Notes

Synthesis of Piperazinylalkylisoxazoline Analogues and Their Binding Affinities for Dopamine Receptor Subtypes

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In recent years, extensive efforts have been made to explore potent ligands for dopamine D_3[1] or D_4[2] receptor for the discovery of antipsychotic drugs.[3] In this connection, some of us have recently reported[4] the design and synthesis of a piperazinylalkylisoxazole library (Figure 1), of which some ligands were found to exhibit high binding affinity and selectivity for the D_3 receptor over the D_2 receptor.[4] In continuation of this research program, we have also been interested in the construction of a structurally similar piperazinylalkylisoxazoline library. We envisaged that the slightly different structural feature of isoxazoline moiety may affect the physicochemical properties of molecules in the library and thus alter their binding affinities with dopaminergic receptors. With this envision in mind and careful scrutiny on binding affinities of piperazinylalkylisoxazole analogues, we designed a focused library of piperazinylalkylisoxazoline derivatives where n = 3 or 4 (Figure 1). Herein, we wish to report the synthesis of piperazinylalkylisoxazoline compounds and their binding affinities for dopamine receptor subtypes.

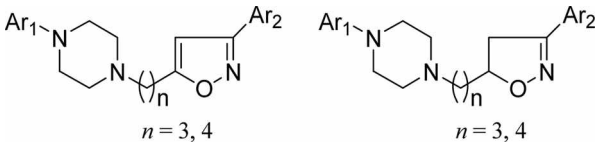
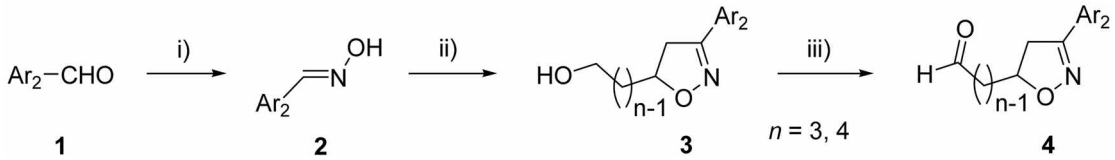


Figure 1

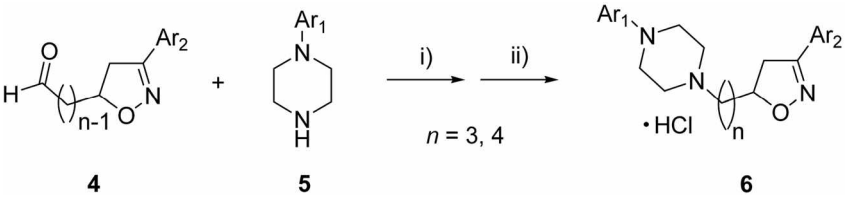
Our synthetic strategy to the construction of a library of piperazinylalkylisoxazolines was quite similar to that adopted for the synthesis of piperzinylalkylisoxazoles.[4] Based on the solution phase combinatorial reductive amination of isoxazoline aldehydes with piperazine derivatives, synthesis of piperazinylalkylisoxazolines was quite straightforward.

The preparation of starting isoxazoline aldehydes was described in Scheme 1. Treatment of aldehydes **1** with hydroxylamine hydrochloride and sodium carbonate in aqueous ethanol (EtOH : H_2O = 2/1) provided the corresponding oximes **2** in 90%-100% yields. Oximes **2** were reacted with *N*-chlorosuccinimide (NCS) in the presence of catalytic amount of pyridine in THF at 60 °C under nitrogen atmosphere. The reaction mixtures were cooled to room temperature over 30 min and then 4-penten-1-ol (for n = 3) or 5-hexen-1-ol (for n = 4) was added slowly. The mixture was treated with triethylamine (Et_3N). In situ generation of nitrile oxides and their 1,3-dipolar cycloadditions[6] proceeded to give the cyclized alcohols **3** in 42%-66% yields. PCC oxidation of alcohols **3** in the presence of silica gel in CH_2Cl_2 afforded isoxazoline aldehydes **4** in 45%-75% yields (Scheme 1).

Combinatorial synthesis[7] of piperazinylalkylisoxazolines was accomplished by the reductive amination of the prepared isoxazoline aldehydes **4** with a variety of commercial phenylpiperazine derivatives **5** using $NaBH(OAc)_3$[8] as outlined in Scheme 2. To solutions of aldehydes **4** and



Scheme 1. *Reagents and Reaction Conditions*: i) NH_2OH·HCl, Na_2CO_3, 60 °C, 1 h, EtOH/H_2O (2/1), 90-100%. ii) pyridine (cat.), NCS, 60 °C, 0.5 h, THF/4-penten-1-ol (for n = 3) or 5-hexen-1-ol (for n = 4), Et_3N , 50 °C ~ r.t., 2 h, 42-66%. iii) PCC, SiO_2, CH_2Cl_2, 5-12 h, 45-75%.



Scheme 2. *Reagents and Reaction Conditions*: i) $NaBH(OAc)_3$ (3 eq.), molecular sieve (3 beads), CH_2Cl_2, r.t., 5 h-12 h. ii) After execution of aqueous workup, the reaction mixture in 2 mL of diethyl ether was treated with ethereal HCl. The precipitant was washed with diethyl ether and dried. Yields were 85%-95%.

amines **5** in CH_2Cl_2 were added $NaBH(OAc)_3$ (3 eq.) and molecular sieve (3 beads). And the reaction mixtures were stirred for 5-12 h at room temperature. After carrying out the aqueous workup, reaction mixtures were dissolved in 2 mL of diethyl ether and followed by treatment with 1 M HCl solution in diethyl ether. The HCl salts of the products **6** were precipitated. The precipitants were filtered, washed with diethyl ether, and dried in *vacuo*. All the products were obtained in good yields (85-95%) and high purities ranging from 85 to 93%. For the solution phase combinatorial reductive amination, phenylpiperazines with electron-withdrawing and electron-donating substituents at *o*-position on the phenyl group such as fluoro- (Ar_15), chloro- (Ar_16), methyl- (Ar_12), methoxy- (Ar_13), and ethoxy- (Ar_14) were mainly employed, considering the binding results of piperazinylalkylisoxazole series. Diphenylmethylpiperazines such as 1-(diphenylmethyl)piperazine (Ar_18), 1-(4-chlorobenzhydryl)piperazine (Ar_19) and 1-[bis(4-fluorophenyl)-methyl]-piperazine (Ar_110) were also employed (Figure 2). The purities and identities of products were confirmed by ^1H NMR, HPLC and HRMS analysis after the conversion of

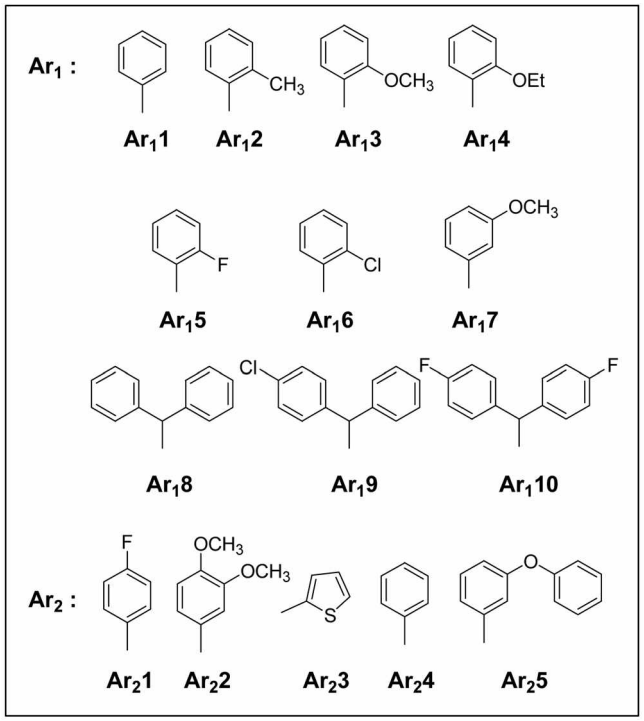


Figure 2

HCl salts of products into the corresponding free amine products. Thus, a small focused library of the well-characterized 100 members was constructed by using reductive amination reaction (Scheme 2, Figure 2).

The constructed piperazinylalkylisoxazoline library members were evaluated *in vitro* for dopamine D_2, D_3, D_4 receptors binding affinity by measuring their ability to displace radioligands ([^{3}H]spiperone for D_2 and D_3, [^{3}H]YM-09151-2 for D_4) from the cloned human dopamine receptors D_{2Long}, D_3 and $D_{4.2}$ which were stably expressed in CHO cells, respectively. The affinity and selectivity of these compounds for the dopamine receptors were also highly dependent on the length of the alkyl chain linker connecting two heterocyles and the substitution pattern at *o*-position on phenylpiperazinyl group as those of piperazinylalkylisoxazole analogues were. In this series, affinities of compounds with the alkyl chain length of $n = 4$ were low and showed low selectivities among receptors in the primary screening, while in piperazinylalkylisoxazole series compounds with a three atom tether ($n = 3$) showed low binding affinities and low selectivities among receptors.[4] In other words binding affinities for two different libraries with the isoxazole[4] and isoxazoline structures showed the reverse pattern for the length of alkyl chain from $n = 3$ to $n = 4$. In addition, the synthesized library was isolated as racemic compounds with the stereogenic center.[9] Table 1 shows the binding data of the selected compounds that exhibited good binding affinity and selectivity among dopamine D_2, D_3, and D_4 receptors. Compounds **6b**, **6c**, and **6d**, with the methyl group at *o*-position of the phenyl group of $Ar_1(Ar_12)$, showed relatively low binding affinity for the D_3 (110-140 nM) and D_4 receptors (310-590 nM). With the introduction of methoxy (Ar_13) and ethoxy (Ar_14) group at *o*-position of phenyl group, the binding affinity to both dopamine D_3 and D_4 receptors increased (compounds **6e-6h**). Compound **6e** showed high affinity for the D_3 receptor (5 nM) with a 288-fold selectivity over the D_2 receptor. Compound **6g** displayed high affinity value of 5 nM at the D_4 receptor with a 22-fold selectivity over the D_2 receptor. Especially, compound **6h** exhibited good binding affinity (4 nM) at both D_3 and D_4 receptors with a 118-fold selectivity over the D_2 receptor. Introduction of electron withdrawing substituents such as fluoro and chloro groups (Ar_15 and Ar_16) at *o*-position of the phenylpiperazine did not give satisfactory binding values. Thus, compounds **6i-6m** showed the moderate binding affinity to the D_3 receptor (21 nM-95 nM). Compound **6n**,

Table 1. Binding Affinities (IC_{50}, nM) for $n = 3$

Compound[a]	Ar_1	Ar_2	D_2	D_3	D_4	D_2/D_3	D_2/D_4
6a	$Ar_1$1	$Ar_2$2	9230	21	71	440	130
6b	$Ar_1$2	$Ar_2$3	9280	110	590	84	16
6c	$Ar_1$2	$Ar_2$4	8920	140	460	64	19
6d	$Ar_1$2	$Ar_2$5	7710	130	310	59	25
6e	$Ar_1$3	$Ar_2$2	1440	5	23	288	63
6f	$Ar_1$3	$Ar_2$3	1930	19	34	102	57
6g	$Ar_1$4	$Ar_2$2	110	31	5	3.5	22
6h	$Ar_1$4	$Ar_2$3	470	4	4	118	118
6i	$Ar_1$5	$Ar_2$1	9150	76	137	120	67
6j	$Ar_1$5	$Ar_2$5	5480	73	105	75	52
6k	$Ar_1$6	$Ar_2$2	5200	21	51	248	102
6l	$Ar_1$6	$Ar_2$3	11560	95	305	122	38
6m	$Ar_1$6	$Ar_2$4	4610	45	86	102	54
6n	$Ar_1$7	$Ar_2$2	8370	18	101	465	83
6o	$Ar_1$8	$Ar_2$2	2860	79	247	36	12
6p	$Ar_1$9	$Ar_2$1	3070	350	390	8.8	7.9
6q	$Ar_1$10	$Ar_2$2	1710	19	91	90	19
Haloperidol			80	57	65	1.4	1.2

[a]All compounds gave satisfactory spectral data

with the methoxy group at *m*-position of the phenyl group ($Ar_1$7), showed a slightly lower binding affinity (18 nM) than compound **6e** with the methoxy group at *o*-position of the phenyl group ($Ar_1$3) (5 nM). It seemed that an introduction of alkoxy group at *o*-position of the phenyl group is desirable for high binding affinity and selectivity at the D_3 and D_4 receptors over D_2 receptor. Diphenylmethylpiperazine analogues **6o-6q** displayed moderate to low binding affinities. Among them, compound **6q**, with symmetric 1-[bis(4-fluorophenyl)methyl]piperazinyl group ($Ar_1$10), displayed the slightly higher affinity than compounds **6o** ($Ar_1$8) and **6p** with asymmetric 1-(4-chlorobenzhydryl)-piperazinyl group ($Ar_1$9), to both D_3 and D_4 receptors. In general, most of compounds exhibited the high selectivity of both the D_3 (3.5 to 465-fold) and D_4 receptors (7.9 to 130-fold) over the D_2. However, the selectivity of the D_3 receptor over D_4 receptor was not significant (maximum of 6-fold selectivity). As for substituents at the isoxazoline ring (Ar_2), dimethoxy ($Ar_2$2) and 3-thienyl ($Ar_2$3) groups seem to guarantee high affinity (Table 1 and Figure 2).

In summary, a small focused library of piperazinylalkyl-isoxazolines was constructed through solution phase combinatorial synthesis and observed for binding affinity at dopamine D_2, D_3, and D_4 receptors. With the linker chain length of n = 3 connecting two heterocycles, most of compounds exhibited good binding affinity and selectivity at the desirable target receptors, the D_3 and D_4 receptors over D_2 receptor. It seemed that an introduction of alkoxy group at *o*-position of the phenyl group (Ar_1) guaranteed high binding affinities for the D_3 and D_4 receptors and high selectivity at the D_3 and D_4 receptors over D_2 receptor. Compounds **6e** and **6h** showed IC_{50} values of 5 nM and 4 nM for the D_3 receptor with 288-fold and 118-fold selectivity over D_2 receptor, respectively. For the D_4 receptor, they displayed binding affinities of 23 nM and 4 nM with 63-fold and 118-fold selectivity over D_2 receptor, respectively.

Experimental Section

Typical procedure for the construction of library members (6): To a solution of 3-[3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl]propanal (25.8 mg, 0.096 mmol) and 1-(2-methoxyphenyl)piperazine (20.0 mg, 0.087 mmol) in dry CH_2Cl_2 (2 mL) were added $NaBH(OAc)_3$ (55.5 mg, 0.262 mmol) and molecular sieve (3 beads). And the reaction mixtures were stirred for 12 h at room temperature. Saturated $NaHCO_3$ solution was added and the mixture was extracted with diethyl ether. Organic extracts were dried over anhydrous $MgSO_4$ and was concentrated. The residue was dissolved in 2 mL of diethyl ether and followed by treatment with 1M HCl solution in diethyl ether. The HCl salt of the product **6e** was precipitated. The precipitant was filtered, washed with diethyl ether, and dried in *vacuo*. In this way the HCl salt of the product **6e** was obtained as white solid (32.7 mg, 86%). Other compounds were synthesized analogously and the spectroscopic data of selected compounds were as follows.

Compound **6e**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (m, 6H), 2.53 (t, 2H), 2.73 (d, 3H), 2.99 (dd, 1H, J = 8.4 Hz, J = 10.8 Hz), 3.13 (s, 3H), 3.41 (dd, 1H, J = 10.4 Hz, J = 16.2 Hz), 3.87 (s, 3H), 3.93 (s, 6H), 4.78 (m, 1H), 6.96 (m, 6H), 7.41 (s, 1H); IR ($CHCl_3$) 2924, 2822, 1602, 1512, 1448, 1358, 1240, 1144, 1074, 918, 820, 761, 696 cm^{-1}.

Compound **6g**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (q, 2H), 1.44 (t, 3H), 1.73 (br, 4H), 2.27 (t, 2H), 2.61 (d, 3H), 2.98 (dd, 1H, J = 7.8 Hz, J = 10.8 Hz), 3.14 (s, 6H), 3.28 (m, 1H), 3.92 (s, 6H), 4.11 (t, 2H), 4.77 (m, 1H), 6.94 (m, 6H), 7.40 (s, 1H); IR ($CHCl_3$) 2938, 2816, 1600, 1518, 1458, 1424, 1370, 1340, 1242, 1153, 1142, 1028, 1008, 916, 808, 752, 664, 628 cm^{-1}.

Compound **6h**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, 1H), 1.47 (t, 3H), 1.78 (br, 5H), 2.49 (t, 1H), 2.70 (br, 3H), 3.02 (m, 1H), 3.15 (s, 4H), 3.44 (m, 1H), 4.08 (q, 2H), 4.79 (m, 1H), 6.97 (m, 4H), 7.07 (t, 1H), 7.20 (d, 1H), 7.39 (d, 1H); IR ($CHCl_3$) 2940, 2814, 1592, 1500, 1446, 1378, 1304, 1240, 1124, 1044, 908, 834, 750, 710 cm^{-1}.

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9. The related topic for the synthesis and stereochemistry of chiral ligands from isoxazoline library will be published as a title of 'Asymmetric Synthesis of Chiral Piperazinylpropylisoxazoline Ligands for Dopamine Receptors'.