DOI 10.5012/bkcs.2011.32.7.2449

Synthesis and Biological Evaluation of Focused Isoxazolylpiperidinylpiperazine Library for Dopamine D_3 and D_4 Receptor Antagonists

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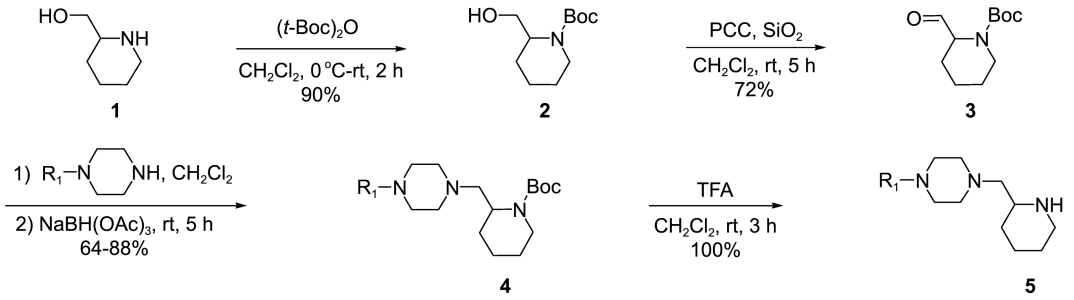
Received April 7, 2011, Accepted May 9, 2011

Key Words : Dopamine receptor, Isoxazolylpiperidinylpiperazine, Antagonist, GPCR

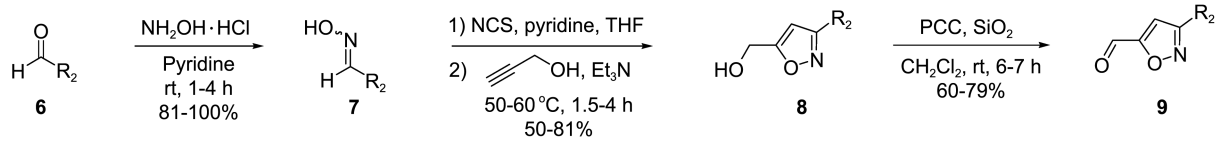
The dopamine receptor system plays a key role in numerous neuropsychiatric and neurological disorders. The D_3 receptor, first described in 1990,[1] is a member of the D_2-like receptor family, and possesses about 50% overall homology to the D_2 receptor subtype.[2] D_3 receptors are localized in the limbic areas of the brain, specifically in the group of neural granule cells located within the ventral striatum in the brains of most animals.[3] In the field of medicinal chemistry extensive efforts have been made to explore potent and selective ligands for dopamine D_3[1] or D_4[4] receptor for the discovery of antipsychotic drugs.[5] Currently, much research effort is being focused on the discovery of highly selective dopamine D_4 receptor ligands.[6] The reason for the interest in this area is derived from the possible involvement of D_4 receptor in schizophrenia.[7-10] In connection with search for selective ligands for various GPCRs, we have recently reported[11] the design and synthesis of arylpiperazine derivative libraries as dopamine receptor antagonist and serotonine receptor antagonist with isoxazole rings. In continuation of our efforts to search for selective GPCR ligands, we envisaged a library of piperidinylpiperazine derivatives with isoxazole rings as a plausible candidate for the purpose. Herein we wish to report the construction of a small focused library of isoxazolylpiperidinylpiperazines and their binding affinities for dopamine D_3 and D_4 receptors.

The synthesis of isoxazolylpiperidinylpiperazine derivatives is shown in Schemes 1, 2 and 3. The synthetic strategy to these compounds involved reductive amination between two building blocks **5** and **9**.

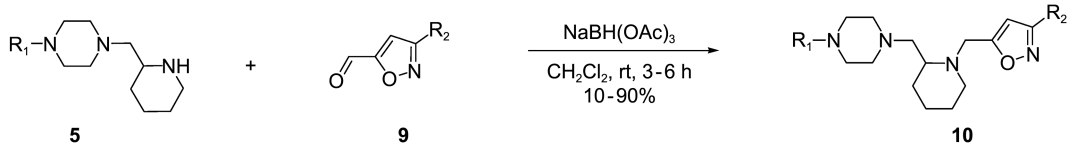
For the acquisition of building block **5**, the amino group of



Scheme 1. Synthesis of building block **5**.

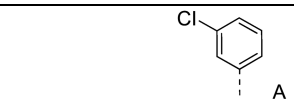
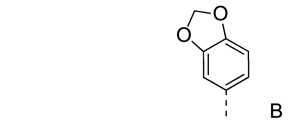
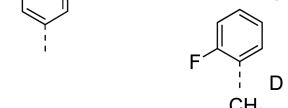
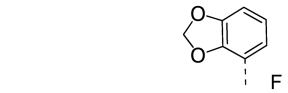
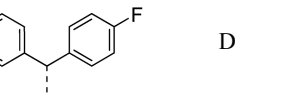


Scheme 2. Synthesis of building block **9**.



Scheme 3. Synthesis of scaffold (isoxazolylpiperidinylpiperazine).

Table 1.

Entry	R^1	R^2	Compound	Yield[a] %
1		A	**10-1**	53.5
2		B	**10-2**	56.8
3		C	**10-3**	64.6
4		D	**10-4**	18.2
5		E	**10-5**	67.9
6		F	**10-6**	75.0
7		A	**10-7**	54.9
8		B	**10-8**	51.3
9		C	**10-9**	65.4
10		D	**10-10**	28.9
11		E	**10-11**	36.7
12		F	**10-12**	20.8
13		B	**10-13**	13.7
14		D	**10-14**	11.0
15		A	**10-15**	13.0
16		B	**10-16**	58.0
17		C	**10-17**	70.8
18		D	**10-18**	16.0
19		E	**10-19**	61.9
20		F	**10-20**	13.0
21		D	**10-21**	73.7
22		A	**10-22**	45.2
23		B	**10-23**	10.0
24		D	**10-24**	23.3
25		D	**10-25**	87.1
26		D	**10-26**	63.5
27		D	**10-27**	71.9

[a]Isolated yield after column chromatography.

piperidin-2-ylmethanol **1** was first protected by $(t\text{-Boc})_2O$ in dichloromethane at 0 °C and then at rt to give compound **2** in 90% yield. Compound **2** was then oxidized with PCC and SiO_2 in dichloromethane at rt for 5 h into aldehyde **3** in 72% yield. The aldehyde **3** was reacted with piperazine derivatives using $NaBH(OAc)_3$ in presence of 4 Å molecular sieve over 5 h to afford compounds **4** in 64-88% yields. Finally building block **5** was obtained in quantitative yield by a simple deprotection reaction with CF_3CO_2H in dichloromethane at rt.

With building block **5** in hand, we moved to our next building block **9**. Treatment of aldehydes **6** with hydroxylamine hydrochloride and pyridine at rt for 1-4 h gave oximes **7** in 81-100% yields. Isoxazole nucleus was generated by two-step sequence of nitrile oxide cycloaddition reaction which involved (1) chlorination of **7** with *N*-chlorosuccinimide and (2) *in situ* generation of nitrile oxide and cycloaddition with propargyl alcohol. In this way isoxazole alcohols **8** were obtained in 50-81% yields. Subsequent PCC oxidation of **8** afforded building block **9** in 60-79% yields.

Table 2. *In vitro* biological data of isoxazolylpiperidinylpiperazine compounds

Entry	Compound	IC_{50}(μM) for D_3 receptor	IC_{50}(μM) for D_4 receptor
1	**10-1**	2.6	4.8
2	**10-2**	> 10	3.8
3	**10-3**	3.1	1.1
4	**10-4**	7.3	6.9
5	**10-5**	8.2	3.5
6	**10-6**	4.1	3.2
7	**10-7**	4.8	8.5
8	**10-8**	> 10	> 10
9	**10-9**	6.2	2.7
10	**10-10**	8.6	6.8
11	**10-11**	5.3	1.4
12	**10-12**	0.7	1.4
13	**10-13**	3.0	3.2
14	**10-14**	4.8	7.8
15	**10-15**	2.2	> 10
16	**10-16**	> 10	> 10
17	**10-17**	5.0	> 10
18	**10-18**	4.8	> 10
19	**10-19**	> 10	8.9
20	**10-20**	> 10	5.5
21	**10-21**	> 10	2.0
22	**10-22**	> 10	> 10
23	**10-23**	4.8	> 10
24	**10-24**	9.5	9.1
25	**10-25**	1.6	> 10
26	**10-26**	1.9	9.5
27	**10-27**	6.3	2.0
	Haloperidol	0.062	-
	Spiperone	-	0.013

Employing the developed combinatorial protocol,[12] synthesis of isoxazolylpiperidinylpiperazine derivatives **10** was finally accomplished by simple reductive amination reaction between building blocks **5** and **9** using $NaBH(OAc)_3$[13] (Scheme 3). Thus prepared isoxazolylpiperidinylpiperazine products are shown in Table 1. Structures and purities of products were confirmed by ^1H NMR, ^{13}C NMR, IR, MS spectroscopic analysis and HPLC.

Thus, a small focused library of diverse compounds **10** was constructed and tested for binding affinities[14] for dopamine D_3 and D_4 receptors. As shown in Table 2 most of the compounds exhibited IC_{50} values below 5 μM which will be the key factor for further research in this field. Compound **10-3** (entry 3, when R_1 is phenyl and R_2 is 2-methoxyphenyl) showed a good activity for dopamine D_3 (3.1 μM) and D_4 (1.1 μM). Among the prepared compounds, compound **10-12** (entry 12, when R_1 is 2-fluorophenyl and R_2 is benzo-1,3-dioxole) showed best binding affinity for dopamine D_3 receptor (0.7 μM) and good activity towards D_4 receptor (1.4 μM). Compound **10-11** (entry 11), in which R_2 of compound **10-12** is replaced by 4-methylphenyl, also exhibited good binding activities but selective affinity for D_4 receptor (1.4 μM) over D_3 receptor (5.3 μM). When R_1 is 3-chlorophenyl a dramatic change of activity was observed as follows: While compound **10-15** (entry 15, when both R_1 and R_2 are 3-chlorophenyl) showed good activity only towards dopamine D_3 receptor (2.2 μM) compared to dopamine D_4 receptor (>10 μM), compound **10-20** (entry 20, when R_1 is 3-chlorophenyl but R_2 is benzo-1,3-dioxole) exhibited completely opposite results (>10 μM for dopamine D_3 receptor and 5.5 μM for dopamine D_4 receptor). It is also noteworthy that compound **10-25** (entry 25), in which R_1 is bis(fluorophenyl)methyl and R_2 is 2-fluorophenyl, showed good activity (1.6 μM) for dopamine D_3 receptor. In our previous research[11a] some piperazinylalkylisoxazole compounds had excellent binding affinities. We envisaged that introduction of any heterocycle with nitrogen in between piperazine and isoxazole may increase the binding affinity. However such notion proved unsuccessful in case of the heterocycle being piperidine as the binding results indicated. Nonetheless it is interesting to note that fluoro compounds show better binding affinities for dopamine D_3 and dopamine D_4 receptors than other derivatives.

In summary we have synthesized a small focused library of isoxazolylpiperidinylpiperazine compounds and tested them *in vitro*[14] for dopamine D_3 and D_4 receptor binding affinities. The results might be helpful for the future research in the design and synthesis of potent and selective ligands for dopamine D_3 or D_4 receptors.

Experimental Section

General Procedure for Synthesis of Building Block 5 (when R_1 = Ph). To a solution of compound **3** (481 mg, 2.25 mmol) in dichloromethane (20 mL) at rt, 1-phenylpiperazine (444 μL, 2.92 mmol) and 4 Å molecular sieve were added. After the mixture being stirred for 5 min, $NaBH(OAc)_3$ (1.43 g, 6.74 mmol) was added and the mixture was stirred for 5 h. Saturated $NaHCO_3$ solution was added and the mixture was extracted with ethyl acetate (50 mL × 3). The organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (Hexane:EtOAc = 4:1) to give the compound **4** (when R_1 = Ph, 531 mg, 66%).

To a solution of thus prepared compound **4** (447 mg, 1.24 mmol) in dichloromethane at rt, trifluoroacetic acid (958 μL, 12.4 mmol) was added. After the mixture being stirred for 3 h, saturated $NaHCO_3$ solution was added and the mixture was extracted with ethyl acetate (50 mL × 3). The organic layers were dried over anhydrous $MgSO_4$ and concentrated. Removal of organic solvents from the residue gave the building block **5** (320 mg, 100%).

^1H NMR (300 MHz, MeOD) δ 7.23 (t, 2H, J = 8.0 Hz), 6.96 (d, 2H, J = 7.8 Hz), 6.83 (t, 1H, J = 7.3 Hz), 3.38-3.35 (m, 1H), 3.25-3.21 (m, 1H), 3.20 (t, 4H, J = 5.0 Hz), 2.98-2.87 (m, 1H), 2.82-2.72 (m, 2H), 2.63-2.49 (m, 3H), 2.46 (dd, 1H, J = 13.5 Hz, J = 4.0 Hz), 1.88 (d, 3H, J = 10.4 Hz), 1.73-1.28 (m, 3H). ^{13}C NMR (75 MHz, MeOD) δ 152.7, 130.0, 121.0, 117.5, 61.1, 54.9, 54.6, 50.4, 45.9, 28.5, 24.1, 23.1. IR (KBr) 1687, 1598, 1505, 1235, 1200, 1172, 1128, 756 cm^{-1}. ESI-MS 260.39 (M+1).

Other [piperazin-1-ylmethyl]piperidine derivatives were synthesized analogously and identified with the ^1H NMR, ^{13}C NMR, IR, and MS spectroscopies.

General Procedure for Synthesis of Building Block 9 (when R_2 = 2-methoxyphenyl). To a solution of 2-methoxybenzaldehyde oximes **7** (when R_2 = 2-methoxyphenyl, 302 mg, 2.00 mmol) in THF (7 mL) at rt under nitrogen atmosphere, *N*-chlorosuccinimide (534 mg, 2.00 mmol) and pyridine (16.0 μL, 0.20 mmol) were added. After being stirred for 1 h at 60 °C, the mixture was cooled to rt and a solution of propargyl alcohol (116 μL, 2.00 mmol) in THF (2 mL) and triethylamine (335 μL, 2.40 mmol) in THF (2 mL) were added dropwise successively. After the mixture being stirred at 50 °C for 3 h, saturated $NaHCO_3$ solution was added and the mixture was extracted with ethyl acetate (50 mL × 3). The organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (Hexane:EtOAc = 1:1) to give the isoxazole alcohol **8** (333 mg, 81%).

To a solution of thus prepared alcohol **8** (when R_2 = 2-methoxyphenyl, 261 mg, 1.27 mmol) in dichloromethane (10 mL) at rt, pyridinium chlorochromate (548 mg, 2.54 mmol) and silica gel (230-400 mesh, 548 mg) were added. After being stirred for 6 h, the mixture was filtered through Florisil column. The filtrate was concentrated and the residue was purified by flash column chromatography (Hexane:EtOAc = 2:1) to give the building block **9** (when R_2 = 2-methoxyphenyl, 195 mg, 76%).

^1H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.88 (dd, 1H, J = 7.7 Hz, J = 1.7 Hz), 7.44 (s, 1H), 7.41-7.35 (m, 1H), 7.01-6.92 (m, 2H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 165.3, 160.7, 157.2, 132.1, 129.4, 121.1, 116.4, 111.5, 110.9, 55.6. IR (KBr) 1697, 1602, 1501, 1472, 1296, 1267,

1252, 1023, 763, 749 cm^{-1}.

Other isoxazole aldehyde derivatives were synthesized analogously and identified with the ^1H NMR, ^{13}C NMR, and IR spectroscopies.

General Procedure for Synthesis of Isoxazolylpiperidinylpiperazine derivatives 10 (when R_1 = Ph and R_2 = 2-methoxyphenyl). To a solution of building block **5** (when R_1 = Ph, 45.0 mg, 0.17 mmol) and building block **9** (when R_2 = 2-methoxyphenyl, 32.0 mg, 0.16 mmol) in dichloromethane (3 mL) at rt, 4 Å molecular sieve was added. After the mixture being stirred for 10 min, $NaBH(OAc)_3$ (100 mg, 0.47 mmol) was added and the mixture was stirred for 5 h. Saturated $NaHCO_3$ solution was added and the mixture was extracted with ethyl acetate (5 mL × 5). The organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (EtOAc) to give the isoxazolylpiperidinylpiperazine **10** (when R_1 = Ph and R_2 = 2-methoxyphenyl, 45.6 mg, 65%).

^1H NMR (400 MHz, MeOD) δ 7.77 (dd, 1H, *J* = 7.7 Hz, *J* = 1.7 Hz), 7.42 (t, 1H, *J* = 7.0 Hz), 7.19 (dd, 2H, *J* = 8.7 Hz, *J* = 7.3 Hz), 7.08 (d, 1H, *J* = 8.3 Hz), 7.01 (td, 1H, *J* = 7.5 Hz, *J* = 0.9 Hz), 6.91 (d, 2H, *J* = 7.9 Hz), 6.83-6.77 (m, 2H), 4.20 (d, 1H, *J* = 15.4 Hz), 4.00 (d, 1H, *J* = 15.3 Hz), 3.82 (s, 3H), 3.11 (t, 4H, *J* = 5.0 Hz), 3.93-3.85 (m, 1H), 2.79 (dd, 1H, *J* = 13.0 Hz, *J* = 5.8 Hz), 2.65-2.57 (m, 2H), 2.57-2.44 (m, 3H), 2.33 (td, 1H, *J* = 11.3 Hz, *J* = 3.0 Hz), 2.55 (dd, 1H, *J* = 13.0 Hz, *J* = 5.1 Hz), 1.84-1.73 (m, 1H), 1.73-1.63 (m, 1H), 1.63-1.54 (m, 1H), 1.54-1.45 (m, 1H), 1.36-1.23 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 161.3, 158.7, 152.2, 132.6, 130.2, 130.0, 121.9, 120.9, 118.9, 117.3,112.9, 106.5, 63.8, 58.3, 56.1, 55.0, 54.4, 50.5, 50.0, 32.0, 26.2, 24.6. IR (KBr) 2923, 2818, 1599, 1505, 1495, 1471, 1456, 1252, 1152, 1117, 1025, 1010, 756, 739 cm^{-1}. ESI-MS 447.43 (M+1).

Other isoxazolylpiperidinylpiperazine derivatives were synthesized analogously and identified with the ^1H NMR, ^{13}C NMR, IR, and MS spectroscopies.

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14. The generated isoxazolylpiperidinylpiperazine analogues were evaluated *in vitro* for dopamine D_3-D_4 receptors binding affinity by measuring their ability to displace radioligands ([^{3}H]spiperone for D_4, [^{3}H]YM-09151-2 for D_3) from the cloned human dopamine receptors D_3 and $D_{4.2}$ which were expressed in CHO cells, respectively.