Design, Synthesis, and Evaluation of Indolebutylamines as a Novel Class of Selective Dopamine D3 Receptor Ligands

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A series of indolebutylamine derivatives were designed, synthesized, and evaluated as a novel class of selective ligands for the dopamine 3 receptor. The most potent compound 11q binds to dopamine 3 receptor with a Ki value of 124 nM and displays excellent selectivity over the dopamine 1 receptor and dopamine 2 receptor. Investigation based on structural information indicates that site S182 located in extracellular loop 2 may account for high selectivity of compounds. Interaction models of the dopamine 3 receptor-11q complex and structure-activity relationships were discussed by integrating all available experimental and computational data with the eventual aim to discover potent and selective D3R ligands.

Key words: dopamine 3 receptor, indolebutylamine, pharmacophore model, selectivity, structure-activity relationship

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Since the discovery by Sokoloff et al. in 1990 (1), dopamine 3 receptor (D3R) has been proved to be a promising therapeutic target for drug discovery. Dopamine 3 receptor antagonist was shown to play a key role in the treatment of schizophrenia (2,3) and drug addiction (4). Although considerable efforts have been devoted to the design and development of D3R antagonists (5–9), function study of D3R in vivo is still limited due to the lack of highly selective antagonists.

One of the important reasons for the difficulty in developing selective antagonist for D3R is attributed to the high sequence homology among the D2-like dopamine receptor subtypes (D2, D3, and D4). For instance, hD3R and hD2R share 78% sequence homology within the seven transmembrane domains and 94% sequence homology within the active site (10,11). To date, most of D3R selective ligands are 4-Phenylpiperazines and their close analogs (12,13). Considering the importance of the D3R in the treatment of addiction and other neuropsychological disorders, it is meaningful to discover novel chemical entities to enrich the structural diversity of potent and selective D3R ligands. Using a strategy that combines synthetic chemistry, binding assays, and a set of computational approach (integrating active site mapping, pharmacophore-based virtual screening, and automated molecular docking), we designed a series of IBA derivatives as a new type of highly selective D3R antagonists. Furthermore, the molecular determinants critical to the binding specificity and selectivity of D3R were identified and the structure-activity relationships (SAR) was investigated.

Methods and Materials

Structure-based pharmacophore model generation
Dopamine 3 receptor was obtained from the Protein Data Bank (PDB ID: 3PBL) (11). The GRID22 program (14) was employed to map the active sites of the optimized X-ray structure of D3R with five types of chemical probes, that is, negative ionizable (COO⁻), positive ionizable (N1⁺), hydrogen-bond acceptor (O), hydrogen-bond donor (N1), and hydrophobic probes (DRY). For each of the five probes used in the grid calculations, grid points were superimposed to identify clusters of positions. The members of each identified clusters were combined into one pharmacophore feature, and the centers of each pharmacophore features were set at the geometric centers of the members in each clusters (15). Finally, a four-feature pharmacophore model was generated.

Virtual screening
The obtained pharmacophore model was used to screen the Asinex GOLD and Maybridge collection database which contain 238 000 compounds. The Ligand Pharmacophore Mapping protocol embedded in DISCOVERY STUDIO 3.5A was employed to retrieve molecules, which can well
match our pharmacophore model. For each molecule in the database, a maximum of 250 conformations with an energy threshold of 20 kcal/mol were generated using FAST algorithm. Only compounds with a fit value greater than three were retained. Then Lipinski’s Rule of Five was applied to reject non-drug-like compounds. The hits obtained were overlaid on the active site of the D3R and those creating steric clashes were discarded. GoldScore was used to rank the hits. The interaction analyses in combination with scoring function was used to guide the final selection.

**Molecular docking**

Molecular docking was carried out using GOLD 5.0.1(16). The binding site was defined to include all residues within a 15.0 Å radius of the conserved D3.32C^2 carbon atom. A hydrogen-bond constraint was set between the protonated nitrogen atom (N1) of ligand and D3.32 side chain. Ten conformations were produced for each ligand, and GoldScore was used as scoring function. Other parameters were set as standard default. High-scoring complexes were inspected visually to select the most reasonable solution.

**Biological evaluation**

**Binding assays**

All the synthesized new compounds were subjected to competitive binding assays for the human dopamine (D1, D2, and D3) receptors, using membrane preparation obtained from HEK293 cells stably transfected respective receptor. [3H] SCH23390 (D1) and [3H]-Spiperone (D2 and D3) were used as standard radioligands. The percentage displacement of radioligand and IC50 values of these compounds is reported in Table 1. Duplicated tubes were incubated at 30 °C for 50 min with increasing concentrations (1 nM–100 μM) of respective compound and with 0.7 nM [3H]SCH23390 (for D1R), or [3H]-Spiperone (for D2R and D3R) in a final volume of 200 μL binding buffer containing 50 mM Tris, 4 mM MgCl2, pH 7.4. Non-specific binding was determined by parallel incubations with either 10 μM SCH23390 for D1 or Spiperone for D2, D3 dopamine receptors, respectively. The IC50 and Ki values were calculated by non-linear regression (PRISM; Graphpad, San Diego, CA, USA) using a sigmoidal function.

**Experimental Section**

Chemicals and solvents were purchased and used without further purification. 1H and 13C NMR spectra were recorded on a Bruker AMX-400 instrument. The chemical shifts were referenced to the solvent peak, namely δ = 7.26 ppm for CDCl3 using TMS as an internal standard. Proton-coupling patterns were described as singlet, doublet, triplet, quartet, multiplet, and broad. Mass spectra were given with an electric ionization (ESI) produced by HP5973 N analytical mass spectrometer. All tested compounds had a minimal purity of 95% assessed by HPLC method (Schemes 1 and 2).

**General procedures for the preparation of compounds 11a–11q**

*N-cyclohexyl-2-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-phenylacetamide* (11a)

(i) Chloroacetyl chloride (1.47 mL, 18.43 mmol) was added to a solution of *N*-cyclohexylalaniline (3.23 g, 18.43 mmol) and Et3N (1.86 g, 18.43 mmol) in anhydrous CH2Cl2 at 0 °C under N2 atmosphere and then stirred at room temperature for 5 h. The reaction was diluted with CH2Cl2 and washed with brine, and the organic layer was dried over Na2SO4, evaporated, and purified by flash chromatography (PE/EtOAc, 10:1) to yield 2-chloro-*N*-cyclohexyl-*N*-phenylacetamide **8** as an off-white solid (3.9 g, yield 84.2%), (ii) To a suspension of compound **8** (3.0 g, 11.95 mmol), K2CO3 (2.48 g, 17.94 mmol) and a catalytic amount of KI (40 mg) in acetonitrile (40 mL) was added tert-butyl piperazine-1-carboxylate (2.22 g, 11.95 mmol). The reaction

**Table 1: The results of virtual screening and corresponding binding assays**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>HBA</th>
<th>HBD</th>
<th>AlogP</th>
<th>Fit value</th>
<th>Gold score</th>
<th>Binding affinity Ki ± SEM (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>476.6</td>
<td>5</td>
<td>1</td>
<td>5.84</td>
<td>3.51</td>
<td>58.5</td>
<td>2161 ± 25</td>
</tr>
<tr>
<td>12</td>
<td>496.0</td>
<td>4</td>
<td>1</td>
<td>3.73</td>
<td>3.29</td>
<td>69.0</td>
<td>2203 ± 9</td>
</tr>
<tr>
<td>13</td>
<td>364.5</td>
<td>3</td>
<td>2</td>
<td>2.93</td>
<td>3.30</td>
<td>59.1</td>
<td>2814 ± 35</td>
</tr>
<tr>
<td>Spiperone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.48 ± 0.1</td>
</tr>
</tbody>
</table>

mixture was refluxed for 8 h, and the reaction mixture was evaporated to dryness. The residue was dissolved in EtOAc (100 mL), washed with H₂O (50 mL), dried, and evaporated to obtain the crude product, which was purified by flash chromatography (CH₂Cl₂/MethOH, 40:1) to afford tert-butyl 4-[(2-cyclohexyl(pHENyL)amiNo)-2-oxoethyl] piperazine-1-carboxylate 9 as a yellow liquid (4.58 g, yield 95.4%), (iii) To a solution of compound 9 (3.0 g, 8.68 mmol) in CH₂Cl₂ (15 mL) was added trifluoroacetic acid (15 mL). The mixture was stirred at room temperature for 12 h, then concentrated, washed with PE and Et₂O separately to yield N-cyclohexyl-N-phenyl-2-piperazin-1-yl acetamide 10 as an off-white solid (2.68 g, yield 90%). (iv) A solution of compound 10 (0.467 g, 1.17 mmol), compound 3 (0.3 g, 1.17 mmol), and K₂CO₃ (0.324 g, 2.34 mmol) in acetonitrile (20 mL) was stirred at 80 °C for 12 h, then the reaction mixture was evaporated to dryness, water added, and the mixture was extracted with CH₂Cl₂ (3 × 30 mL), washed with brine, dried over anhydrous Na₂SO₄, and concentrated. The residue was purified with flash chromatography on silica gel (CH₂Cl₂/MeOH, 40:1) to afford compound 11a (0.2 g, yield 35.7%) (17). ¹H NMR (400 MHz, CDCl₃) δ 8.11 (s, 1H, NH), 7.40 (d, J = 5.1 Hz, 3H, Ar-H), 7.24 (dd, J = 13.6, 8.6 Hz, 2H, Ar-H), 7.11–7.09 (m, 2H, Ar-H), 7.02 (s, 1H, Ar-H), 6.92 (t, J = 9.0 Hz, 1H, Ar-H), 4.59 (t, J = 12.0 Hz, 1H, NCH). 2.75 (s, 2H, CH₂CO), 2.70 (t, J = 7.5 Hz, 2H, CH₂), 2.44 (m, J = 22.1, 14.7 Hz, 10H, CH₂), 1.91–1.81 (m, 6H, CH₂), 1.72 (d, J = 13.6 Hz, 3H, Ar-H), 1.57 (d, J = 12.6 Hz, 1H), 1.39 (dd, J = 26.0, 12.9 Hz, 2H), 0.98 (m, 4H).¹C NMR (100 MHz, CDCl₃) δ 170.38, 159.94, 157.63, 139.33, 134.79, 131.56, 130.45, 129.86, 129.10, 129.01, 124.99, 116.09, 116.04, 112.96, 112.87, 110.38, 110.12, 104.34, 103.81, 61.17, 59.27, 56.03, 53.73, 53.69, 32.62, 27.99, 26.92, 26.52, 23.88. ESI-MS m/z 477.2 [M + H]+.

2-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-phenylacetamide (11b) White solid (150 mg, yield 40.5%). ¹H NMR (400 MHz, CDCl₃) δ 9.13 (s, 1H), 8.06 (s, 1H), 7.58–7.56 (m, 2H), 7.34 (t, J = 7.9 Hz, 2H), 7.27–7.23 (m, 2H), 7.11 (t, J = 7.4 Hz, 1H), 7.03 (d, J = 1.8 Hz, 1H), 6.93 (td, J = 9.1, 2.4 Hz, 1H), 3.14 (s, 2H), 2.75 (t, J = 7.5 Hz, 3H), 2.68 (s, 4H), 2.56 (s, 4H), 2.47 (t, J = 8.0 Hz, 2H), 1.94–1.87 (m, 2H).¹C NMR (100 MHz, CDCl₃) δ 168.42, 158.86, 156.53, 137.64, 132.88, 129.05, 128.03, 127.94, 124.22, 123.06, 119.47, 116.51, 116.46, 116.69, 111.60, 110.37, 110.10, 103.93, 103.70, 61.97, 58.01, 53.52, 53.40, 27.24, 22.79. ESI-MS m/z 395.2 [M + H]+.

N-cyclohexyl-2-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-(2-methoxyphenyl)acetamide (11e) White solid (154 mg, yield 43.4%). White solid (160 mg, yield 43.3%). ¹H NMR (400 MHz, CDCl₃) δ 6.41 (t, J = 7.8 Hz, 1H), 7.25 (dd, J = 9.3, 4.7 Hz, 1H), 7.17–7.07 (m, 3H), 7.07 (s, 1H), 7.01 (t, J = 7.8 Hz, 1H), 6.82 (td, J = 9.0, 2.4 Hz, 1H), 4.42 (tt, J = 12.1, 3.5 Hz, 1H), 3.82 (m, 3H), 2.81–2.67 (m, 4H), 2.48–2.01 (m, 10H), 1.92–1.74 (m, 5H), 1.67 (d, J = 11.4 Hz, 1H), 1.57 (d, J = 12.9 Hz, 1H), 1.42–1.30 (m, 2H), 0.96–0.80 (m, 3H).¹C NMR (100 MHz, CDCl₃) δ 170.86, 159.84, 157.54, 157.47, 134.70, 132.28, 131.46, 129.01, 128.91, 127.89, 124.94, 121.86, 115.97, 115.92, 113.13, 112.90, 112.80, 110.31, 110.04, 103.98, 103.75, 60.64, 59.26, 56.68, 55.90, 53.73, 53.61, 33.10, 30.82, 27.96, 26.89, 26.86, 26.60, 23.84. ESI-MS m/z 507.4 [M + H]+.

2-(4-(3-(5-chloro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-phenylacetamide (11f) White solid (160 mg, yield 43.3%). ¹H NMR (400 MHz, CDCl₃) δ 8.50 (s, 1H), 7.41–7.40 (m, 3H), 7.27–7.24 (m, 2H), 7.12 (d, J = 7.9 Hz, 1H), 7.06–7.03 (m, 3H), 6.90 (td, J = 9.0, 1.9 Hz, 1H), 4.53 (t, J = 12.1 Hz, 1H), 2.95–2.59 (m, 14H), 2.08–2.00 (m, 2H), 1.79 (d, J = 10.7 Hz, 2H), 1.71 (d, J = 13.1 Hz, 2H), 1.55 (d, J = 12.6 Hz, 1H), 1.41–1.31 (m, 2H), 1.06–0.84 (m, 3H).¹C NMR (100 MHz, CDCl₃) δ 167.94, 158.80, 156.47, 137.65, 132.83, 130.07, 129.49, 128.78, 127.47, 127.37, 123.55, 114.07, 112.00, 111.91, 110.47, 110.21, 103.42, 103.19, 59.38, 56.91, 54.57, 51.87, 50.61, 31.34, 29.68, 25.66, 25.23, 24.29, 22.13. ESI-MS m/z 477.2 [M + H]+.
**2-(4-(3-(5-chloro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-(2-methoxyphenyl)acetamide (11g)**

White solid (137 mg, yield 28.8%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.14\) (s, 1H), 7.34 (t, \(J = 9.5, 1.8\) Hz, 1H), 7.25–7.20 (m, 2H), 7.06–6.88 (m, 5H), 4.55–4.49 (m, 1H), 3.78 (s, 3H), 2.79–2.67 (m, 4H), 2.48–2.37 (m, 10H), 1.96–1.83 (m, 3H), 1.71 (d, \(J = 14.9\) Hz, 1H), 1.63 (d, \(J = 13.0\) Hz, 1H), 1.54 (d, \(J = 13.3\) Hz, 1H), 1.42–1.27 (m, 3H), 1.16 (ddd, \(J = 24.6, 12.3, 3.7\) Hz, 4H), 0.95–0.76 (m, 2H), \(^1^3\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta 169.18, 158.67, 156.34, 156.21, 132.83, 131.39, 129.68, 127.88, 127.78, 127.36, 123.17, 120.53, 116.14, 111.72, 111.63, 111.52, 110.08, 109.81, 103.80, 103.56, 59.80, 58.22, 55.19, 54.97, 53.28, 53.03, 32.05, 29.63, 27.10, 25.79, 25.76, 25.49, 22.85. ESI-MS m/z 507.2 [M + H].

**2-(4-(3-(5-bromo-1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-phenylacetamide (11h)**

White solid (110 mg, yield 20.5%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.46\) (s, 1H), 7.70 (s, 1H), 7.34 (t, \(J = 7.8\) Hz, 1H), 7.22 (s, 2H), 7.04 (d, \(J = 7.4\) Hz, 1H), 6.97–6.93 (m, 3H), 4.53 (t, \(J = 11.7\) Hz, 1H), 3.78 (s, 3H), 2.79–2.67 (m, 4H), 2.47–2.36 (m, 10H), 1.93–1.78 (m, 4H), 1.71 (d, \(J = 12.9\) Hz, 1H), 1.63 (d, \(J = 12.1\) Hz, 1H), 1.54 (d, \(J = 12.1\) Hz, 1H), 1.42–1.26 (m, 2H), 1.20–1.14 (m, 1H), 0.95–0.78 (m, 2H), \(^1^3\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta 168.59, 138.24, 134.86, 130.39, 129.28, 129.09, 128.31, 124.43, 122.53, 121.49, 115.80, 112.53, 112.20, 60.32, 58.09, 54.06, 53.28, 52.98, 31.43, 27.20, 25.69, 25.29, 22.69. ESI-MS m/z 539.2 [M + H].

**2-(4-(3-(5-chloro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-(2-methoxyphenyl)acetamide (11i)**

White solid (129 mg, yield 18.8%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.56\) (s, 1H), 7.70 (s, 1H), 7.38 (s, 3H), 7.19 (s, 1H), 7.08 (s, 1H), 7.07 (s, 1H), 6.95 (s, 1H), 4.58 (t, \(J = 12.1\) Hz, 1H), 2.74 (s, 2H), 2.68 (t, \(J = 7.4\) Hz, 2H), 2.46–2.35 (m, 10H), 1.83–1.80 (m, 4H), 1.70 (d, \(J = 12.5\) Hz, 2H), 1.55 (d, \(J = 12.9\) Hz, 1H), 1.42–1.32 (m, 2H), 1.05–0.83 (m, 3H), \(^1^3\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta 169.18, 156.18, 134.92, 131.35, 129.69, 129.29, 127.29, 124.36, 122.65, 121.47, 120.54, 115.67, 112.61, 112.18, 111.52, 59.76, 58.13, 55.20, 54.97, 53.25, 52.98, 32.03, 29.61, 27.26, 25.77, 25.73, 25.47, 22.71. ESI-MS m/z 569.2 [M + H].

**2-(4-(3-(5-chloro-1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-phenylacetamide (11j)**

White solid (125 mg, yield 33.2%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.08\) (s, 1H), 7.59 (d, \(J = 7.8\) Hz, 1H), 7.40 (dd, \(J = 5.0, 1.6\) Hz, 3H), 7.34 (d, \(J = 8.1\) Hz, 1H), 7.17 (t, \(J = 7.4\) Hz, 1H), 7.12–7.08 (m, 3H), 7.01 (s, 1H), 4.59 (tt, \(J = 12.2, 3.5\) Hz, 1H), 2.99–2.95 (m, 2H), 2.77–2.54 (m, 12H), 1.82 (d, \(J = 10.6\) Hz, 2H), 1.72 (d, \(J = 13.4\) Hz, 2H), 1.56 (d, \(J = 12.5\) Hz, 1H), 1.43–1.33 (m, 3H), 1.03 (ddd, \(J = 25.1, 12.5, 3.4\) Hz, 2H). ESI-MS m/z 445.4 [M + H].

**2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-N-cyclohexyl-N-(2-methoxyphenyl)acetamide (11k)**

White solid (130 mg, yield 44.4%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.55\) (d, \(J = 7.9\) Hz, 1H), 7.48 (dd, \(J = 11.4, 4.4\) Hz, 1H), 7.33 (d, \(J = 8.1\) Hz, 1H), 7.21 (dd, \(J = 13.7, 5.3\) Hz, 2H), 7.14–7.04 (m, 3H), 7.00 (tt, \(J = 7.4\) Hz, 1H), 4.43 (tt, \(J = 11.8, 3.3\) Hz, 1H), 3.87 (d, \(J = 8.0\) Hz, 13H), 3.28–3.17 (m, 2H), 2.89 (tt, \(J = 7.0\) Hz, 2H), 2.24–2.14 (m, 2H), 1.95 (d, \(J = 11.2\) Hz, 1H), 1.80 (t, \(J = 10.7\) Hz, 2H), 1.69 (d, \(J = 13.1\) Hz, 1H), 1.58 (d, \(J = 12.8\) Hz, 1H), 1.43–1.19 (m, 3H), 1.05–0.80 (m, 2H). ESI-MS m/z 489.4 [M + H].
Results and Discussion

Pharmacophore-based virtual screening

The obtained pharmacophore model was shown in Figure 1A. As a result of our virtual screening protocol, 16 compounds were selected to purchase and submitted to pharmacological experiments (Tables S1 and S2). To our delight, three of them revealed moderate D3R activities. Their chemical structures and corresponding binding assays were summarized in Figure 1B and Table 1. Compound 11a, with a high fit value and a core structure of indolepropylamine and N-phenylacetamide, matches the pharmacophore model quite well (Figure S1) and represents a novel class of D3R ligands. It was identified to bind hD3R with 2161 nM affinity and was thus chosen as the lead compound for further optimization.

Rational design and structure-activity relationships

The structural analysis and the ligand-receptor interaction elucidated by molecular docking were investigated to guide the structure modification and optimization of compound 11a. Compound 11a is characterized by an indole head, a linear alkyl linker and the N-phenylacetamide tail connected to a piperazine moiety. To rationalize the design of the derivatives, the structural model of the complex D3R-11a was constructed by combining molecular docking and all available experimental data (Figure 2A). Three important interactions were identified in the D3R-11a model: the conserved salt bridge interaction between the protonated nitrogen atom (N1) of 11a and the carbonylate group of D3R; the cation-π contact between the protonated nitrogen atom and F6.51; and the hydrogen bond formed by the oxygen atom of carboxyl group in 11a and Y7.35 in D3R. It indicates that the piperazine ring and the carboxyl group are critical to the activity, as these indispensable interactions determined the binding orientation of the head down into the orthosteric binding site (OBS; enclosed by TM-III, -V, -VI, -VII) and the tail up to the second binding pocket (SBP; comprised of ECL2 and the extracellular segments of TM-III, -VII) (18). The hollow space was found in the OBS and SBP (Figure 2A), suggesting that 11a could be optimized by appending larger groups in the head and tail or lengthening the linker. Therefore, a series of IBA derivatives were designed, synthesized, and bioassayed for D3R activity with the aim to improve the potency of this series of ligands (Table 2).

As our lead compound 11a already carries an aromatic head and a bulky tail, the length of the linker was first considered to be incremented to fill the hollow space in the active site and 11p was obtained, providing a delightful improvement in the binding affinity (Ki = 636 nM). To verify our predicted binding mode, the effect of the length of the linker (n = 2-4) on affinity was further examined. Indeed, the affinity of 11o with a 4-carbon linker is superior to those of 11m with 2-carbon and/or 11k with 3-carbon linkers. As predicted, it proved that the longer linker could...
allow the molecule to extend to the hollow space in the OBS and SBP, thus the affinity was increased. In addition, the affinity of 11a with larger cyclohexyl group on the tail was higher than those of 11b, 11c, and 11d with smaller groups. It further confirms that the tight binding in OBS and SBP by introducing proper functional groups to the molecules could improve the affinity to D3R.

Compounds 11f, 11h, and 11g were next prepared to evaluate the influence of different halogens at 5-position of AB.
the indole head on binding affinity. The results indicated that all halogenated derivatives displayed improved binding affinity, and the chlorinated compound performs better than the fluorinated or brominated one (11f > 11a > 11h) when the length of linker is 3 (n = 3). In addition, when n = 4, the binding affinity of 11p with a 5-fluorine on indole is superior to 11n with a hydrogen, demonstrating the important role played by halogens in D3R ligand design.

We noted that the o-methoxy group in the phenyl ring of the tail plays a positive role in the binding of D3R (19,20). Therefore, a set of o-methoxy substituted derivatives (11e - g, i, k, m, o, q) of compounds 11a, - f, - h, - j, - l, - n, - p were designed and synthesized. Generally, introduction of the o-methoxy group positively contributed to the affinity to D3R. Close examination indicated that the o-methoxy substituent in the phenyl ring played an optimal role when a 4-carbon linker was present. Among these molecules, 11q emerged as the most potent ligand, displaying fourfold increase in binding affinity (Ki = 124 nm) compared with 11p. Of interest, [35S]GTPγS binding assay of compound 11q showed that it produced antagonistic activity at D3R, and the calculated antagonistic potencies (IC50) of 11q were 828 nm for the D3R (Table 3).

Molecular docking study was promptly conducted to investigate the binding mode of 11q with D3R to reveal the role played by the o-methoxy group and the structural features responsible for the increased affinity (Figure 2B). Consistent with the binding mode of D3R with the lead 11a (without the o-methoxy group), the salt bridge formed with D3.32, the cation-π interaction with F6.51, and the hydrogen bond with Y7.35 were maintained. When n = 4, 11q extends to the hollow space of the OBS and SBP, and the hydrophobic interactions in the hydrophobic cavity in OBS, in contact with hydrophobic residues V3.33, V5.39, W6.48, F6.51, F6.52, H6.55 (Figure 3B). In the meantime, the N-phenylacetamide tail extends to the SBP and participates in the hydrophobic interactions with I183 in ECL2 and V2.61, L2.64, F3.28, V3.29, Y7.43 (Figure 3B). Our predicted binding mode is consistent with the site-directed mutagenesis studies, which indicated that the mutation of F6.51 and V2.61 caused significant reduction in binding affinities of D3R ligands (22,23). Thus, the hydrophobic interactions in the OBS and SBP, and the hydrogen-bond interactions formed with the key residues D3.32 and Y7.35 mostly contribute to the activity to D3R.

It is worth noting that the designed series of compounds have high selectivity to D3R. As D2R and D3R share high sequence identity in the active site, we sought to identify the structural determinants for such a high selectivity over the other dopamine receptor subtypes. Sequence alignment of the key residues involved in the binding of 11q was conducted within D3R, D2R, and D1R (Figure 3A). As D1R and D3R share less sequence identity, the non-conserved residues at positions 7.35, 7.43, and 6.55 in D1R make collision with 11q, hence, it is not difficult to understand that this series of compounds display no activity for hD1R. Whereas in the case of D2R and D3R, which have almost identical residues in the binding pocket, what contributes to their selectivity? Only three of 22 residues are not conserved in the 11q-contact binding region of D3R/erts (21–23) and well account for the higher affinity of 11q.

Taking the SAR analyses together, we looked deep into the D3R-11q model to find the intrinsic factor that gives this novel series of compounds a unique pharmacological activity toward D3R. The D3R-11q model shows that the interactions formed by the protonated nitrogen of piperazine and the negatively charged D3.32, and the amide moiety with Y7.35 (Figure 2B) anchor the binding orientation of 11q. It makes the indole head down to the hydrophobic cavity in OBS, in contact with hydrophobic residues V3.33, V5.39, W6.48, F6.51, F6.52, H6.55 (Figure 3B). In the meantime, the N-phenylacetamide tail extends to the SBP and participates in the hydrophobic interactions with I183 in ECL2 and V2.61, L2.64, F3.28, V3.29, Y7.43 (Figure 3B). Our predicted binding mode is consistent with the site-directed mutagenesis studies, which indicated that the mutation of F6.51 and V2.61 caused significant reduction in binding affinities of D3R ligands (22,23). Thus, the hydrophobic interactions in the OBS and SBP, and the hydrogen-bond interactions formed with the key residues D3.32 and Y7.35 mostly contribute to the activity to D3R.

Table 3: [35S]GTPγS binding assays of compound 11q for the D3R

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11q</td>
<td></td>
<td>827.63</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>–</td>
<td>248.02</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>25.673</td>
<td>–</td>
</tr>
</tbody>
</table>

*35S]GTPγS binding activity could not be detected.
selectivity of this series of D3R ligands over D2R. Heinrich et al., reported a series of indolebutylamines (IBAs) and phenylpiperazine derivatives showing high activities against 5-HT1AR and D2R (24,25). Our lead compound 11a obtained from virtual screening bears N-phenylacetamide tail, and it displays different pharmacological profile from phenylpiperazine derivatives and shows high selectivity toward D3R instead. The introduction of amide group causes the lost of D2R activity.

**Conclusions**

In summary, a new type of compounds with the core structure of IBA and N-phenylacetamide were identified as high selective D3R ligands over D1R and D2R by combining a series of computational approaches, the synthetic chemistry, and binding assays. Compound 11q displays relatively high binding affinity ($K_i = 124\, \text{nM}$), and it was evaluated as D3R antagonist by $[^{35}\text{S}]\text{GTP}_S$ binding assay. The results indicated that a fluorine substituted indole head, a 4-carbon linker, an $\alpha$-methoxy of the phenyl ring, and a cyclohexyl substituent on the amide mainly contributed to the affinity to D3R. In-depth analysis of the binding mode of 11q with D3R constructed by molecular docking not only elucidated SARs in detail, but also recognized the molecular determinants critical for D3R affinity as well as selectivity. The carboxylate group of D3.32 formed conserved salt bridge interaction with the protonated nitrogen atom (N1) of the piperazine ring, which is a common feature among D3R ligands. Y7.43 is engaged in the hydrogen-bond interaction with $\alpha$-methoxy of the phenyl ring. F6.52 forms $\pi-\pi$-stacking interaction with indole ring. And together with D3.32, Y7.35, and F6.51, they were recognized as key residues for binding of the designed compounds. S182 in ECL2 is involved in the ligand’s entrance to D3R and was found to be the molecular structural determinant for the selectivity of D3R over D2R. As these three residues have not obtained much attention in the previously reported studies on selective D3R ligands, this study may give a hint in the design of novel selective D3R ligands. Further optimization of this series of compounds is ongoing.

**Figure 3:** (A) The sequence alignment in the binding pocket of D1R, D2R, and D3R; (B) Predicted binding mode of compound 11q (yellow sticks) with D3R (blue cartoon), and D2R (gray cartoon) is superimposed to D3R.

**Scheme 1:** Syntheses of important intermediates 3a-d and 6b. Reagents and conditions: (a) dihydropyran, DMA/4% H$_2$SO$_4$; (b) CBr$_4$, PPh$_3$, DCM; (c) TMSCN, TBAF, THF; (d) NaOH/EtOH; (e) LiAlH$_4$, THF; (f) CBr$_4$, PPh$_3$.

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References


Scheme 2: Syntheses of compounds 11a–q. Reagents and conditions: (a) chloroacetylchloride, TEA, DCM; (b) Boc-piperazine, K2CO3, CH3CN; (c) TFA/DCM; (d) 3a–3d, 6b, K2CO3, CH3CN.


Note


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Compound 11a fitted to the four-feature pharmacophore model.

Figure S2. Key residues in the binding pocket of D3R (a) and D2R (b) were shown in spheres.

Table S1. Summary of the molecular properties, fit values, and gold scores of the selected 16 compounds.

Table S2. Binding affinities of the selected 16 compounds for dopamine D1, D2, and D3 receptors.

Synthesis, Biological Evaluation, and Molecular Modeling

