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Synthesis and antidepressant activity of a series of arylalkanol and aralkyl piperazine derivatives targeting SSRI/5-HT_{1A}/5-HT_7

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Abstract

A series of arylalkanol and aralkyl piperazine derivatives have been synthesized and evaluated for 5-HT reuptake inhibitory abilities and binding affinities at the 5-HT_{1A}/5-HT_7 receptors. Antidepressant activities of the compounds *in vivo* were screened using the forced swimming test (FST). The results indicated that the compound **8j** exhibited high affinities for the 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, k_i = 0.84 nM; 5-HT_7, k_i = 12 nM) coupling with moderate 5-HT reuptake inhibitory activity (RUI, IC_{50} = 100 nM) and showed a marked antidepressant-like activity in the FST model.

Key words: Antidepressant; arylalkanol and aralkyl piperazine; SSRI/5-HT_{1A}/5-HT_7.

Depression is a chronic, debilitating disease that can disturb thoughts, feelings, behaviors, even sometimes leads to suicide. According to the World Health Organization, Major depressive disorder (MDD) will become the second leading cause of disability worldwide by the year 2020, affecting 121 million people[1]. Various chemical drugs have been developed to treat patients with depression since the late 1950s, such as the monoamine oxidase inhibitors, the tricyclic antidepressants, the selective serotonin reuptake inhibitors, the serotonin and norepinephrine reuptake inhibitors etc.[2-3] However, there are still high unmet needs in the treatment of depression，including therapeutics delayed onset, treatment resistance and adverse effects such as sexual dysfunction. [4-5]

To enhance drug efficacy and suppress unwanted effects, different strategies have been developed, such as the triple reuptake inhibitors (SSRI/SNRI/SDARI) and novel compounds with the combination of serotonin reuptake inhibition and various 5-HT receptor subtypes affinity.[6-7] For example, vilazodone (Chart **1**), a novel serotonin reuptake inhibitor with 5-HT_{1A} partial agonistic activity, displays fast antidepressant efficacy with minimal undesirable effects, especially sexual dysfunction. [8-9] Besides, the blockade of 5-HT_7 receptors has been shown to enhance the effects of SSRIs on serotonin levels *in vivo*. The combined administration of low doses of an SSRI with selective 5-HT_7 receptor antagonist (SB-269970) was found active in behavioral models of depression. [10-11] Recently, a study showed that the novel multimodal antidepressant vortioxetine has the potential of improving cognitive function partly via its 5-HT_7 receptor antagonism.[12]

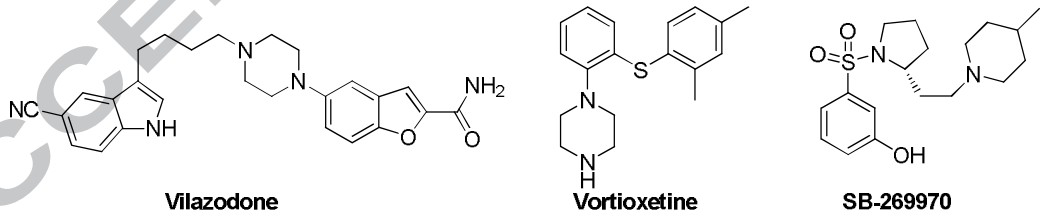
To the best of our knowledge, the effect of compounds with the combination of serotonin reuptake inhibition and 5-HT_{1A}/5-HT_7 receptors in animal models of depression has not been studied so far. Herein we described the synthesis and biological evaluation of novel arylalkanol and aralkyl piperazine derivatives showing high affinities for the 5-HT_{1A}/5-HT_7 receptors coupled with 5-HT reuptake inhibitory activity.

The compounds with dual SSRI and 5-HT_{1A} activity have been reported. Earlier studies by Monge et al. demonstrated that the aryl piperazine benzo[b]thiophene

derivatives **I** and **II** (Chart **2**) showed high affinity for both 5-HT_{1A} receptor and 5-HT transporter.[13-14] The agents acting on 5-HT_{1A} and 5-HT_7 receptors have also been reported in recent decades. Leopoldo et al. reported that 1-(2-Biphenyl)piperazine derivatives **III** and **IV** (Chart **2**) displayed high affinity for 5-HT_7 and 5-HT_{1A} receptors.[15-16] In our previous study, we evaluated the aryl piperazine benzo[b][1,4]oxazine derivative **V** that exhibited potent functional activities at both 5-HT_{1A} receptor [K_i = 1.48 nM] and serotonin transporter [IC_{50} = 195.02 nM] (Chart **3**).[17]

In this study, we firstly investigated whether 5-HT reuptake inhibition could be improved by replacing the benzo[b][1,4]oxazine moiety with 3,4-dichlorophenyl moiety which is a preferred substituent in some SSRI antidepressants (Chart **3**).[18] Subsequently, we replaced the 2-methoxyphenyl moiety at the N4 position with other aromatic substituents to explore the effect of different aromatic ring substitutions on the affinity for dual 5-HT_{1A} and 5-HT_7 receptors (Chart **3**).

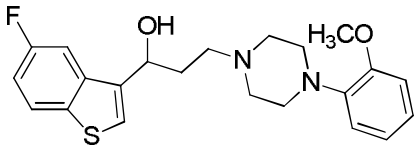
Compounds were evaluated for binding affinities for 5-HT_{1A}/5-HT_7 receptors and inhibiting 5-HT reuptake *in vitro*. The selected compounds were tested for microsomal stabilities *in vitro* and antidepressant-like activities *in vivo*. Preliminary results indicated the target compounds exhibited high potency for 5-HT_{1A}/5-HT_7 receptors and moderate 5-HT reuptake inhibitory and produced marked antidepressant-like effects *in vivo*.



Vilazodone **Vortioxetine** **SB-269970**

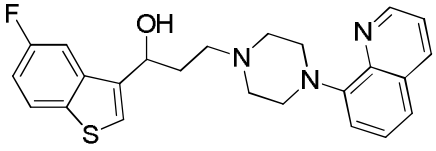
Chart 1. Chemical structures of representative antidepressant compounds

SSRI/5-HT$_{1A}$ agents



I

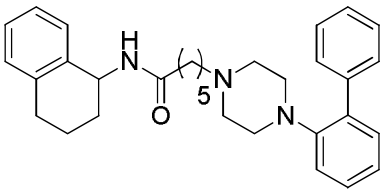
K_i (5-HT$_{1A}$) = 2.3 nM
K_i (SERT) = 12 nM



II

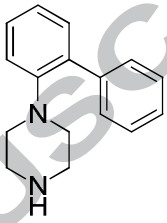
K_i (5-HT$_{1A}$) = 2.7 nM
K_i (SERT) = 7.5 nM

5-HT$_{1A}$/5-HT$_7$ agents



III (LP-12)

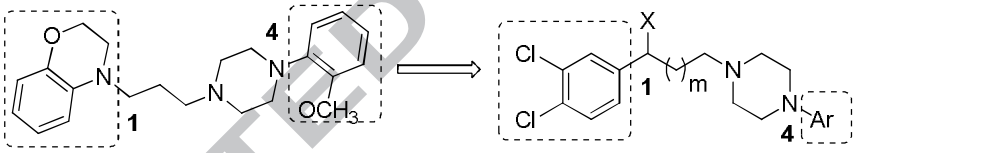
K_i (5-HT$_{1A}$) = 60.9 nM
K_i (5-HT$_7$) = 0.13 nM



IV

K_i (5-HT$_{1A}$) = 99 nM
K_i (5-HT$_7$) = 1.4 nM

Chart 2. Structures of SSRI/5-HT$_{1A}$ agents and 5-HT$_{1A}$/5-HT$_7$ agents



V

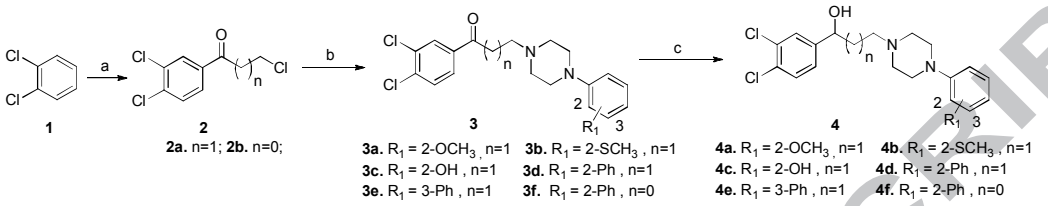
VI

Modification of aromatic substitution

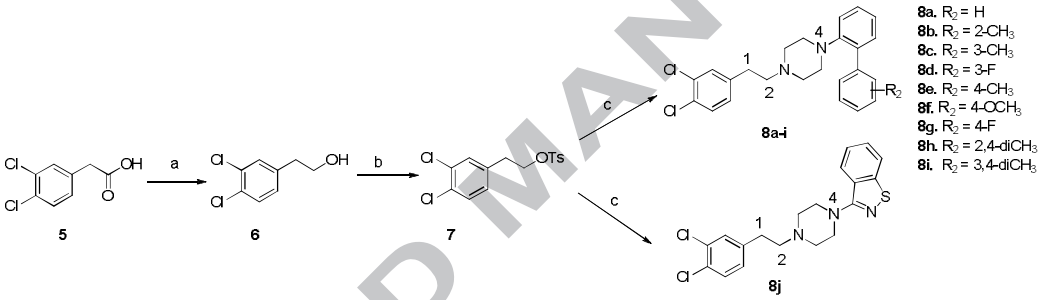
Chart 3. Rational design of novel SSRI/5-HT$_{1A}$/5-HT$_7$ activity compounds

The synthesis of all target compounds was prepared as outlined in schemes **1-2**. In Scheme **1**, a series of linear-chain arylalkanol piperazine derivatives (**4a-4f**) was prepared. Briefly, 3-chloropropanoyl chloride or chloroacetyl chloride with 1,2-dichlorobenzene in the presence of aluminum choride and dichloromethane by the Friedel-Crafts reaction afforded the intermediates **2**. Then the compounds **2** were reduced by sodium borohydride to provide the intermediates **3**. Finally, the compounds **3** were treated with 4-arylpiperazine derivatives via S_N2 mechanism, yielding target compounds **4a-4f**. According to Scheme **2** , a series of linear-chain aralkyl piperazine derivatives (**8a-8j**) were prepared. In general, compound **5** was

reduced with lithium aluminum hydride into the corresponding alcohol **6**. Then the activation of alcohol **6** with 4-toluenesulfonyl chloride in the presence of triethylamine at room temperature provided intermediate **7**. Compound **7** was then reacted with 4-arylpiperazine derivatives to give the target compounds **8a-8j**.



Scheme 1. Reagents and conditions: (a) $AlCl_3$, 3-chloropropanoyl chloride (or chloroacetyl chloride), 70 ^oC; (b) K_2CO_3, CH_3CN, 80 ^oC; (c) $NaBH_4$, MeOH, room temperature.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 0-25 ^oC ; (b) TsCl, Et_3N, DCM, 25 ^oC; (C) K_2CO_3, CH_3CN, 80 ^oC.

All of compounds were tested for their inhibition of 5-HT reuptake and binding affinities for the 5-HT_{1A} and 5-HT_7 receptors. The binding affinity for 5-HT_{1A} receptor was determined by investigating the displacement of [^{3}H]-8-OH-DPAT to HEK-293 cell membrane homogenates, [19] and the affinity for the 5-HT_7 receptor was determined by displacement binding to CHO cell membrane homogenates using [^{3}H]-LSD to previously procedures reported. [20] The inhibition of uptake of [^{3}H]-5-HT into rat brain synaptosomes through the serotonin transporter was also evaluated. [21] All compounds were initially screened at 10 μM concentration, and potent compounds (inhibition >90%) were then assayed to obtain their IC_{50} values. The detail results were summarized in Table **1-2**. The metabolic stability of the test compounds was measured *in vitro* using liver microsomes, a system widely used to evaluate the

susceptibility to first-pass oxidative metabolism (Table **3**).[22]

We first focused on the R_1 substituent in the 2- or 3- position of phenylpiperazine ring in **4** (Scheme **1**). Compounds **4a-4c** showed more than 90% inhibition for dual 5-HT_{1A} and 5-HT_7 receptors but expressed less than 90% inhibition for 5-HT reuptake even at a concentration of 10 μM. Interestingly, the 1-(2-biphenyl)piperazine derivative **4d** exhibited moderate inhibitory for 5-HT reuptake (IC_{50} = 590 nM) and showed more than 90% inhibition for 5-HT_{1A}/5-HT_7 receptors, whereas the 1-(3-biphenyl)piperazine counterpart **4e** remained selectively inhibitory for 5-HT reuptake and 5-HT_{1A} receptor (inhibition ratio >90%). These results showed that 2-phenyl was essential as 5-HT_{1A} /5-HT_7 pharmacophore moiety.

The influence of the length of the linker on 5-HT reuptake inhibition in relation to 5-HT_{1A} and 5-HT_7 receptor affinity was also explored. The two-carbon chain analog **4f** displayed outstanding affinity for 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, k_i = 2.4 nM; 5-HT_7, k_i = 3.2 nM) and better 5-HT reuptake inhibitory activity (IC_{50} = 340 nM).

In our next series, we investigated the effects of removing the hydroxyl group at the C1 position in arylalkanol-piperazine derivatives (Scheme **2**). As shown in Table **1-2**, compound **8a** showed potent inhibition for 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, k_i = 6.3 nM; 5-HT_7, k_i = 0.75 nM) and lower inhibition for 5-HT reuptake (IC_{50} = 840 nM). Meanwhile, the role of various substituted phenyl groups was also explored. We first introduced a methyl into the ortho-, meta- and pair- position of the phenyl group. Compounds **8b, 8c** and **8e** showed selectively inhibition for 5-HT_{1A}/5-HT_7 receptors (inhibition ratio > 90%) . The 2,4-dimethyl and 3,4-dimethyl substituted derivatives **8h** and **8i** were also weak inhibitors of 5-HT reuptake. The replacement of methyl group with methoxy group did not improve the activity of 5-HT reuptake inhibition (compare **8f** with **8e**). Besides, the 3-fluorophenyl **8d** (RUI, IC_{50} = 880 nM) and 4-fluorophenyl **8g** (RUI, IC_{50} = 950 nM) analogs showed lower inhibition for 5-HT reuptake. Those results indicated that the introduction of various substitutions on the phenyl ring led to reduce 5-HT reuptake inhibition activity. To explore the effect of aromatic heterocycle at the N4 position on 5-HT reuptake inhibitory activity, the biphenyl moiety was replaced by benzo[d]isothiazole to produce compound **8j**.

Surprisingly, compound **8j** exhibited higher inhibition for 5-HT reuptake than the other biphenyl substituted compounds (RUI, IC_{50} = 100 nM), and also displayed excellent affinity for 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, k_i = 0.84 nM; 5-HT_7, k_i = 12 nM).

The metabolic stability of potent compounds **4f** and **8j** was tested herein. The data indicated that the two compounds displayed short half-life ($t_{1/2}$ values were 6.1 min and 5.3 min, respectively), which was a little faster than vortioxetine ($t_{1/2}$ = 9 min).

Compounds **4f** and **8j** was selected for further profiling in the mouse forced swim test (FST). The compounds were administered orally once daily for 7 days at doses of 10, 20 and 40 mg/kg/day (PO), respectively. For comparative purposes, vortioxetine was acted as a positive control (20 mg/kg/day, PO). The results are shown in Figure **1**. Compared with vehicle, the selected compounds **4f** and **8j** reduced immobility times in the FST in a dose-dependent manner that was statistically significant at 20 and 40 mg/kg (PO). The positive control, vortioxetine, also produced a statistically significant reduction of immobility time at 20 mg/kg dose.

In summary, a novel series of arylalkanol-piperazine derivatives **4a-4f** and aralkyl piperazine derivatives **8a-8j** were designed and synthesized. Several compounds showed potent binding affinities at the 5-HT_{1A}/5-HT_7 receptors and moderate inhibition of 5-HT reuptake, and exhibited marked antidepressant-like activity in the FST. Especially, compound **8j** showed more potent efficacy in the animal test, and could act as a potential candidate for a promising antidepressant. The preliminary results lay a foundation for future development of novel derivatives targeting SSRI/5-HT_{1A}/5-HT_7 as potent multi-model antidepressant agents. The analogs possessing an improved pharmacokinetic property will be the focus of future disclosures.

Table 1

5-HT_{1A} and 5-HT_7 Receptor Binding and 5-HT Reuptake Inhibition of target compounds [a]

Compd.	RUI (Inhibition ratio)	5-HT_{1A} (Inhibition ratio)	5-HT_7 (Inhibition ratio)
4a	88.8%	99.3%	100.9%
4b	89.04%	99.9%	101.1%
4c	84.9%	99.4%	102.4%
4d	98.9%	98.9%	101.5%
4e	105.3%	91.5%	69.8%
4f	112.0%	98.5%	99.8 %
8a	103.7%	97.8%	101.1%
8b	61.6%	93.9%	97.3%
8c	72.3%	98.4%	94.7%
8d	97.2%	99.7%	96.4%
8e	65.8%	99.5%	98.1%
8f	66.1%	98.7%	97.5%
8g	92.1%	98.8%	96.7%
8h	31.5%	83.2%	93.5%
8i	17.6%	96.8%	93.2%
8j	101.6%	98.7%	96.0%

[a] Percent inhibition measured at a concentration of 10 μM.

Table 2

5-HT_{1A} and 5-HT_7 Receptor Binding and 5-HT Reuptake Inhibition of selected compounds [a]

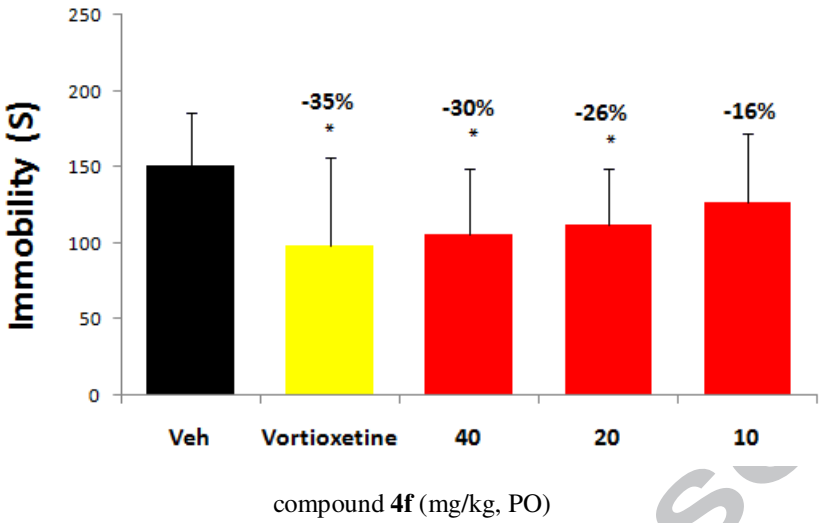
Compd.	RUI (IC_{50}, nM)	5-HT_{1A}(K_i, nM)	5-HT_7 (K_i, nM)
4d	590	-	-
4f	340	2.4	3.2
8a	840	6.3	0.75
8d	880	-	-
8g	950	-	-
8j	100	0.84	12
Vortioxetine	2.9	9.5	26

[a] IC_{50} and K_i values were obtained from 8 concentrations of the compound, each in duplicate. (Binding assays were conducted by Eurofins Cerep SA, Celle L'Evescault, France).

Table 3

Rat Liver Microsomal Metabolic Stability Assay

Compd.	$t_{1/2}$ (min)	CL (μL/min/mg)
4f	6.1	225.6
8j	5.3	261.7
Vortioxetine	9	153.2
Omeprazole	8.5	162.6



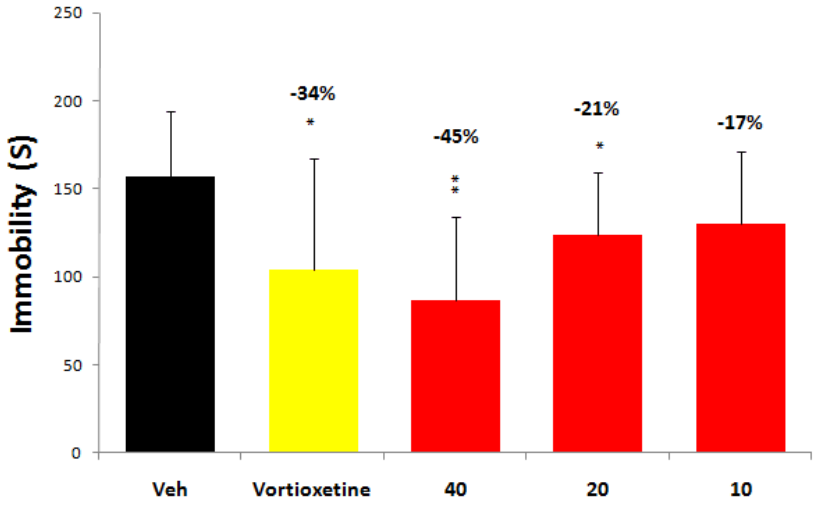


Figure 1. Effect of treatment of mice with compounds **4f** and **8j** at graded doses on the immobility time in the forced swim test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

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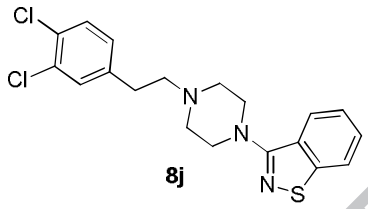
Graphical abstract



IC_{50} (nM)	K_i (nM)	
RUI	5-HT$_{1A}$	5-HT$_7$
100	0.84	12