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Design and Synthesis of a Piperazinylalkylisoxazole Library for Subtype Selective Dopamine Receptor Ligands

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Abstract—A piperazinylbutylisoxazole libary was designed, synthesized and screened for the binding affinities to dopamine D_2, D_3, and D_4 receptors. Several ligands were identified to possess high binding affinity and selectivity for the D_3 and D_4 receptors over the D_2 receptor. Compounds **6s** and **6t** showed K_i values of 2.6 nM and 3.9 nM for the D_3 receptor with 46- and 50-fold selectivity over the D_2 receptor, respectively.

Introduction

Recently we have reported[1] a simple and efficient way of constructing libraries of heterocycles focused on the potential GPCR(G-Protein Coupled Receptor) binding capability.[2] In a classical pharmacology two types of dopamine receptors acting through G-proteins had been defined: the D_1 receptor which mediates the activation of adenylyl cyclase[3] and the D_2 receptor which mediates its inhibition.[4] Today at least five dopamine receptors were identified through molecular pharmacology and they were grouped into two subfamilies, the D_1-like (D_1[5] and D_5[6]) and the D_2-like group (D_2,[7] D_3[8] and D_4[9]), based on amino acid homology and similar pharmacologic properties. The D_2-like receptors show high affinities for the most of the drugs used to treat schizophrenia and Parkinson's disease. Classical dopamine D_2 antagonists are effective in treating schizophrenia. However, they cause severe extrapyramidal side-effects[10] that are thought to result from the blockade of D_2 receptors in the striatum of brain. The distribution of the D_3 and D_4 receptors in the limbic areas of brain suggests that these receptors may be particularly an attractive target for the design of potent and selective antipsychotic drugs without causing extrapyramidal side effects.[11]

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Design of the Library

Based on SAR (structure–activity relationships) analysis of lignads for various GPCR's, especially dopamine D_3 and D_4 receptors antagonists and privileged structures for GPCRs,[12] the major aspects of the structural design to generate potent and selective ligands were divided into three parts: part **A** containing a biogenic amine, part **B** with alkyl chain as a linker and part **C** as electrostatically similar groups to carbonyl group or aromatic group at Q. Part **A** generally includes heterocyclic rings such as piperazine, piperidine or pyrrolidine. Part **B** serves as a linker between the biogenic amine and the hydrophobic aromatic group will be alkyl chain with varying length or conformationally rigid carbocyclic group. The chain length presumably reflects relative position of aromatic groups and the biogenic amine in spatial arrangement for binding to receptors, and could impose selectivity among receptors. The Q position of part **C** will both rigidify the part of the linker chain and be used as alternatives to bioisosteres such as amide,[13] carbonyl,[14] oxygen,[15] secondary alcohol,[16] and heterocycles.[17] The substituents, S_1 and S_2 occupy the hydrophobic binding sites for aromatic residues. In practice, we selected a scaffold with an arylpiperazine and an isoxazole connected by a linker for the library. We designed a synthetic scheme of piperazinylalkylisoxazole library by combination of diverse arylpiperazines with

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the substituted phenylisoxazole aldehydes where $n=3$ or $n=4$. (Fig. 1).

Chemistry

The synthetic strategy for obtaining a series of piperazinylalkylisoxazoles was adopted from the solution phase combinatorial synthesis,[1,18] where the amine functionality in the scaffold provides particularly rapid and easy purification of products. In practice, purification was achieved by forming HCl salt of target molecules without using chromatographic purification in the last step.

The preparation of starting materials was outlined in Scheme 1. The phenylalkylisoxazole aldehydes were prepared from the substituted phenylaldehydes (**1**). Reaction of hydroxylamine HCl with aldehydes **1** in ethanolic aqueous solution ($EtOH:H_2O=2/1$) gave the corresponding oximes in 90–100% yields. Oximes **2** were treated with NCS(N-chlorosuccinimide) and a catalytic amount of pyridine in THF at 60 °C under nitrogen atmosphere. The reaction mixtures were stirred for 30 min at room temperature and then 5-hexyn-1-ol (for $n=4$) was added slowly. The reaction mixtures were treated with Et_3N in THF over 10 min. After executing the aqueous workup, the alcohols **3** were obtained in 55–80% yields. Reaction of alcohols **3** with PCC/SiO_2 in CH_2Cl_2 gave aldehydes **4** in 45–85% yields (Scheme 1).

Construction of solution phase combinatorial library was described in Scheme 2. The combinatorial synthesis was accomplished by the reductive amination of the prepared aldehydes **4** with commercially available phenylpiperazine derivatives **5** using $NaBH(OAc)_3$.[19] Solutions of amines **5** and aldehydes **4** in CH_2Cl_2 was added $NaBH(OAc)_3$ (3 equiv) and molecular sieve (3 beads). The reaction mixtures were stirred for 5–12 h at room temperature. After executing of the aqueous workup, reaction mixtures dissolved in 2 mL of diethyl ether followed by treatment with 1M HCl solution in diethyl ether gave HCl salts of the products **6** as solid. The solvent was filtered off and the precipitant was washed with diethyl ether, and dried in vacuo. All the compounds were obtained in good yields (85–100%) and high purities ranging from 85–96%. Purity and identity of the products were confirmed through ^1H NMR, HPLC and HRMS after the solid products were converted into the corresponding free bases. A small library with well-characterized 300 compounds was constructed by using reductive amination, precipitation sequence (Scheme 2).

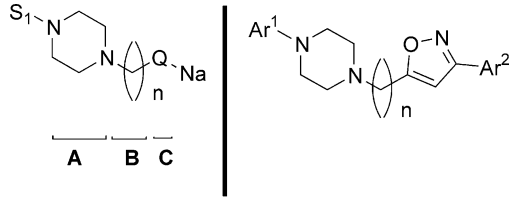
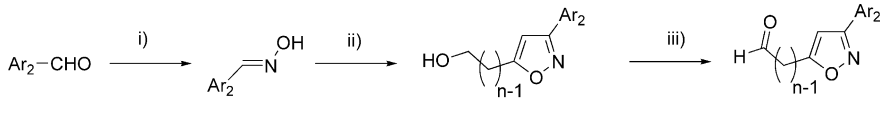


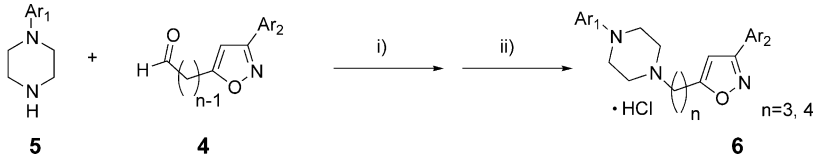
Figure 1.

Results and Discussion

The generated phenylpiperazinylalkylisoxazole analogues were evaluated in vitro for dopamine D_2–D_4 receptors binding affinity by measuring their ability to displace radioligands ([^{3}H]spiperone for D_2 and D_4, [^{3}H]YM-09151–2 for D_3) from the cloned human dopamine receptors D_{2long}, D_3 and $D_{4.2}$ which were expressed in CHO cells, respectively. The affinity and selectivity of this class of compounds for the dopamine receptors were largely dependent on substitution pattern at *o*-position on phenylpiperazinyl group and the length of the alkyl chain linker. The extension of alkyl chain from $n=3$ to $n=4$ affected the binding affinity greatly since the affinities of compounds with a three atom tether were very low and showed no selectivity among receptors in the primary screening. Table 1 shows the binding data of the selected compounds that showed good activity and selectivity among dopamine D_2, D_3, and D_4 receptors. With a methoxy group at *o*-position of phenyl group of $Ar_1(Ar_13)$, the binding affinity to dopamine D_3 receptor increased (compounds **6k**–**6q**). Extension of methoxy group at *o*-position of phenyl group (Ar_14) to ethoxy group (compounds **6r**–**6u**) gave the further improvement of affinity for the D_3 receptor (2.6–19 nM). On the other hand, with a methyl group (compounds **6h**–



Scheme 1. Reagents and reaction conditions: (i) $NH_2OH\cdot HCl$, 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-pentyn-1-ol (for $n=3$) or 5-hexyn-1-ol (for $n=4$), Et_3N, 50 °C—rt, 2 h, 55–80%; (iii) PCC, silica gel, CH_2Cl_2, 5–12 h, 45–85%.



Scheme 2. Reagents and reaction conditions: (i) $NaBH(OAc)_3$ (3 equiv), molecular sieves (3 beads), CH_2Cl_2, rt, 5–12 h; (ii) After executing 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, 85–100%.

Table 1. Binding affinity (K_i, nM)[20] for n = 4

Entry[a]	Substituent		Receptor			Selectivity	
	Ar_1	Ar_2	D_2	D_3	D_4	D_2/D_3	D_4/D_3
6a	$Ar_1$1	$Ar_2$1	2530	12	96	211	8
6b	$Ar_1$1	$Ar_2$2	1470	60	1697	25	28
6c	$Ar_1$1	$Ar_2$3	3388	24	774	141	32
6d	$Ar_1$1	$Ar_2$4	2196	16	76	137	5
6e	$Ar_1$1	$Ar_2$5	4136	15	117	276	8
6f	$Ar_1$1	$Ar_2$6	3366	18	541	187	30
6g	$Ar_1$1	$Ar_2$7	9724	27	346	360	13
6h	$Ar_1$2	$Ar_2$3	1401	38	413	37	11
6i	$Ar_1$2	$Ar_2$5	2108	163	465	13	3
6j	$Ar_1$2	$Ar_2$6	6512	292	683	22	2
6k	$Ar_1$3	$Ar_2$1	321	23	33	14	1.4
6l	$Ar_1$3	$Ar_2$2	295	6	50	49	8
6m	$Ar_1$3	$Ar_2$3	418	5.2	257	80	49
6n	$Ar_1$3	$Ar_2$4	440	6.5	34	68	5
6o	$Ar_1$3	$Ar_2$5	726	4.7	21	154	4
6p	$Ar_1$3	$Ar_2$6	1727	9.5	198	181	21
6q	$Ar_1$3	$Ar_2$7	964	15	88	64	6
6r	$Ar_1$4	$Ar_2$2	95	19	51	5	3
6s	$Ar_1$4	$Ar_2$3	119	2.6	28	46	11
6t	$Ar_1$4	$Ar_2$5	194	3.9	12	50	3
6u	$Ar_1$4	$Ar_2$6	341	9	34	38	4
6v	$Ar_1$5	$Ar_2$3	5148	20	233	257	12
6w	$Ar_1$5	$Ar_2$7	585	10	58	59	6
6x	$Ar_1$6	$Ar_2$1	1461	30	1346	49	45
6y	$Ar_1$6	$Ar_2$2	1522	19	2314	80	121
6z	$Ar_1$6	$Ar_2$3	2618	20	2438	130	122
6aa	$Ar_1$6	$Ar_2$4	3828	16	1118	239	70
6bb	$Ar_1$6	$Ar_2$5	620	52	2145	12	41
6cc	$Ar_1$6	$Ar_2$6	486	15	2243	32	150
6dd	$Ar_1$6	$Ar_2$7	491	40	603	12	15
Haloperidol			18	25	42	0.72	1.7

[a]All compounds gave satisfactory analytical and/or mass spectral data.

6j) at *o*-position of phenylpiperazine, the binding affinities decreased (38–292 nM). *o*-Fluorophenylpiperazine analogues (compounds **6v** and **6w**) also gave good binding affinities (10 and 20 nM). Among phenylpiperazine analogues, compounds **6s** and **6t** showed high binding affinity with K_i values of 2.6 and 3.9 nM, respectively. Though diphenylmethylpiperazine analogues (compounds **6x**–**6dd**) showed rather lower binding affinity (15–52 nM) than arylpiperazines for the D_3, compounds **6x** to **6dd** exhibited high selectivity for dopamine D_3 receptor over the D_2 and D_4 receptors regardless of Ar_2. For compounds **6z** and **6aa**, 130- to 239-fold higher selectivity of the D_3 receptor over the D_2 receptor was observed. Compounds **6e**, **6g** and **6v** showed the high selectivity for the D_3 over the D_2 receptor (276-, 360- and 257-fold, respectively). Compound **6x**–**6aa** showed a good selectivity for the D_3 over the D_4 receptor. Generally, high affinity appeared to be guaranteed by 3,4-dimethoxyphenyl- ($Ar_2$3), 2,4-dimethoxyphenyl- ($Ar_2$4) and 2-thienyl- ($Ar_2$5) groups and high selectivity was exerted either by *o*-alcoxy group or an extra phenyl group (Table 1; Fig. 2).

Summary

A small library of piperazinylbutyl(propyl)isoxazoles designed as GPCR lignads was constructed through solution phase combinatorial synthesis. The variation imposed on the two aromatic groups and the length of the spacer that connected two parts generated an array of activity and selectivity for the dopamine receptors. Also, the isoxazole ring as an alternative of bioisosteres play a important role in binding with receptors. With the chain length of four-carbon tether, most of the compounds showed good binding affinity and selectivity at the desirable target receptors, the D_3 and D_4 receptors over D_2 receptor. Compounds **6s** and **6t** showed K_i values of 2.6 and 3.9 nM for the D_3 receptor with 46-fold and 50-fold selectivity over the D_2 receptor, respectively. Further QSAR (Quantitative Structure and Activity Relationships) studies of these series are in progress.

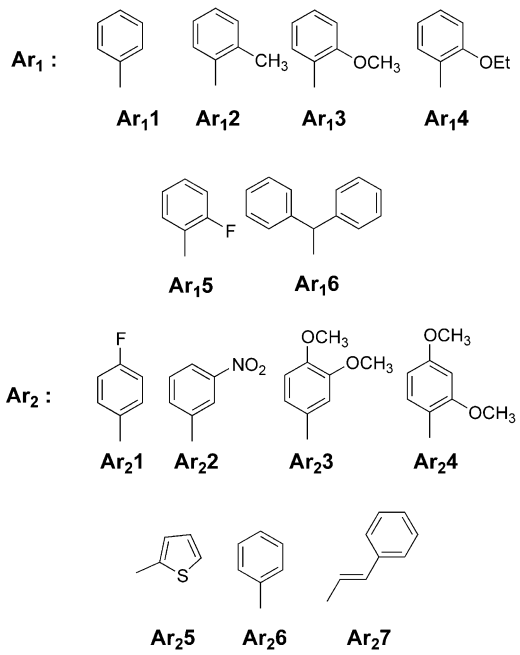


Figure 2.

Acknowledgements

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20. Assay protocol: Sf-9 membranes expressing either dopamine hD_{2L}, dopamine rD_3 or $hD_{4.2}$ receptors were purchased from PerkinElmer Life Sciences (Boston, MA). Radioligands used were [^{3}H]Spiperone (D_2 and D_3 dopamine binding assays, 1 nM), and [^{3}H] YM-09151–2 (D_4 dopamine binding assays, 0.06 nM). [^{3}H]Spiperone and [^{3}H] YM-09151-2 bindings were performed by the protocol provided by supplier of Sf-9 membranes for both hD_{2L} and rD_3, and $hD_{4.2}$ receptors, respectively. Briefly, the buffer used in hD_{2L} or rD_3 receptor binding assay was 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, or 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 120 mM NaCl, respectively. In [^{3}H] YM-09151–2 receptor binding assays, the buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl and 1.5 mM $CaCl_2$ was used. Nonspecific binding was determined with haloperidol (10 uM) and clozapine (10 uM) for D_2 and D_3, and D_4 receptors, respectively. Competition binding studies were carried out with 8 concentrations of the test compound run in duplicate tubes, and isotherms from three assays were calculated by computerized nonlinear regression analysis (GraphPad Prism Program, San Diego, CA) to yield inhibition constant (K_i) values.