http://dx.doi.org/10.5012/bkcs.2012.33.12.4227

Isoxazolylpyrrolidinylpiperazine Ligands, a New Class for Dopamine D_3 and D_4 Receptor Antagonists

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Received August 29, 2012, Accepted September 13, 2012

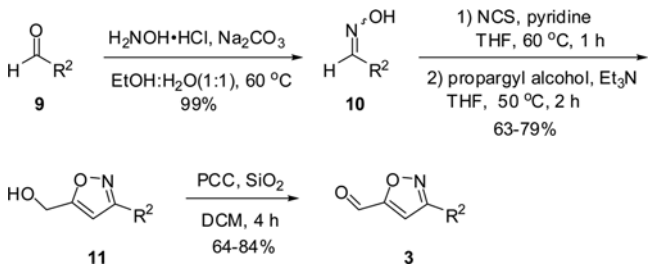
Key Words : Dopamine receptor, Antagonist, Isoxazolylpyrrolidinylpiperazine, Reductive amination

Since the dopamine receptor system plays an important role in the development of neurological disorders like schizophrenia,[1-5] extensive efforts have been made to explore potent and selective ligands for dopamine receptors for the discovery of antipsychotic drugs.[6-10] In connection with search for potent ligands for dopamine receptors, we have studied the construction and biological activities of libraries of arylpiperazine derivatives with isoxazole rings.[11,12] Recently we reported the design and synthesis of isoxazolylpiperidinylpiperazine derivatives having 6-membered nitrogen heterocycle, piperidine in between piperazine and isoxazole.[13] To investigate the structure-activity relationship, the new isoxazolylpyrrolidinylpiperazine derivatives, which possess 5-membered ring system, pyrrolidine in between piperazine and isoxazole, were designed and synthesized. In this paper, we report the construction of a small focused library of isoxazolylpyrrolidinylpiperazines and their binding affinities for dopamine D_3 and D_4 receptors.

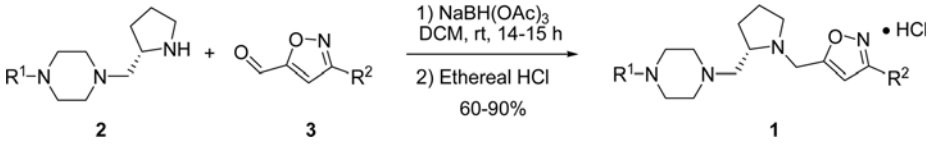
The synthesis of isoxazolylpyrrolidinylpiperazine derivatives is shown in Schemes 1, 2 and 3. The key synthetic strategy to these compounds involved coupling between two building blocks **2** and **3** using reductive amination reaction.

Preparation of building block **2** is described in Scheme 2. Protection of the amino group of pyrrolidin-2-ylmethanol **4** with $(t\text{-Boc})_2O$ in dichloromethane at rt for 1 h provided compound **5** in 90% yield. Oxidation of **5** with PCC and SiO_2 in dichloromethane at rt for 3 h gave aldehyde **6** in 71% yield. Reductive amination of the aldehyde **6** with piperazine derivatives **7** using $NaBH(OAc)_3$ in presence of 4 Å molecular sieve over 6 h afforded compounds **8** in 60-92% yields. Deprotection of **8** with CF_3CO_2H in dichloromethane at rt furnished building block **2** in 87-99% yields.

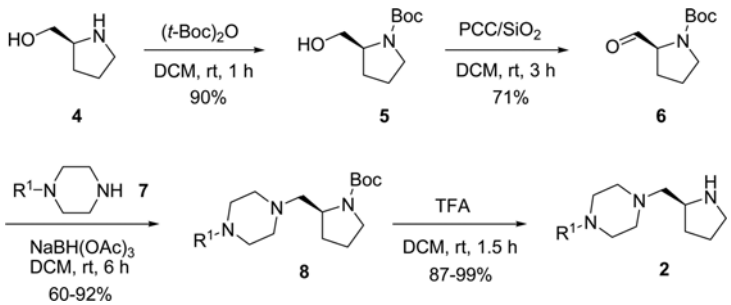
Synthesis of building block **3** is shown in Scheme 3. Treatment of aldehydes **9** with hydroxylamine hydrochloride and Na_2CO_3 at 60 °C for 1-6 h gave oximes **10** in 99% yields. Isoxazole ring system was then installed by two-step sequence of nitrile oxide cycloaddition reaction which



Scheme 3. Synthesis of building block **3**.



Scheme 1. Synthesis of isoxazolylpyrrolidinylpiperazine derivatives.



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

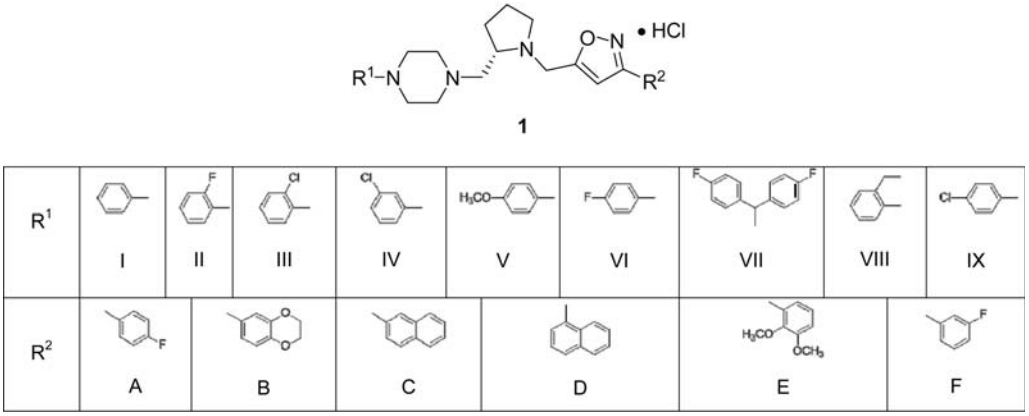


Figure 1. Library of isoxazolylpyrrolidinylpiperazine derivatives.

involved (1) chlorination of **10** with *N*-chlorosuccinimide and (2) *in situ* generation of nitrile oxide and cycloaddition with propargyl alcohol. In these ways isoxazole alcohols **11** were obtained in 63-79% yields. PCC oxidation of **11** afforded building block **3** in 64-84% yields.

Final assembly of isoxazolylpyrrolidinylpiperazine derivatives **1** was conducted by simple reductive amination reaction between building blocks **2** and **3** using $NaBH(OAc)_3$[14] and subsequent treatment with ethereal HCl, employing the developed combinatorial protocol[12] (Scheme 1 & Table 1). Thus, a small focused library of well-characterized isoxazolylpyrrolidinylpiperazine members was constructed (Figure 1). Structures and purities of products were confirmed by ^1H NMR, ^{13}C NMR and IR spectroscopic analysis.

The constructed isoxazolylpyrrolidinylpiperazine library members were tested for binding affinities[15] towards dopamine D_3 and D_4 receptors. As shown in Table 1, most of the compounds exhibited IC_{50} values less than 10 μM. For example, compound **1-17** (entry 17, when R^1 is 2-chlorophenyl and R^2 is 2,3-dimethoxyphenyl) showed a good activity for both dopamine D_3 and D_4 receptors (1.1 μM and 2.2 μM, respectively). Among the library members, compound **1-42** (entry 42, when R^1 is bis(4-fluorophenyl)methyl and R^2 is 3-fluorophenyl) displayed best binding affinity for dopamine D_3 receptor (0.22 μM) and compound **1-10** (entry 10, when R^1 is 2-fluorophenyl and R^2 is 1-naphthyl) exhibited best binding affinity for dopamine D_4 receptor (1.2 μM). As far as the selectivity of binding affinity for receptors is concerned, compound **1-42** (entry 42, *vide supra*) showed highest selectivity of binding affinity for D_3 receptor (0.22 μM) over D_4 receptor (> 10 μM). Compounds **1-36** and **1-37** (entries 36 and 37, when R^1 and R^2 are 4-fluorophenyl and 3-fluorophenyl, and bis(4-fluorophenyl)methyl and phenyl, respectively) also displayed high selectivity (0.46 μM and 0.25 μM, respectively) for dopamine D_3 receptor over D_4 receptor (> 10 μM). As for the selectivity of binding affinity for D_4 receptor over D_3 receptor, only compound **1-25** (entry 25, when R^1 is 4-methoxyphenyl and R^2 is 4-fluorophenyl) exhibited slightly better activity towards dopamine D_4 receptor (3.5 μM) than to dopamine D_3 receptor (> 10 μM). In addition, compounds **1-10**, **1-16**, and **1-28** (entries 10, 16, and 28, when R^1 are 2-fluorophenyl, 2-chlorophenyl, and 4-methoxyphenyl, respectively and R^2 is 1-naphthyl) showed the potent activity towards both dopamine D_3 and D_4

Table 1. Yields of Reductive Amination and Binding Affinities of Isoxazolylpyrrolidinylpiperazines towards Dopamine D_3 and D_4 Receptors

Entry	R^1	R^2	Compound	Time (h)	Yield (%)	IC_{50} (μM) for D_3 receptor	IC_{50} (μM) for D_4 receptor
1	I	A	**1-1**	14	71	> 10	8.7
2		B	**1-2**	15	82	> 10	> 10
3		C	**1-3**	14	80	> 10	> 10
4		D	**1-4**	15	76	7.3	> 10
5		E	**1-5**	14	81	4.9	> 10
6		F	**1-6**	14	79	4.0	> 10
7	II	A	**1-7**	14	70	5.3	2.3
8		B	**1-8**	15	63	6.3	2.5
9		C	**1-9**	14	75	1.6	6.8
10		D	**1-10**	15	83	3.4	1.2
11		E	**1-11**	14	80	2.1	> 10
12		F	**1-12**	14	75	1.4	9.4
13	III	A	**1-13**	14	73	3.7	2.5
14		B	**1-14**	15	71	2.8	3.5
15		C	**1-15**	14	78	1.6	> 10
16		D	**1-16**	15	74	1.0	4.5
17		E	**1-17**	14	70	1.2	2.2
18		F	**1-18**	14	60	1.8	6.6
19	IV	A	**1-19**	14	63	3.9	3.8
20		B	**1-20**	15	71	9.1	9.4
21		C	**1-21**	14	71	> 10	> 10
22		D	**1-22**	15	79	1.7	> 10
23		E	**1-23**	14	83	> 10	> 10
24		F	**1-24**	14	65	3.0	> 10
25	V	A	**1-25**	14	64	> 10	3.5
26		B	**1-26**	15	88	> 10	> 10
27		C	**1-27**	14	70	1.1	> 10
28		D	**1-28**	15	68	5.1	2.4
29		E	**1-29**	14	79	4.8	6.9
30		F	**1-30**	14	81	1.0	> 10

Table 1. Continued

Entry	R^1	R^2	Compound	Time (h)	Yield (%)	IC_{50} (μM) for D_3 receptor	IC_{50} (μM) for D_4 receptor
31	VI	A	**1-31**	14	91	5.5	2.3
32		B	**1-32**	15	89	> 10	> 10
33		C	**1-33**	14	84	2.9	> 10
34		D	**1-34**	15	71	7.0	> 10
35		E	**1-35**	14	80	> 10	> 10
36		F	**1-36**	14	77	0.46	> 10
37	VII	A	**1-37**	14	68	0.25	> 10
38		B	**1-38**	15	66	3.6	> 10
39		C	**1-39**	14	70	> 10	> 10
40		D	**1-40**	15	85	4.0	> 10
41		E	**1-41**	14	77	1.5	> 10
42		F	**1-42**	14	81	0.22	> 10
43	VIII	A	**1-43**	14	77	0.60	4.3
44		B	**1-44**	15	83	8.2	> 10
45		C	**1-45**	14	78	2.1	8.4
46		D	**1-46**	15	80	2.2	7.3
47		E	**1-47**	14	82	2.8	> 10
48		F	**1-48**	14	84	0.27	4.6
49	IX	A	**1-49**	14	84	8.7	4.9
50		B	**1-50**	15	67	> 10	> 10
51		C	**1-51**	14	76	> 10	> 10
52		D	**1-52**	15	86	> 10	> 10
53		E	**1-53**	14	76	4.0	7.7
54		F	**1-54**	14	63	2.9	8.0
Haloperidol						0.062	-
Spiperone						-	0.013

receptors (3.4 μM and 1.2 μM, 1.0 μM and 4.5 μM, 5.1 μM and 2.4 μM, respectively). It is also worthwhile to note that compounds **1-27** and **1-30** (entries 27 and 30, when R^1 is 4-methoxyphenyl and R^2 is 2-naphthyl and 3-fluorophenyl, respectively) also showed high selectivity of binding affinities for D_3 receptor (1.1 and 1.0 μM, respectively) over D_4 receptor (> 10 μM).

In summary a small focused library of isoxazolylpyrrolidinylpiperazine compounds was constructed and was tested for *in vitro*[15] binding affinities towards dopamine D_3 and D_4 receptors. In general, the selectivity of binding affinity for D_3 receptor over D_4 receptor tends to be higher for compounds when R^2 substituent is 3-fluorophenyl. The results might be good guide to the future research in the development of potent and selective ligands for dopamine D_3 or D_4 receptor.

Experimental Section

General Procedure for Synthesis of Building Block 2 (when R^1 = Ph). To a solution of compound **6** (94 μL, 0.50 mmol) in dichloromethane (3.0 mL) at room temperature under nitrogen atmosphere was added 1-phenylpiperazine (when R^1 = Ph, 97 μL, 0.65 mmol). After the mixture being stirred for 3 min, $NaBH(OAc)_3$ (410 mg, 1.95 mmol) and 4 Å molecular sieve were added and the mixture was stirred for 6 h. Saturated $NaHCO_3$ solution was added and the mixture was extracted with ethyl acetate (50 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (Hexane:EtOAc = 2:3) to give the compound **8** (when R^1 = Ph, 140 mg, 80%).

To a solution of thus prepared compound **8** (1.33 g, 3.86 mmol) in dry dichloromethane (13 mL) at room temperature was added trifluoroacetic acid (7.0 mL) and the mixture was stirred for 1.5 h. Distilled water was added and the mixture was washed with ethyl acetate. The aqueous layer was treated with $NaHCO_3$ and was extracted with $CHCl_3$. The organic layers were dried over anhydrous $MgSO_4$ and concentrated. Further removal of organic solvents from the residue *in vacuo* gave the building block **2** (when R^1 = Ph, 0.95 g, 99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.0, 7.5 Hz, 2H), 6.91 (d, *J* = 8.0 Hz, 2H), 6.86 (t, *J* = 7.5 Hz, 1H), 5.23 (br, 1H), 3.67 (m, 1H), 3.09-3.23 (m, 6H), 2.76 (m, 2H), 2.53-2.66 (m, 3H), 2.49 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.06 (m, 1H), 1.90-1.94 (m, 2H), 1.57 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9, 129.8, 118.3, 113.5, 60.0, 58.2, 57.5, 56.1, 44.8, 29.7, 25.2.

Other [piperazin-1-ylmethyl]pyrrolidine derivatives were synthesized analogously and identified with ^{1}H NMR and ^{13}C NMR spectroscopies.

Gerneral Procedure for Synthesis of Building Block 3 (when R^2 = 2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl). To a solution of 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde oximes **10** (2.69 g, 15.0 mmol) in THF (70 mL) at room temperature under nitrogen atmosphere, *N*-chlorosuccinimide (2.40 g, 18.0 mmol) and pyridine (120 μL, 1.50 mmol) were added. After being stirred for 40 min at 60 °C, the mixture was cooled to rt and solutions of propargyl alcohol (700 μL, 12.0 mmol) in THF (2 mL) and triethylamine (2.50 mL, 18.0 mmol) in THF (4 mL) were added dropwise successively. After the mixture being stirred at 50 °C for 2 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 = 2:1) to give the isoxazole alcohol **11** (when R^2 = 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl, 1.87 g, 67%).

To a solution of thus prepared alcohol **11** (1.16 g, 5.00 mmol) in dichloromethane (15 mL) at room temperature, pyridinium chlorochromate (2.16 g, 10.0 mmol) and 4 Å molecular sieve (4.00 g) were added. After being stirred for 4 h, the mixture was concentrated and the residue was purified by flash column chromatography (hexane:EtOAc = 3:1) to give the building block **3** (when R^2 = 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl, 740 mg, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.31 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.13 (s, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 4.22-4.27 (m, 4H).

Other isoxazole aldehyde derivatives were synthesized analogously and identified with the ^{1}H NMR spectroscopy.

General Procedure for Synthesis of Isoxazolylpyrrolidinylpiperazine Derivatives 1 (1-2, when R^1 = Ph and R^2 = 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl). To a solution of building block **2** (when R^1 = Ph, 49 mg, 0.20 mmol) and building block **3** (when R^2 = 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl, 65 mg, 0.28 mmol) in dichloromethane (3 mL) at room temperature, 4 Å molecular sieve (7 beads) was added. After the mixture being stirred for 3 min, $NaBH(OAc)_3$ (140 mg, 0.66 mmol) was added and the mixture was stirred for 15 h. The mixture was filtered through basic alumina. After the removal of organic solvent *in vacuo*, the residue was dissolved in diethyl ether (1.0 mL) and was added ethereal HCl slowly. The precipitated HCl salt of the product was collected using centrifuge. The white precipitant was then washed with diethyl ether and dried *in vacuo* to give the desired product (76 mg, 82%). The structure was characterized after treating the HCl salt with saturated $NaHCO_3$ solution and usual workup.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.24-7.32 (m, 3H), 6.93 (d, *J* = 9.0 Hz, 3H), 6.85 (t, *J* = 7.5 Hz, 1H), 6.40 (s, 1H), 4.36 (d, *J* = 14.8 Hz, 1H), 4.28 (m, 4H), 3.87 (d, *J* = 14.8 Hz, 1H), 3.20 (t, *J* = 5.0 Hz, 4H), 3.14 (m, 1H), 2.84 (m, 1H), 2.68-2.73 (m, 2H), 2.58-2.64 (m, 3H), 2.39-2.47 (m, 2H), 1.95 (m, 1H), 1.79 (m, 1H), 1.73 (m, 1H), 1.59 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 162.0, 151.6, 145.4, 144.0, 129.3, 122.8, 120.4, 119.9, 118.0, 116.3, 116.1, 101.2, 64.7, 64.5, 64.4, 59.8, 54.8, 54.2, 49.4, 49.3, 30.4, 23.0. IR (KBr): 2943 (C-H), 2887 (C-H), 2814 (C-H), 1617 (aromatic C=C), 1527 (aromatic C=C), 1237 (C-N), 1067 (C-N), 1153 (C-O), 1142 (C-O), 1028 (C-O), 698, 720 cm^{-1}.

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

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