Synthesis and Biological Evaluation of Isoxazoline and Isoxazole Derivatives as 5-HT_{2A} and 5-HT_{2C} Receptor Ligands

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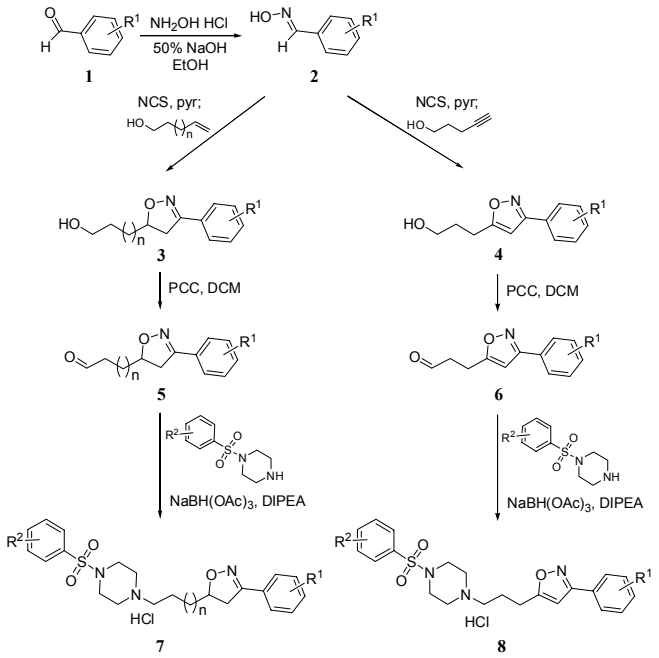
Antidepressants have been available since 1950s. The first generation drugs are monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs).[1,2] The second generation antidepressants are selective serotonin reuptake inhibitors (SSRIs)[3] and serotonin norepinephrine reuptake inhibitors (SNRIs),[4] which are more selectively bound to the targets such as serotonin transporter and/or norepinephrine transporter resulting in reduced side effects. However, all antidepressants in clinical use show limited efficacy, slow onset of action, and variable degree of side effects such as changes in sexual performance.[5] To fulfill these unmet needs of the antidepressants, there have been efforts to find new mechanisms that can be exploited for developing faster-acting and more efficient antidepressants. Several potential mechanisms have been reported, which include antagonism of the serotonin autoreceptors (5-HT_{1A}), postsynaptic serotonin receptors (5-HT_{2A} and 5-HT_{2C}), neurokinin receptors, and corticotrophin-releasing factor (CRF).[6,7,8] It is worth to note that the effect of citalopram, one of the SSRIs, can be augmented by pre-treatment with either 5-HT_{2A} antagonist or 5-HT_{2C} antagonist.[6b] Therefore, 5-HT_{2A} and 5-HT_{2C} receptors represent reasonable targets for the improved treatment of depression. In this study, in order to discover 5-HT_{2A} and 5-HT_{2C} receptor antagonists, we synthesized a series of isoxazoline and ioxazole derivatives and evaluated their binding affinities to 5-HT_{2A} and 5-HT_{2C} receptors.

Isoxazoline and isoxazole derivatives were synthesized from substituted benzaldehydes in 4 steps (Scheme 1). Benzaldehydes **1** were treated with hydroxylamine-HCl salt in EtOH under basic conditions to give the corresponding oximes **2** in 44% ~ 95% yields.[9] The oximes **2**, thus obtained, underwent [2+3] cycloaddition using NCS and pent-4-en-1-ol (or but-3-en-1-ol) to give isoxazolines **3** (n = 0 or 1) in 35% ~ 73% yields.[10] On the other hand, treatment of benzaldehyde oxime **2** with NCS and pent-4-yn-1-ol afforded isoxazoles **4** in 39% yield. The compounds **3** and **4** were oxidized with PCC to the corresponding aldehydes **5** and **6** in 31% ~ 85% yields. The aldehydes **5** and **6** underwent reductive amination with arylsufonylpiperazines with R^2 substituent such as hydrogen, fluorine, chlorine, methyl and dimethyl groups by using $NaBH(OAc)_3$ and Hünig base at room temperature to give the final products **7** and **8** in 20% ~ 75% yields.[11]

Total 37 compounds were synthesized and biologically evaluated against 5-HT_{2A} and 5-HT_{2C} receptors stably expressed by CHO-K1 cell lines through [^{3}H]-ketanserin and [^{3}H]-mesulergine binding assay, respectively.[12] The synthesized isoxazolines **7** with R^1 = H and n = 0 or 1 were tested against 5-HT_{2A} and 5-HT_{2C} receptors and their binding affinities are summarized as IC_{50} values in Table 1. The isoxazolines **7-1** ~ **7-10** with n = 0 show no binding affinity to the 5-HT_{2A} receptor except **7-7** (R^1 = *o*-Me) of which an IC_{50} value to the 5-HT_{2A} receptor was 2279 nM. The binding affinities of isoxazolines **7-11** ~ **7-22** with n = 1 were slightly improved compared with those of the isoxazolines **7-1** ~ **7-10** with n = 0. Like **7-7**, isoxazolines with *ortho*-substitutents, **7-15** and **7-18**, show the best binding affinities to 5-HT_{2A} receptor with IC_{50} values of 942 nM and



Scheme 1. Synthesis of isoxazoline and isoxazole derivatives

Table 1. Binding affinities to 5-HT_{2A} and 5-HT_{2C} receptors of ioxazoline derivatives **7** with R^1 = H

en.	compd	R^1	n	R^2	5-HT_{2A} IC_{50} (nM)	5-HT_{2C} IC_{50} (nM)
1	**7-1**	H	0	H	> 10000	> 10000
2	**7-2**	H	0	*o*-F	> 10000	> 10000
3	**7-3**	H	0	*m*-F	> 10000	> 10000
4	**7-4**	H	0	*p*-F	> 10000	> 10000
5	**7-5**	H	0	*m*-Cl	> 10000	> 10000
6	**7-6**	H	0	*p*-Cl	> 10000	> 10000
7	**7-7**	H	0	*o*-Me	2279	> 10000
8	**7-8**	H	0	*m*-Me	> 10000	> 10000
9	**7-9**	H	0	*p*-Me	> 10000	> 10000
10	**7-10**	H	0	2,3-dimethyl	> 10000	> 10000
11	**7-11**	H	1	H	8243	> 10000
12	**7-12**	H	1	*o*-F	7239	> 10000
13	**7-13**	H	1	*m*-F	> 10000	> 10000
14	**7-14**	H	1	*p*-F	> 10000	> 10000
15	**7-15**	H	1	*o*-Cl	942	3162
16	**7-16**	H	1	*m*-Cl	> 10000	> 10000
17	**7-17**	H	1	*p*-Cl	9216	> 10000
18	**7-18**	H	1	*o*-Me	566	897
19	**7-19**	H	1	*m*-Me	2618	> 10000
20	**7-20**	H	1	*p*-Me	4942	> 10000
21	**7-21**	H	1	2,3-dimethyl	7383	> 10000
22	**7-22**	H	1	*m*-OMe	> 10000	> 10000
23	ketanserin				2.5	--[a]
24	methiothepin				--[a]	1.6

[a]not determined

Table 2. Binding affinities to 5-HT_{2A} and 5-HT_{2C} receptors of isoxazole derivatives **8** with R^1 = H

en.	compd	R^1	R^2	5-HT_{2A} IC_{50} (nM)	5-HT_{2C} IC_{50} (nM)
1	**8-1**	H	H	8088	> 10000
2	**8-2**	H	*o*-F	8763	8030
3	**8-3**	H	*m*-F	4297	> 10000
4	**8-4**	H	*p*-F	> 10000	> 10000
5	**8-5**	H	*o*-Cl	3005	4261
6	**8-6**	H	*m*-Cl	> 10000	> 10000
7	**8-7**	H	*p*-Cl	> 10000	> 10000
8	**8-8**	H	*o*-Me	3106	2098
9	ketanserin			2.5	--[a]
10	methiothepin			--[a]	1.6

[a]not determined

Table 3. Binding affinities to 5-HT_{2A} and 5-HT_{2C} receptors of isoxazoline derivatives **7** with R^2 = *o*-Me and n = 1

en.	compd	R^1	n	R^2	5-HT_{2A} IC_{50} (nM)	5-HT_{2C} IC_{50} (nM)
1	**7-23**	*m*-Me	1	*o*-Me	321	1573
2	**7-24**	2,3-dimethyl	1	*o*-Me	868	1417
3	**7-25**	3,4-dimethyl	1	*o*-Me	204	2029
4	**7-26**	3,5-dimethyl	1	*o*-Me	603	2910
5	**7-27**	*m*-Cl	1	*o*-Me	790	756
6	**7-28**	3,5-dichloro	1	*o*-Me	72	150
7	**7-29**	2,3-dimethoxy	1	*o*-Me	444	1549
8	ketanserin				2.5	--[a]
9	methiothepin				--[a]	1.6

[a]not determined

566 nM, respectively. Also, **7-15** and **7-18** show binding affinities to 5-HT_{2C} receptor with IC_{50} values of 3162 nM and 897 nM, respectively. Among the series of the isoxazoline compounds in Table 1 (R^1 = H), **7-18** (R^2 = *o*-Me) shows the best binding affinity against both 5-HT_{2A} and 5-HT_{2C}.

The synthesized isoxazole derivatives **8-1** ~ **8-8** with R^1 = H were also biologically evaluated against 5-HT_{2A} and 5-HT_{2C} receptors (Table 2). Isoxazoles with *ortho*-substitutents like *o*-Cl and *o*-Me show most significant binding affinities to 5-HT_{2A} and 5-HT_{2C} receptors. Thus, the IC_{50} values of **8-5** (R^2 = *o*-Cl) and **8-8** (R^2 = *o*-Me) were evaluated as 3005 nM and 4261 nM, respectively, against 5-HT_{2A} receptor, and 3106 nM and 2098 nM, respectively, against 5-HT_{2C} receptor.

Taken together, two interesting structure-activity relationships could be derived: (i) compounds with *o*-Me substituent at the R^2 position show potent binding affinities to both 5-HT_{2A} and 5-HT_{2C} receptors; (ii) isoxazoline derivatives show better binding affinities than the corresponding isoxazoles.

Based on this information, a series of isoxazoline derivatives with fixed R^2 (*o*-Me), fixed chain length (n = 1), and various R^1 such as methyl, dimethyl, chloro, dichloro and dimethoxy groups were synthesized and biologically evaluated against 5-HT_{2A} and 5-HT_{2C} receptors (Table 3). The isoxazoline **7-23** with R^1 as *m*-Me shows binding affinity with IC_{50} values of 321 nM to 5-HT_{2A} receptor and 1573 nM to 5-HT_{2C} receptor. Among the dimethyl isoxazolines (**7-24** ~ **7-26**), **7-25** shows the most potent binding affinity with an IC_{50} value of 204 nM to 5-HT_{2A} receptor. The *m*-Cl substituent as R^1 was not as effective as *m*-Me substituent (**7-23**) in increasing binding affinity of the isoxazoline derivative **7-27** to 5-HT_{2A} receptor. However, it is noteworthy that the chlorine substituent significantly improved the binding affinity of the chlorinated isoxazoline derivatives (**7-27** and **7-28**) to 5-HT_{2C} receptor. In particular, the dichloro-isoxazoline derivative **7-28** (R^1 = 3,5-dichloro) was proved to be the most active against both 5-HT_{2A} and 5-HT_{2C} receptors with IC_{50} values of 72 nM and 150 nM, respectively. In the case of the dimethoxy-isoxazoline derivative (**7-29**, R^1 = 2,3-dimethoxy), however, the binding affinities were almost the same as those of **7-23**. These results indicate that stereoelectronic effect might be at work in modulating the binding affinities of the isoxazoline derivatives to the two target receptors. Electron-donating R^1 groups such as methyl, dimethyl and dimethoxy affect negatively to bind to 5-HT_{2C} receptor and positively to bind to 5-HT_{2A} receptor, while electron-withdrawing R^1 groups such as chloro and dichloro affect positively to bind to both 5-HT_{2A} and 5-HT_{2C} receptors.

The antidepressant effect of the most active compound **7-28** was tested. The compound **7-28** was orally injected at a dose of 100 mg/kg to mice in the forced swimming test[13] and

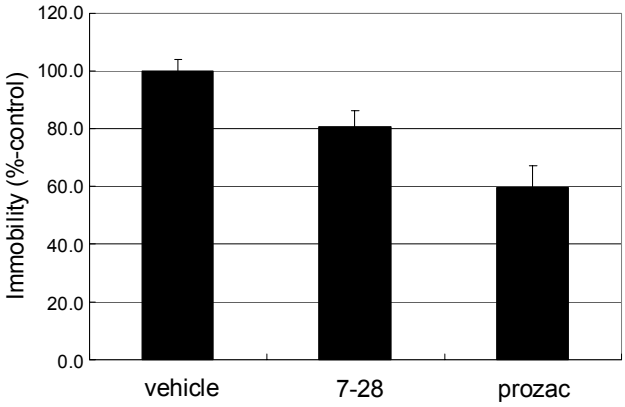


Figure 1. Antidepressant effects of Prozac® and the synthesized compound **7-28** on immobility in forced swimming test in mice. Prozac® and the tested compound (100 mg/kg) were injected orally (PO) 60 min before the testing, and the total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± SEM.

the total duration of immobility of the mice was monitored. After administration of the compound **7-28**, the total duration of immobility was reduced to 80.6% (Figure 1). In the case of Prozac® as positive control, the duration of immobility was reduced to 59.5%.

In summary, in order to find antagonists of 5-HT_{2A} and 5-HT_{2C} receptors, which are known as good targets for the improved treatment of depression, a series of isoxazoline and isoxazole derivatives were synthesized and biologically evaluated. The structure-activity relationships derived from this study indicate that *o*-Me group is favored as R^2 substituent and the stereoelectronic effects of the R^1 substituents might modulate the binding affinities of the isoxazoline derivatives. In particular, an isoxazoline **7-28** with *o*-Me group as an R^2 substituent and 3,5-dichloro substituent at R^1 shows the most potent binding affinities to both 5-HT_{2A} and 5-HT_{2C}, of which IC_{50} values were 72 nM and 150 nM, respectively. In the depression animal model, the most potent compound **7-28** shows the antidepressant effect. To find more potent isoxazoline derivatives as potential antidepressants, intensive structure-activity relationship study and *in vivo* study with animal models are in progress which will be published in due course.

Experimental Section

3,5-Dichlorobenzaldehyde oxime (2, R^1 = 3,5-dichloro). To a solution of 3,5-dichlorobenzaldehyde (3.0 g, 5.7 mmol) in 6 mL of water and 5 mL of EtOH were successively added NH_2OH·HCl (1.44 g, 19.4 mmol) and 2 mL of 50% aqueous NaOH solution at room temperature. After 1 h, the reaction was completed. The reaction mixture was extracted with EtOAc (200 mL) and the organic layer was dried over $MgSO_4$, filtered and concentrated to give the desired product (1.03 g, 5.4 mmol) in 95% yield: ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (s, 1H), 7.39 (s, 2H), 7.29 (s, 1H).

3-(3-(3,5-Dichlorophenyl)-4,5-dihydroisoxazol-5-yl)propan-1-ol (3, R^1 = 3,5-dichloro). A solution of 3,5-dichlorobenzaldehyde oxime (1.5 g, 7.88 mmol) in 30 mL of THF was treated with NCS (1.3 g, 9.46 mmol) and pyridine (64 μL, 0.79 mmol) and the resulting mixture was stirred at 60 °C for 30 min. A solution of 4-penten-1-ol (0.65 mL, 6.3 mmol) and TEA (1.3 mL, 9.5 mmol) in 10 mL of THF was added to the reaction mixture at 60 °C. The resulting mixture was stirred at 55 °C for 1 h. After reaction was completed, the mixture was cooled to room temperature and concentrated. The residue was purified by column chromatography to afford the desired product (0.76 g, 2.7 mmol) in 34% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.55 (s, 1H), 7.40 (s, 1H), 4.83-4.88 (m, 1H), 3.74 (t, 2H, *J* = 5.5 Hz), 3.35-3.44 (m, 1H), 2.91-3.00 (m, 1H), 1.67-1.89 (m, 4H).

3-(3-(3,5-Dichlorophenyl)-4,5-dihydroisoxazol-5-yl)propanal (5, R^1 = 3,5-dichloro). A solution of the compound **3** (R^1 = 3,5-dichloro, 0.76 g, 2.7 mmol) in 11 mL of DCM was treated with PCC (0.90 g, 4.16 mmol) and SiO_4 (0.9 g) at room temperature. The reaction mixture was stirred for 4 h. After reaction was completed, the mixture was filtered through silica gel pad with ethyl ether to give the desired product (0.47g, 1.7 mmol) in 63% yield: ^{1}H NMR(300 MHz, $CDCl_3$) δ 9.77 (s, 1H), 7.61 (s, 1H), 7.47 (s, 1H), 7.33 (s, 1H), 4.75-4.80 (m, 1H), 3.29-3.38 (m, 1H), 2.84-2.92 (m, 1H), 2.64 (t, *J* = 7.16 Hz, 2H), 1.85-1.99 (m, 2H).

3-(3,5-dichlorophenyl)-5-(3-(4-(o-tolylsulfonyl)piperazin-1-yl)propyl)-4,5-dihydroisoxazole hydrogen chloride (7-28). A solution of the aldehyde **5** (50 mg, 0.18 mmol) in 4 mL of DCM was treated with 1-(*o*-tolylsulfonyl)piperazine (62 mg, 0.30 mmol), DIPEA (86 mL, 0.49 mmol) and 100 mg of molecular sieve (4Å) at room temperature. The resulting mixture was stirred at room temperature for 1 h and then, $NaBH(OAc)_3$ (0.16 g, 0.74 mmol) was added to the reaction mixture. After 10 h, the resulting mixture was quench with 0.3 mL of water and filtered through celite pad. The filtrate was concentrated and purified by column chromatography, and then treated with ethereal HCl to afford the desired product (46 mg, 0.086 mmol) in 48% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.19 (br s, 1H), 7.89 (d, *J* = 1.9 Hz, 2H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.78 (t, *J* = 1.9 Hz, 1H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.58-7.40 (m, 2H), 7.09 (s, 1H), 3.75-3.63 (m, 2H), 3.63-3.45 (m, 2H), 3.25-3.02 (m, 6H), 2.91 (t, *J* = 7.3 Hz, 2H), 2.57 (s, 3H), 2.20-2.04 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.7, 160.4, 138.0, 135.4, 134.8, 134.2, 133.6, 132.5, 130.3, 130.1, 127.2, 125.5, 100.7, 54.9, 50.7, 42.6, 40.8, 23.7, 21.4, 20.7; HR-MS (EI+, M+H) calc for $C_{23}H_{25}N_3O_3Cl_2S$ 493.0994, found 493.0968.

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