D^{20}$ −3.4 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 210 (4.48), and 287 (4.14) nm. IR (KBr): ν_{max} 2941, 2820, 1638, 1578, 1506, 1452, 1368, 1299, 1165, 826, and 743 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.03 (br s, 1H), 7.36 (d, 2H, J=8.1 Hz), 7.26 (d, 2H, J=7.8 Hz), 7.05–6.87 (m, 4H), 6.08 (d, 1H, J=2.7 Hz), 6.06 (br s, 1H, J=2.4 Hz), 5.40 (dd, 1H, J=12.9, 3.0 Hz), 4.03 (t, 2H, J=6.3 Hz), 3.88 (s, 3H), 3.16–3.06 (m, 5H), 2.82 (dd, 1H, J=17.1, 3.0 Hz), 2.69 (m, 4H), 2.49 (t, 2H, J=7.2 Hz), 2.40 (s, 3H), 1.87–1.80 (m, 2H), and 1.76–1.68 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 195.8, 167.5, 164.1, 162.9, 152.3, 141.4, 138.8, 135.5, 129.5, 126.2, 122.9, 121.0, 118.2, 111.3, 103.1, 95.6, 94.6, 79.1, 68.3, 58.1, 55.4, 53.4, 50.6, 43.3, 27.0, 23.2, and 21.2. ESIMS *m/z* 517.2 $[M + H]^+$; HR-Q-TOFMS: 517.2766 $[M + H]^+$ (calcd for $C_{31}H_{36}N_2O_5$, 517.2697).

5-hydroxy-2-(fluorophenyl)-7-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butoxy)chroma n-4-one (7c):

Yellow sticky residue, yield: 27%. $[\alpha]_D^{20}$ −1.9 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 209.5 (4.67), and 285.5 (4.31) nm. IR (KBr): ν_{max} 2938, 1641, 1576, 1501, 1449, 1373, 1304, 1242, 1163, 835, and 743 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.01 (br s, 1H), 7.47–7.43 (m, 2H), 7.16–7.10 (m, 2H, *J*=7.8 Hz), 7.05–6.87 (m, 4H), 6.10 (d, 1H, *J*=2.4 Hz), 6.06 (d, 1H, *J*=2.1 Hz), 5.40 (dd, 1H, *J*=13.2, 3.0 Hz), 4.03 (t, 2H, *J*=6.0 Hz), 3.88 (s, 3H), 3.12–3.02 (m, 5H), 2.82 (dd, 1H, *J*=17.1, 3.3 Hz), 2.68 (m, 4H), 2.49 (t, 2H, *J*= 7.5 Hz), 2.40 (s, 3H), 1.87–1.80 (m, 2H), and 1.75–1.68 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 195.3, 167.6, 164.2, 162.6, 152.3, 141.4, 134.4, 134.3, 128.0, 122.9, 121.0, 118.2, 116.0, 115.7, 111.3, 103.0, 95.7, 94.7, 78.5, 68.3, 58.1, 55.4, 53.5, 50.6, 43.4, 27.0, and 23.2. ESIMS *m/z* 520.8 $[M + H]^+$; HR-Q-TOFMS: 521.2506 $[M + H]^+$ (calcd for $C_{30}H_{33}FN_2O_5$, 521.2446).

Procedure for the preparation of 7d and 7e

For the preparation of **7d** and **7e**, 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole (2.5 mmol) and a catalytic amount of KI were added to a suspension of **6a** and **6c** (2 mmol) and K_2CO_3 (1.22 mmol) in acetonitrile (15 mL). The resulting mixture was refluxed for 1 h. After filtration, the resulting filtrate was evaporated to dryness under reduced pressure. The residue was evaporated under reduced pressure, and the crude product was purified by means of chromatography (petroleum ether/EtOAc 2:1) to yield **7d** and **7e**.

5-hydroxy-2-(4-methoxyphenyl)-7-(4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)chroman-4-one (7d):

Yellow solid, yield 33%. $[\alpha]_D^{20}$ −8.6 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 202.5 (4.73), 229 (4.43), and 285.5 (4.46) nm. IR (KBr): ν_{max} 2947, 2806, 1638, 1576, 1506, 1371, 1302, 1267, 1163, and 827 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.05 (br s, 1H), 7.40 (dd, 1H, *J*=8.7, 5.1 Hz), 7.44 (d, 2H, *J*=8.7 Hz), 7.25 (dd, 2H, *J*=8.7, 2.1 Hz), 7.07 (dt, 1H, *J* =9.0, 2.1 Hz), 6.97 (d, 2H, *J*=8.7 Hz), 6.08 (d, 1H, *J*=2.1 Hz), 6.06 (br s, 1H, *J*=2.4 Hz), 5.40 (dd, 1H, *J*=13.2, 3.0 Hz), 4.03 (t, 2H, *J*=6.0 Hz), 3.85 (s, 3H), 3.17–3.07 (m, 4H), 2.81 (dd, 1H, *J*=17.1, 3.0 Hz), 2.47 (t, 2H, *J*=7.2 Hz), 2.21–2.04 (m, 6H), 1.89–1.80 (m, 2H, H-2''), and 1.76–1.68 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 195.9 , 167.5, 164.1, 162.9, 161.1, 160.1, 130.5, 127.7, 122.7, 122.5, 117.3, 114.4, 112.5, 112.1, 103.1, 97.6, 97.2, 95.5, 94.6, 79.0, 68.3, 58.3, 55.4, 53.5, 43.2, 34.6, 30.5, 27.0, and 23.3. ESIMS *m/z* 560.8 $[M + H]^+$; HR-Q-TOFMS: 561.2447 $[M + H]^+$ (calcd for $C_{32}H_{33}FN_2O_6$, 561.2395).

5-hydroxy-2-(4-fluorophenyl)-7-(4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)bu toxy)chroman-4-one (7e):

Yellow solid, yield 33%. $[\alpha]_D^{20}$ −5.2 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 202 (4.71), 226 (4.59), and 285 (4.43) nm. IR (KBr): ν_{max} 3078, 2943, 2803, 1641, 1614, 1580, 1510, 1452, 1368, 1302, 1246, 1171, and 816 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (br s, 1H), 7.72 (dd, 2H, *J*=8.7, 5.1 Hz), 7.47–7.42 (m, 2H), 7.24 (dd, 2H, *J*=8.7, 2.1 Hz), 7.16–7.02 (m, 4H), 6.08 (d, 1H, *J*=2.1 Hz), 6.05 (br s, 1H, *J*=2.4 Hz), 5.40 (dd, 1H, *J*= 12.6, 3.0 Hz), 4.03 (t, 2H, *J*=6.0 Hz), 3.11–3.01 (m, 4H), 2.80 (dd, 1H, *J*=17.1, 3.0 Hz),

2.48 (t, 2H, J=7.2 Hz), 2.22–2.03 (m, 6H), 1.87–1.80 (m, 2H), and 1.75–1.68 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 195.9, 167.5, 164.1, 162.6, 161.0, 134.3, 134.3, 128.0, 122.7, 122.5, 117.3, 115.9, 115.7, 112.5, 112.1, 103.0, 97.2, 95.7, 94.6, 78.5, 68.3, 58.2, 53.4, 30.4, 43.3, 34.5, 26.9, and 23.2. ESIMS *m/z* 548.7 $[M + H]^+$; HR-Q-TOFMS: 549.2273 $[M + H]^+$ (calcd for $C_{31}H_{30}F_2N_2O_5$, 549.2196).

7-(4-Bromopropoxy)-5-hydroxy-2-(4-methoxyphenyl)chroman-4-one (6d):

In the same way as preparation of **6 (a–c)**, 1,3-dibromopropane (10 mmol) was added to a solution of **5a** (5 mmol) and potassium carbonate in acetone (30 mL), and the mixture was refluxed for 3 h. After cooling to room temperature, the mixture was filtered, the solvent was evaporated and the residue was purified by column chromatography (petroleum ether/EtOAc 15:1) to yield 6d as yellow oils. Yield: 54%. ^{1}H NMR δ ppm (300 MHz, $CDCl_3$): 12.03 (br s, 1H), 7.41 (d, 2H, J=8.7 Hz), 7.04–6.88 (m, 6H), 6.09 (d, 1H, J=2.1 Hz), 6.05 (br s, 1H, J=1.8 Hz), 5.37 (dd, 1H, J=12.9, 2.7 Hz), 4.03 (t, 2H, J=6.0 Hz), 3.85 (s, 3H), 3.51 (t, 2H, J=6.0 Hz), 3.11 (dd, 1H, J=17.1, 12.9 Hz), 2.80 (dd, 1H, J= 17.1, 3.0 Hz), and 2.04–1.99 (m, 2H). ESIMS *m/z* 407.2 $[M + H]^+$.

Procedure for the preparation of 7f and 7g

In the same way as in the preparation of **7** (**a**–**c**), arylpiperazine (piperidine) (2.5 mmol) was added to a suspension of **6d** (2 mmol). The resulting mixture was refluxed for 1 h. After filtration, the resulting filtrate was evaporated to dryness under reduced pressure. The residue was evaporated under reduced pressure, and the crude product was purified by means of chromatography (petroleum ether/EtOAc 2:1) to yield **7f** and **7g**.

5-hydroxy-2-(4-methoxyphenyl)-7-(4-(4-(2-methoxyphenyl)piperazin-1-yl)propoxy)chroman-4-one (7f):

Yellow sticky residue, yield 21%. $[\alpha]_D^{20}$ −10.2 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 209.5 (4.63), and 285.5 (4.31) nm. IR (KBr): ν_{max} 2945, 2818, 1643, 1574, 1516, 1501, 1449, 1375, 1304, 1242, 1163, and 829 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (br s, 1H), 7.40 (d, 2H, *J*=8.4 Hz), 7.04–6.87 (m, 6H), 6.09 (br s, 1H), 6.08 (br s, 1H), 5.38 (dd, 1H, *J*=12.9, 2.4 Hz), 4.08 (t, 2H, *J*=6.0 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 3.17–3.07 (m, 5H), 2.80 (dd, 1H, *J*=17.1, 3.0 Hz), 2.69 (m, 4H), 2.58 (t, 2H, *J*=7.2 Hz), and 2.07–1.99 (m, 2H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 196.0, 167.2, 164.1, 162.9, 160.1, 152.2, 140.8, 130.4, 127.7, 123.3, 121.1, 118.4, 114.2, 111.2, 103.2, 95.6, 94.6, 79.0, 66.4, 55.4, 54.8, 53.2, 49.8, 43.2, and 29.7. ESIMS *m/z* 519.0 $[M + H]^+$; HR-Q-TOFMS: 519.2548 $[M + H]^+$ (calcd for $C_{30}H_{34}N_2O_6$, 519.2490).

5-hydroxy-2-(4-methoxyphenyl)-7-(4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)chroman-4-one (7g):

Yellow sticky residue, yield 27%. $[\alpha]_D^{20}$ −4.5 (*c* 0.1, MeOH). UV (MeOH): λ_{max} (log ε) 203 (4.69), 227 (4.64), and 285.5 (4.45) nm. IR (KBr): ν_{max} 2930, 2845, 1639, 1574, 1512, 1450, 1302, 1252, 1165, and 829 cm^{-1}. ^1H NMR δ ppm (300 MHz, $CDCl_3$): 12.04 (br s, 1H), 7.72 (dd, 1H, *J*=8.4, 5.1 Hz), 7.39 (d, 2H, *J*=8.4 Hz), 7.25 (m, 1H), 7.06 (dt, 1H, *J*=9.0, 2.1 Hz), 6.96 (d, 2H, *J*=8.4 Hz), 6.10 (d, 1H, *J*=2.4 Hz), 6.08 (d, 1H, *J*=2.4 Hz), 5.40 (dd, 1H, *J*=12.9, 2.1 Hz), 4.08 (t, 2H, *J*=6.0 Hz), 3.85 (s, 3H), 3.17–3.08 (m, 4H), 2.81 (dd, 1H, *J*=17.1, 2.7 Hz), 2.60 (t, 2H, *J*=7.2 Hz), and 2.33–1.98 (m, 8H). ^{13}C NMR δ ppm (75 MHz, $CDCl_3$): 196.0, 167.4, 164.1, 162.9, 161.0, 160.0, 130.4, 127.7, 122.7,

122.6, 117.3, 114.2, 112.5, 112.2, 103.1, 97.6 , 95.6, 94.6, 79.0, 66.7, 55.4, 55.0, 53.5, 43.2, 34.5, 30.4, 30.0, and 26.5. ESIMS *m/z* 546.9 $[M + H]^+$; HR-Q-TOFMS: 547.2309 $[M + H]^+$ (calcd for $C_{31}H_{31}FN_2O_6$, 547.2239).

In vitro evaluation of biological activity

Measurement of dopamine D_2 receptor activity

The activity of the D2 receptor was detected by the fluorescent method for measuring cytoplasmic calcium ion concentration after dopamine stimulation (23, 24). D2 receptor/G protein α16a co-transfected HEK293 cells (from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences) were seeded in 96-well plates and incubated overnight at 37°C. The media were then removed, and 40 μL of 10 μM Fluo-4/AM (a calcium fluorescent probe) (Invitrogen Corporation, USA) was added. The plates were then incubated for 40 min at 37°C. After the supernatant was discarded and the cells were washed with calcium buffer, 50 μL of calcium buffer containing tested compounds at the dosage range from 0.01 nM to 10 μM were added, and the plates were incubated for 10 min. The fluorescence values at an emission wavelength of 525 nm and an excitation wavelength of 485 nm were detected by the FlexStation II microplate reader (Molecular Devices, USA), starting 15 s after 25 μL of calcium buffer containing 50 nM dopamine (an agonist of the dopamine D2 receptor) (Sigma-Aldrich, USA) was automatically added by the instrument. Data were expressed as: % Response = $(F_{Sample}-F_{Blank})/(F_{Dopamine}-F_{Blank})$. F_{Sample} indicated the fluorescence value of tested compounds, F_{Blank} represented the

fluorescence value completely suppressed by 10^{-5} M eticlopride (an antagonist of the dopamine D2 receptor) (Sigma-Aldrich, USA), and $F_{Dopamine}$ indicated the fluorescence value of the 50 nM dopamine-only group.

Determination of LPS/IFN-γ-induced nitric oxide release from BV2 microglial cells

BV2 mouse microglia (5×10^5 cells/mL) were seeded in 96-well plates and incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air for 5-7 d. After the cells grew to 70-80% confluence, pre-treatment with tested compounds at the dosage range from 0.1 μM to 10 μM, 4 h prior to LPS (1 μg/mL) (Sigma-Aldrich, USA) and IFN-γ (1 ng/mL) (Bio-RAD, USA) stimulation for 24 h. The nitrite content (an indicator of NO production) was measured with the Griess reaction (12,13). The supernatant (100 μL) was mixed with an equal volume of the Griess reagent (Solution A: 222488; Solution B: S438081) (Beyotime, China), and the absorbance of the mixture at 525 nm was determined using an ELISA plate reader (Themo, USA).

In order to eliminate the possibility that the decrease of NO content may be due to the cell death induced by LPS/IFN-γ, cell viability was also determined by a CCK-8 assay before the measurement of NO release. A dose of 100 μL of CCK-8 (Dojindo Laboratories, Japan) solution was added to 1 mL of cell suspension (1×10^5 cells/mL in 96-well plates) and incubated for 4 h. The optical density was measured at 540 nm using an ELISA plate reader.

In vivo evaluation of the effects of synthesized compounds

Animals

Male BALB/c mice weighing 20 ± 2 g (from Vital River Laboratory Animal Technology Co. Ltd., China) were housed under a 12 h light/12 h dark cycle with free access to food and water. Animals were randomly assigned to different experimental groups, and each group was kept in a separate cage. All experiments were performed in accordance with the requirements of the Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation, and approved by the Animal Ethics Committee of Capital Medical University, China.

Open field test in the MK-801-induced mouse model

BALB/c mice were intragastrically administered compounds **7d**–**7g** (50 mg/kg) for 4 d. On the 4th day, 60 min after drug administration, mice were placed in Plexiglas boxes (41.5 cm × 41.5 cm × 41.5 cm) equipped with a video-based Ethovision System (Noldus, Wageningen, The Netherlands) for evaluating locomotor activity. After baseline recording for 60 min, a dose of 0.6 mg/kg of (+)-MK-801 hydrogen maleate (an NMDA receptor antagonist) (Sigma-Aldrich, USA) was intraperitoneally injected, and the locomotor activity of each mouse was recorded for 210 min at intervals of 10 min. The total distance of mouse movement was calculated (25,26).

Climbing behavior assay in an apomorphine-induced mouse model

BALB/c mice were intragastrically administered compounds **7d**–**7g** (25 mg/kg) for 4 d. After drug administration on the 4th day, mice were placed in specially designed cages with

steel sidewalls (35 cm × 35 cm × 55 cm) and a stainless steel floor covered with aspen chips. Immediately after subcutaneous injection of a dose of 2 mg/kg apomorphine (an agonist of dopamine receptor) (Tocris bioscience, Britain), mice were put into the test cage. After a 20-min period of exploratory activity, climbing behaviour (the time of climbing on the sides of top of cage) was recorded every 5 min by an observer who was blind to the drug treatment (27, 28).

Statistical analysis

SPSS 17.0 and Microsoft Excel 2007 were used for the statistical analysis. All data were expressed as the mean ± standard error of mean (S.E.M.). The differences among the groups were analyzed by one-way analysis of variance (ANOVA) with a Tukey *post hoc* test when comparing multiple groups. *P* values lower than 0.05 were regarded as statistically significant.

Results

Synthesized compounds

In the present experiment, seven novel flavanone derivatives (**7a**–**7g)** were synthesized, and the general strategy is summarized in Fig. 1. The chalcones **3a**–**3c** were obtained through the condensation of 2,4-di-MOM phloracetophenone **2** with substituted benzaldehyde. The chalcones then underwent cyclisation in the presence of CH_3COONa in methanol to yield the corresponding dihydroflavonoids **4a**–**4c**. After acidification of **4a**–**4c** to give **5a**–**5c**, the latter were reacted with 1,4-dibromobutane to yield **6a**–**6c,** or with

1,3-dibromopropane to yield **6d**. The corresponding bromine substitutes were reacted with 1-(2-methoxyphenyl)piperazine in acetonitrile, in the presence of K_2CO_3 and a catalytic amount of potassium iodide, to give **7a–7c** and **7f**, or reacted with 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole to give **7d, 7e,** and **7g**.

Effects of compounds 7a–7g on dopamine D_2 receptor activity in vitro

The activity of the D2 receptor was detected by the fluorescent method for measuring cytoplasmic calcium ion concentration after dopamine stimulation. The results showed that the synthesized compounds **7a–7g** decreased D_2 receptor activity in a dose-dependent manner in D2 receptor/G protein α16a co-transfected HEK293 cells (Fig. 2). Their IC_{50} values were 0.051–0.35 μM (Table 1).

Effects of compounds 7a–7g on LPS/IFN-γ-induced NO production in BV2 microglial cells

As shown in Fig. 3, LPS/IFN-γ significantly increased NO content in BV2 microglial cells (P<0.01). Compound **7g** (0.1, 1, 10 μM) inhibited the LPS/IFN-γ-induced over-production of NO in a dose-dependent manner (P<0.05,P<0.01). At a dose of 10 μM, **7a, 7b, 7d, 7e,** and **7f** markedly decreased the levels of NO in LPS/IFN-γ-stimulated BV microglia (P<0.01). The results indicated that these compounds had an anti-neuroinflammatory effect.

To eliminate the possibility that the decrease in NO content might be due to the cell death induced by LPS/IFN-γ, cell viability was determined by the CCK-8 assay in the same

manner as NO measurement. The results showed that **7a**–**7g** (1, 10, 100 μM) alone or in the presence of LPS/ IFN-γ had no significant influence on the cell viability of BV2 microglia (data not shown).

Effects of compounds 7d–7g on locomotor activity in MK-801-induced mouse model of schizophrenia

The open field test was used to detect the locomotor activity in the schizophrenia mouse model induced by MK-801 (an antagonist of NMDA receptors). Fig. 4A displays the total distance traveled and recorded at 10-min intervals for 270 min. There were no significant differences among groups in the total distance traveled in the baseline recording for 60 min. After intraperitoneal injection of MK-801 (0.6 mg/kg), the total distance traveled for 210 min significantly increased in the model mice (P<0.01), indicating the positive symptom of schizophrenia. Intragastric administration of **7d**, **7e**, and **7g** (50 mg/kg) for 4 d decreased the total distance traveled for 210 min, in which the effects of **7d** were significantly different from those observed in the MK-801 model mice (P<0.05) (Fig. 4B). These results indicated that some of the synthesized compounds suppressed the hyperactivity in the NMDA receptor antagonist-induced mouse model of schizophrenia.

Effects of compounds 7d–7g on climbing behavior in apomorphine-induced mouse model of schizophrenia

Apomorphine is an agonist of dopamine receptors, and has been classically linked to motor agitation, one of the positive symptoms of schizophrenia. In the present experiment, the

results showed that the latency in the climbing test was significantly increased by the subcutaneous injection of apomorphine (P<0.01). This increase was reversed by intragastrical administration of **7d**, **7e**, **7f**, and **7g** for 4 d, in which the effects of **7g** were significantly different from that observed in the apomorphine model mice (P<0.05) (Fig. 5). These results demonstrated that some of the synthesized compounds decreased the dopamine receptor agonist-induced hyperactivity in the schizophrenia mouse model, suggesting that the compounds may have a potential antipsychotic effect.

Discussion

It has been reported that natural flavanones possess a broad range of pharmacological properties, including anti-neuroinflammatory and radical-scavenging effects (11−16). In addition, some first-line antipsychotic drugs containing an arylpiperazine (piperidine) group have inhibitive effects on dopamine activity (17−22). In an attempt of creating a multi-targeted antipsychotic drug, we designed and synthesized compounds **7a**–**7g**, which have the skeletal structure of flavanones combined with the arylpiperazine (piperidine) group.

In the biological activity evaluation of synthesized flavanone derivatives, we did *in vitro* study first, and found that 7 target compounds inhibited dopamine 2 receptor activity with different IC_{50}, and 6 compounds suppressed NO release from a microglia inflammatory cell model. According to the results *in vitro*, we chose 4 compounds with better biological activities to continue *in vivo* study. We used two classic animal models of schizophrenia for drug screening, and found that some compounds by intragastrical

administration improved the behavioral impairment in apomorphine-induced mouse model (dopaminergic mechanism) and in MK-801-induced mouse model (glutamatergic mechanism).

The dopamine hypothesis of schizophrenia has been proposed for several decades. The first evidence was gathered from the administration of amphetamine and other compounds, which increase extracellular concentrations of dopamine and can induce psychotic symptoms similar to those seen in schizophrenia (29). Recently, a study including 176 post-mortem samples from patients with schizophrenia has shown an increased expression of the presynaptic D2 autoreceptor (30), and there is a clear association between the D2 receptor gene and schizophrenia (31). In the present study, we evaluated dopamine D2 receptor activity by using D2 receptor/G protein α16a co-transfected HEK293 cells. In this assay, the activation of the dopamine-induced D2 receptor can activate coupled G protein α16, causing the activation of phospholipase C (PLC) and the generation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 proceeds to combine with its receptor in the endoplasmic reticulum and mitochondria of cells, causing the release of calcium ions into the cytoplasm. Thus, the changes of the intracytoplasmic calcium ion concentration can be used to detect the D2 receptor activation state (23,24). Our present study showed that synthesized compounds **7a**–**7g** containing an arylpiperazine (piperidine) group decreased D2 receptor activity in a dose-dependent manner in D2 receptor/Gα16a co-transfected HEK293 cells. Furthermore, mice treated with apomorphine, a mixed D_1/D_2 agonist, tend to adopt a vertical position along the walls of their cage (32,33). This climbing behavior appears to be elicited by stimulation of DA

receptors in the striatum (34). In addition, apomorphine has been used as a good tool drug to induce climbing behavior using a relatively high dose acting as a postsynaptic DA agonist (35,36). Our *in vivo* study exhibited that oral administration of **7d**, **7e**, **7f**, and **7g** decreased the hyperactivity in the climbing test in an apomorphine-induced schizophrenia mouse model. These results suggest that some of the synthesized compounds may have potential antipsychotic effects via the inhibition of dopamine activity.

Glutamatergic neurons represent the primary excitatory afferent and efferent systems innervating the cortex, limbic regions, and striatum. The postsynaptic actions of glutamate are mediated by a family of glutamate-gated ion channels (such as NMDA receptors),which permit the influx of sodium and calcium, thereby depolarizing (exciting) neurons (37,38). Much evidence suggests that hypofunction of NMDA receptors mediates neurotransmission disorder, which is a critical deficit in schizophrenia (39,40). Animals injected with MK 801 (an antagonist of NMDA receptors) exhibit schizophrenia-like behaviors, suggesting that the glutamatergic hypofunction may produce the symptoms that are relevant to schizophrenia (41,42). In the present study, , we found that intragastrical administration of **7d**, **7e**, and **7g** decreased the hyperlocomotor activity in the open field test in MK-801-induced schizophrenia model mice. This result suggests that some of the synthesized compounds may enhance glutamatergic function, which may be useful in the treatment of schizophrenia.

Recently, neuropathological and neuroimaging studies have provided consistent evidence of an association between schizophrenia and microglial activation and proliferation (43–45). Numerous studies have found that people with schizophrenia have

increased blood concentrations of inflammatory cytokines (46–48). The microglia hypothesis of schizophrenia proposes that activated microglia in the central nervous system release pro-inflammatory cytokines and free radicals, which cause abnormal neurogenesis, neuronal degradation, and white matter abnormalities, contributing to the pathogenesis of schizophrenia (49). In the present study, we used LPS/IFN-γ to activate microglia and induce a significant increase in the production of the inflammatory mediator NO. We found that most of the target compounds with the skeletal structure of flavanones inhibited the LPS/IFN-γ-induced over-production of NO in BV microglia. This result suggests that these compounds may have an anti-neuroinflammatory effect, which may be advantageous in treating schizophrenia.

Conclusion

In the current study, a series of novel flavanone derivatives with an arylpiperazine (piperidine) group was synthesized. The evaluation of the biological activity showed that compounds **7a–7g** decreased dopamine D_2 receptor activity and inhibited neuroinflammation *in vitro*. Oral administration of **7d**, **7e**, and **7g** reversed the movement hyperactivity in mice induced by both MK-801 (an antagonist of NMDA receptors) and apomorphine (an agonist of dopamine receptors). These results suggest that some of novel flavanone derivatives have the potential antipsychotic effect, and may be useful in the treatment of schizophrenia.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Nos. 81273498, 81473373), National Science and Technology Major Project for New Drug Research and Development of China (No. 2015ZX09101-016), Beijing New Medical Disciplines Grant (No. XK100270569), and Beijing High-level Health and Technical Personal Plan (Nos. 2011-1-7, 2014-2-014). We thank Shanghai Institute of Materia Medica, Chinese Academy of Sciences for technical assistance in dopamine D_2 receptor test.

Conflict of interest

The authors declare that there are no conflicts of interest associated with this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Table:

Table 1. IC_{50} of 7a–7g on dopamine D_2 receptor activity in D2 receptor/Gα16a co-transfected HEK293 cells

Compound	IC_{50} (M)	95% Confidence limit (M)
7a	2.05×10^{-7}	1.30×10^{-7}~3.24×10^{-7}
7b	3.46×10^{-7}	1.80×10^{-7}~6.65×10^{-7}
7c	5.73×10^{-8}	3.59×10^{-8}~9.13×10^{-8}
7d	5.13×10^{-8}	3.34×10^{-8}~7.89×10^{-8}
7e	8.77×10^{-8}	4.92×10^{-8}~1.56×10^{-7}
7f	2.20×10^{-7}	1.21×10^{-7}~3.99×10^{-7}
7g	1.16×10^{-7}	6.83×10^{-8}~1.95×10^{-7}

Figure Legend

Fig. 1. Synthesis of compounds 7a–7g.

Fig. 2. Effects of 7a–7g on dopamine D_2 receptor activity in D_2 receptor/Gα16a co-transfected HEK293 cells. The activity of the D2 receptor was detected by the fluorescent method for measuring cytoplasmic calcium ion concentration after dopamine stimulation. DRD2: dopamine D_2 receptor. Gα16a: G protein α16a. The data are expressed as mean ± S.E.M. from three independent experiments.

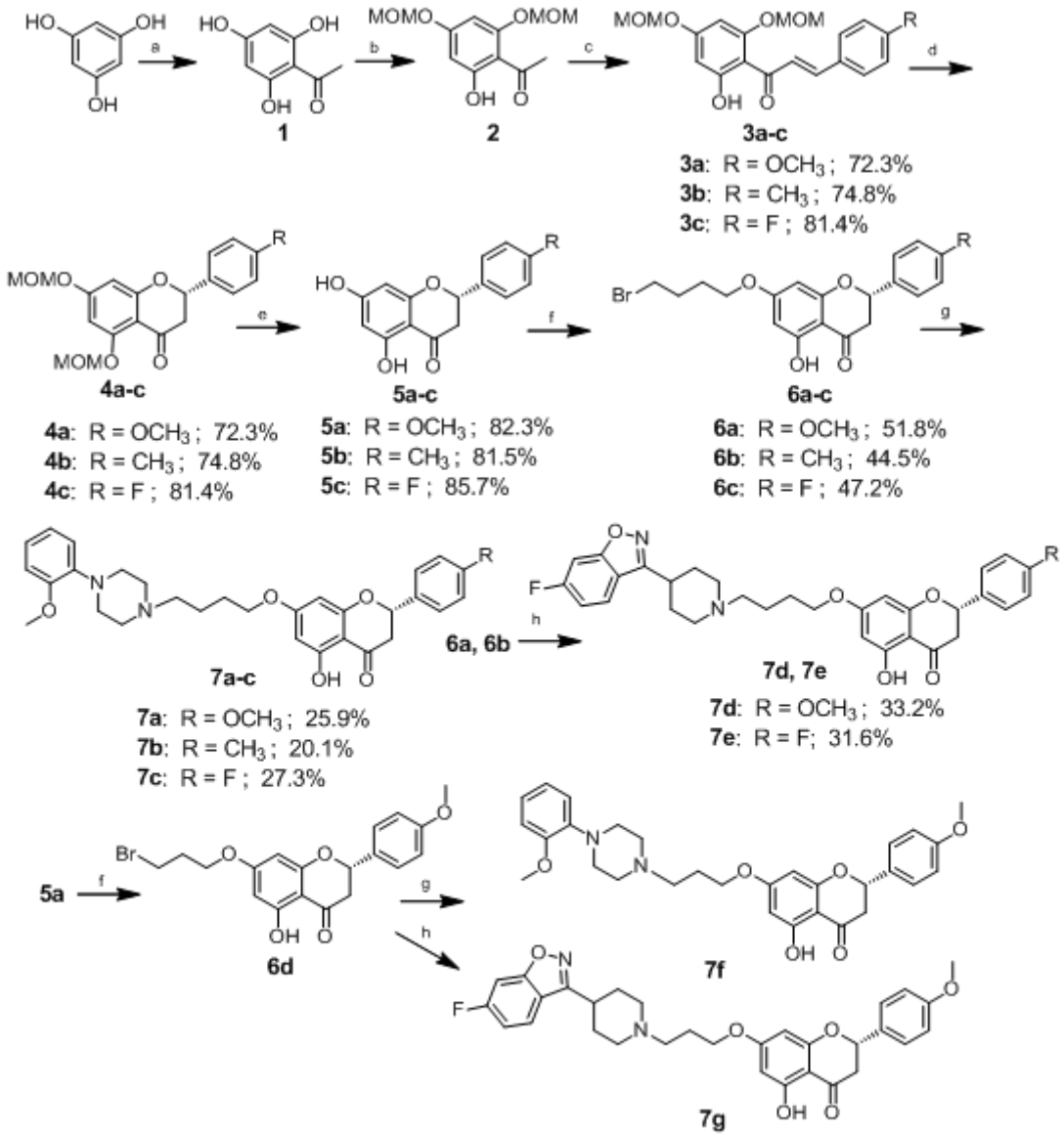
Fig. 3. **Effects of 7a–7g on LPS/IFN-γ-induced NO production in BV-2 microglia.** Cells were pretreated with compounds **7a–7g** for 4 h before LPS/IFN-γ stimulation for 24 h. NO release was measured in the supernatant in cultured BV-2 microglia by using a Griess assay. The data are expressed as the mean ± S.E.M. from three independent experiments. $^{\#\#}P < 0.01$, LPS/IFN-γ model group *vs.* control group; $^{*}P < 0.05$, $^{**}P < 0.01$, drug administration groups *vs.* LPS/IFN-γ model group.

Fig. 4. Effects of 7d–7g on locomotor activity in MK-801-induced schizophrenia mouse model. Compounds **7d–7g** (50 mg/kg) were intragastrically administered to the test mice for 4 d. The open field test was conducted by using a video-based Ethovision System. (A) The total distance curve of movement recorded at 10 min intervals for 270 min. After 60 min of baseline recording, MK-801 (an antagonist of NMDA receptors) was

intraperitoneally injected, and the movement of mice was continually recorded for 210 min. (B) The total distance of movement after MK-801 injection for 210 min. The data represent the mean ± S.E.M., n = 10 per group. $^{\#\#}P < 0.01$, MK-801 model group *vs*. control group; $^{*}P < 0.05$, $^{**}P < 0.01$, drug administration groups *vs.* MK-801 model group.

Fig. 5. Effects of 7d–7g on the latency in apomorphine-induced schizophrenia mouse model. Compounds **7d–7g** (25 mg/kg) were intragastrically administered to the test mice for 4 d. Apomorphine (a dopamine receptor agonist) was subcutaneously injected 30 min before the climbing behavior test started. The latency was recorded every 5 min for 25 min by a stopwatch. The data represent the mean ± S.E.M., n = 10-11 per group. $^{\#\#}P < 0.01$, apomorphine model group *vs*. control group; $^{*}P < 0.05$, drug administration groups *vs*. MK-801 model group.

Fig. 1



Reagents and conditions: (a) CS_2, $PhNO_2$, $AlCl_3$, CH_3COCl, rt,1h; (b) Me_2CO, K_2CO_3, MOM chloride, rt, 1.5h; (c): MeOH, substituted benzaldehyde, NaOH, reflux, 3h; (d): MeOH, CH_3COONa, reflux, 14h; (e): MeOH, HCl, reflux, 0.5h; (f): Me_2CO, K_2CO_3, reflux, 3h, **7a-7c** from 1,4-dibromobutane; and **9a** from 1,3-dibromopropane; (g) CH3CN, K_2CO_3, KI, 1-(2-methoxyphenyl)piperazine; (h) CH_3CN, K_2CO_3, KI, 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole

Fig. 2

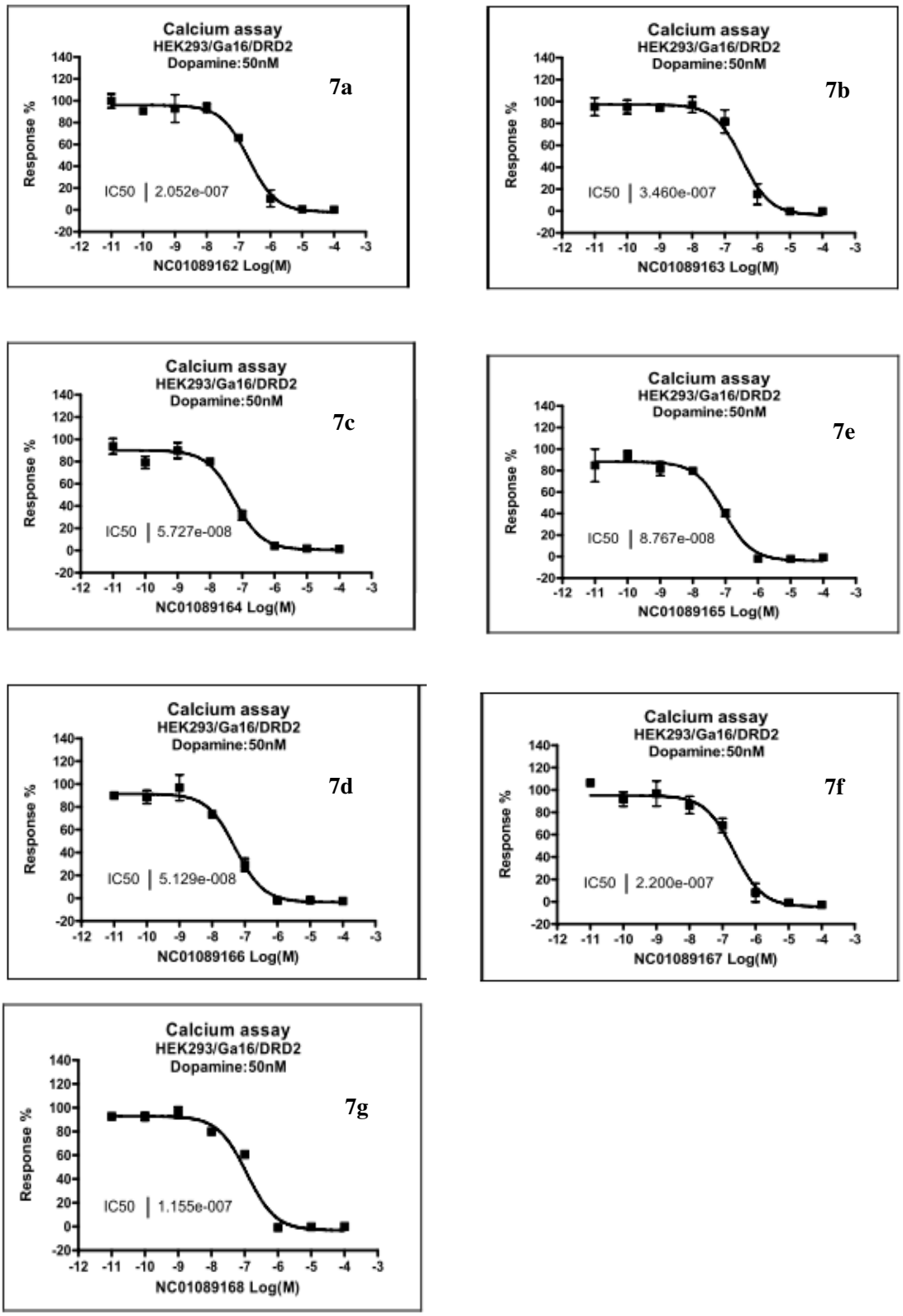
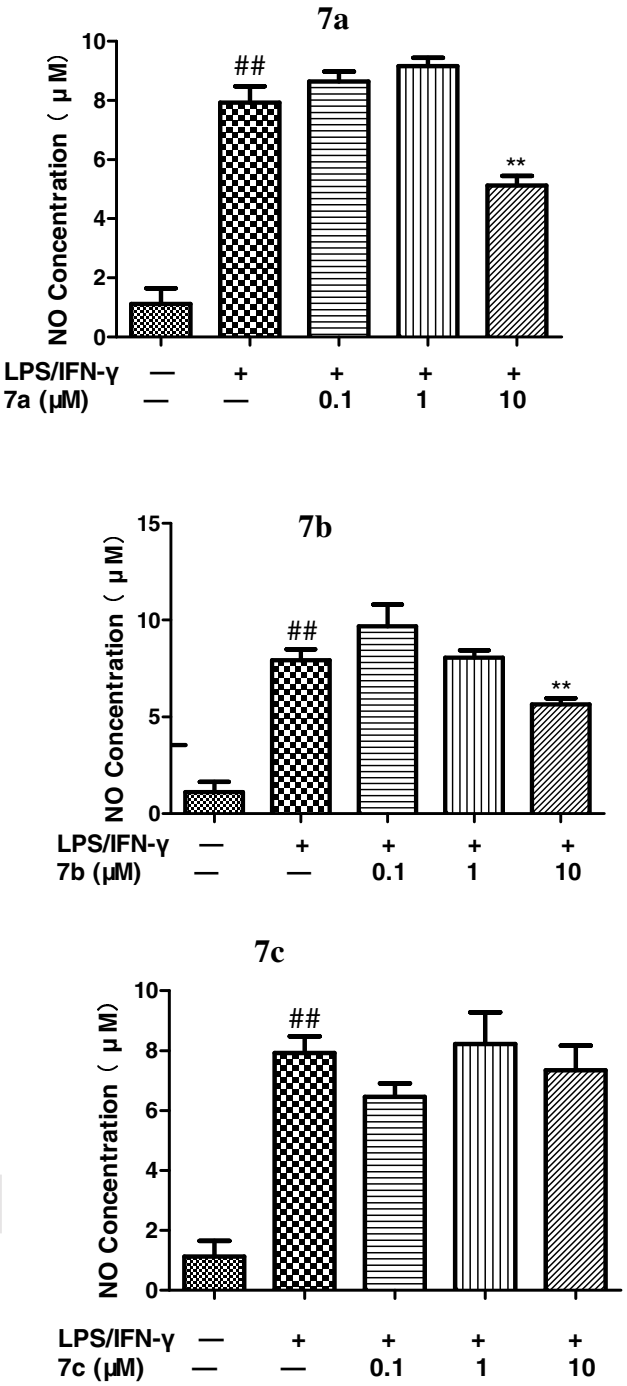
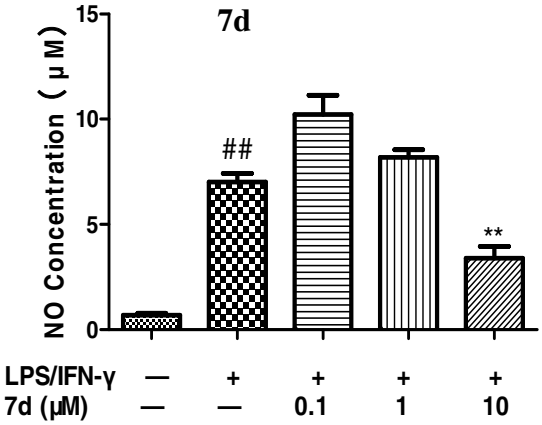
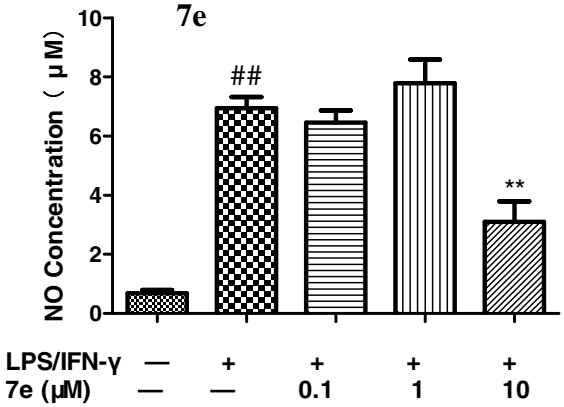
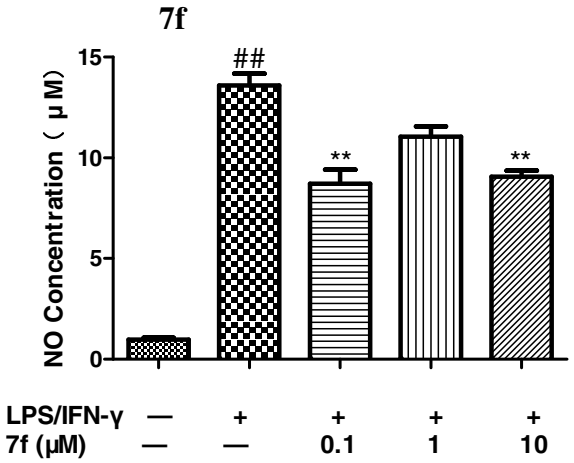


Fig. 3.









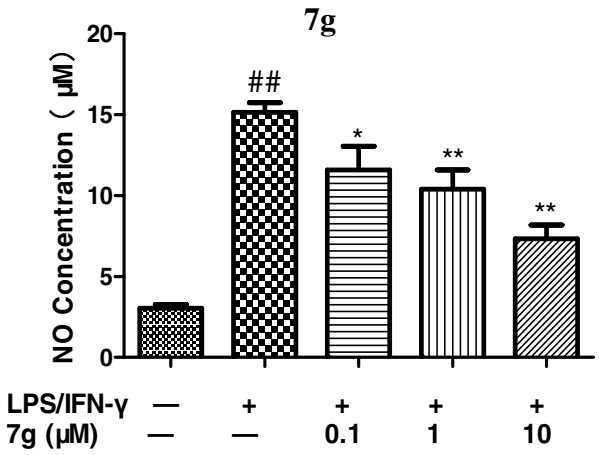


Fig. 4

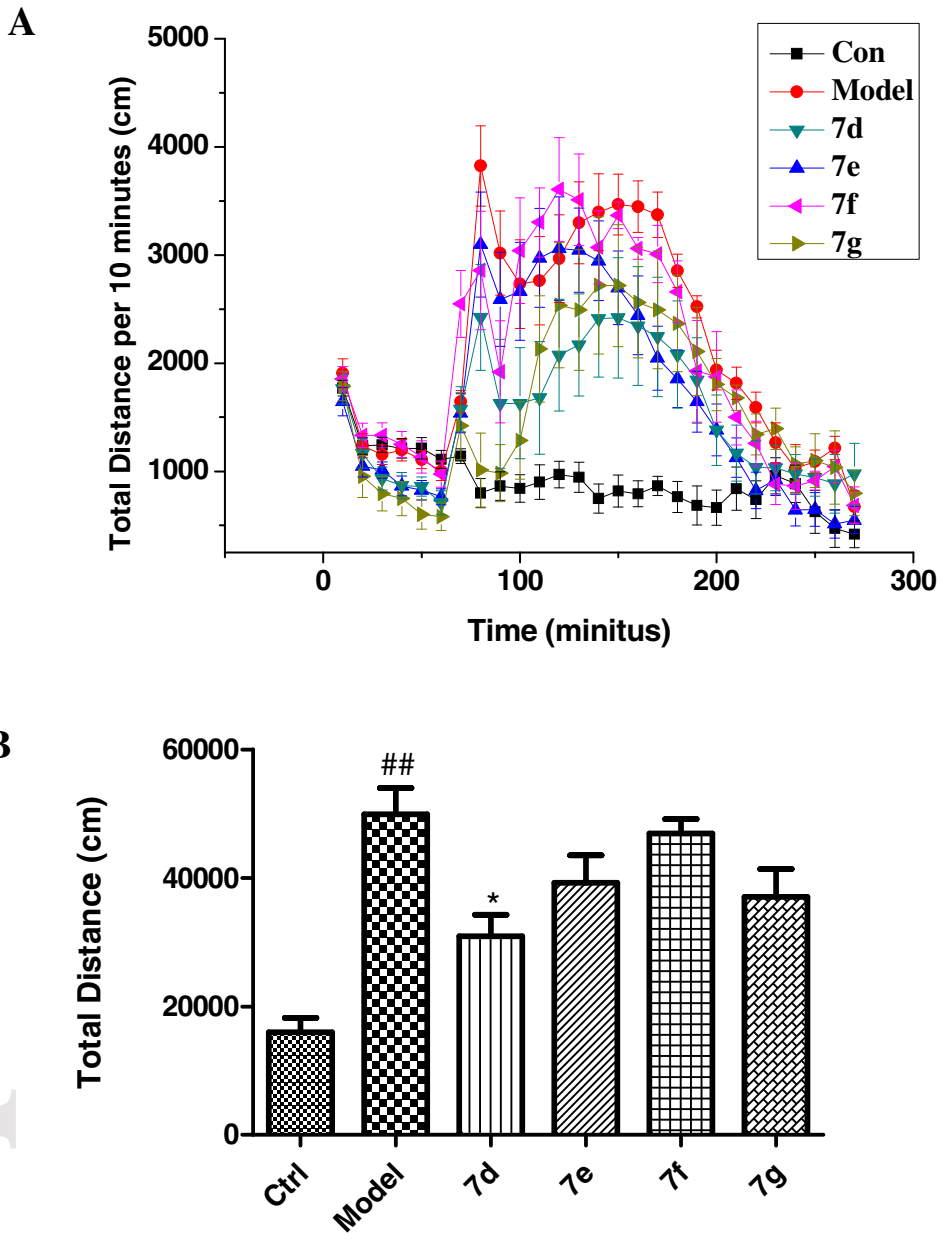


Fig. 5