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Synthesis and antidepressant effect of novel aralkyl piperazine and piperidine derivatives targeting SSRI/5-HT_{1A}/5-HT_7

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[†] *These authors contributed equally to this work.*

Abstract

A series of novel aralkyl piperazine and piperidine derivatives were synthesized, and evaluated for their serotonin reuptake inhibitory and 5-HT_{1A}/5-HT_7 receptors affinities activity. Antidepressant activities *in vivo* of the selective compound were screened using the forced swimming test (FST) and tail suspension test (TST). The results indicated that compound **19a** exhibited high affinities for the 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, K_i = 12 nM; 5-HT_7, K_i = 3.2 nM) coupled with potent serotonin reuptake inhibition (IC_{50} = 14 nM) and showed a marked antidepressant-like effect in the FST and TST models.

Key words

Antidepressant, serotonin reuptake inhibition, 5-HT_{1A} receptor, 5-HT_7 receptor

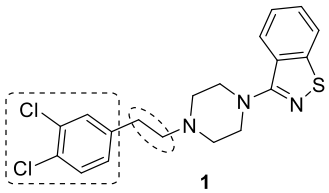
Depression is a common mental disorder characterized by symptoms like anhedonia, decreased energy, impaired cognitive functions and other actions, with an estimated 350 million people affected worldwide.[1] According to the WHO, the serious depressive illness will have become the main cause of disability by 2030.[2] Although a plethora of antidepressants are in market, targeting mainly monoamine serotonin and norepinephrine transporters,[3] there is still a significant unmet medical need for management of this disorder, including therapeutics delayed onset, treatment resistance and adverse effects such as sexual dysfunction.[4]

Currently, it has been suggested that drugs combining activities of selective serotonin reuptake inhibitor and 5-HT receptor subtypes may form a novel strategy for higher therapeutic efficacy of antidepressant.[5] Clinical research has demonstrated that the antidepressant activity of selective serotonin reuptake inhibitor (SSRI) may be augmented by coadministration of pindolol, an effect that has been attributed to pindolol's partial agonistic effect at the 5-HT_{1A} receptor.[6] Meanwhile, 5-HT_7 receptor blockade has been shown to be efficacious in animal models of depression, e.g. the mouse forced swimming test.[7] Furthermore, the combination of an SSRI with 5-HT_7 receptor blockade synergistically augments the levels of 5-HT in the prefrontal cortex, suggesting that 5-HT_7 receptor antagonist in combination with serotonin transporter (SERT) blockade has antidepressant potential.[8] Hence, it stands to reason that agents with dual or multiple acting on SSRI/5-HT_{1A}/5-HT_7 have the potential to fill significant clinical unmet needs in depression.

Previous reports from our laboratories have revealed two novel series (**Chart. 1**) capable of modulating SSRI/5-HT_{1A}/5-HT_7 activities.[9-10] Specifically，the promising compound **2** possessed good oral pharmacokinetic properties and exhibited significant antidepressant activity in *vivo*. Our SAR studies revealed that 3-propyl indole moiety produced 5-HT uptake inhibition pharmacophore moiety, while biphenyl piperazine moiety served as 5-HT_{1A}/5-HT_7 pharmacophore moiety. Meanwhile, the introduction of a fluoro group into the para-position of the piperazine ring could improve metabolic stability significantly. In order to expand upon the type of structures that can potentially improve SSRI/5-HT_{1A}/5-HT_7 activities and to probe the structure–

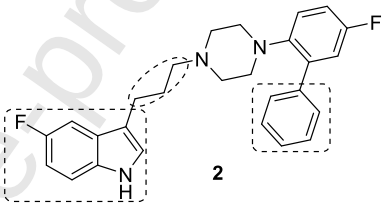
activity relationship (SAR), we replaced the biphenyl portion of **2** with other aromatic substituents. We herein describe the synthesis and biological evaluation of a series of novel aralkyl piperazine and piperidine derivatives. The general structure of target compounds **I** and **II** is shown in **Chart 2**.

All 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 several target compounds exhibited high affinities for 5-HT_{1A}/5-HT_7 receptors coupled with potent 5-HT reuptake inhibition and produced marked antidepressant-like effects *in vivo*.



1

IC_{50} (nM)	K_i (nM)	
RUI	5-HT_{1A}	5-HT_7
100	0.84	12



2

IC_{50} (nM)	K_i (nM)	
RUI	5-HT_{1A}	5-HT_7
25	28	3.3

Chart 1. Structures of SSRI/5-HT_{1A}/5-HT_7 agents

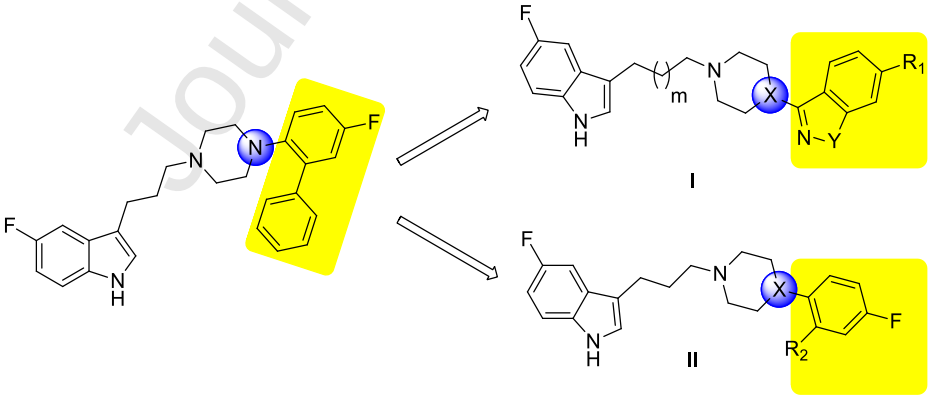


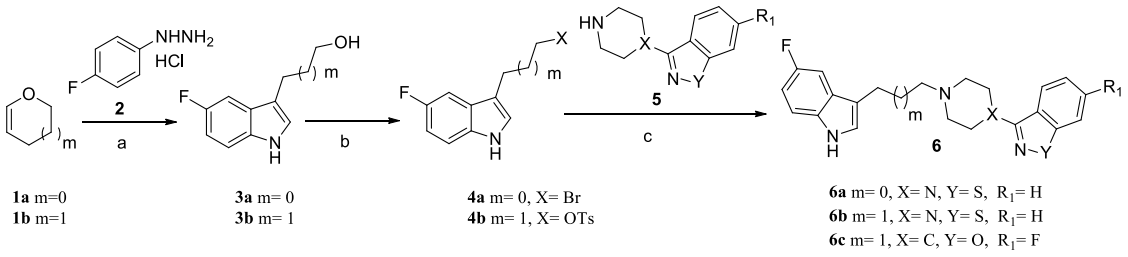
Chart 2. Rational design of novel SSRI/5-HT_{1A}/5-HT_7 activity compounds

The synthesis of target compounds was prepared as outlined in Schemes **1-4**. In Scheme **1**, a series of 5-fluoro-1H-indole piperazine and piperidine derivatives **6** were prepared. The alcohols **3a** and **3b** were obtained by the Fischer indole synthesis

reaction of 4-Fluorophenylhydrazine hydrochloride **2** with 2,3-dihydrofuran **1a** and 3,4-dihydro-2H-pyran **1b**, respectively. Bromination of compound **3a** with tetrabromomethane and triphenylphosphine provided compound **4a**. Activation of alcohol **3b** with 4-toluenesulfonyl chloride in the presence of triethylamine at room temperature provided compound **4b**. The desired compounds **6** were offered by nucleophilic substitution reaction of intermediates **4a-b** with aryl piperazine and piperidine derivatives **5**. The four carbon linker compound **11** was prepared (Scheme **2**). The acylation of the aromatic ring **7** with 4-chlorobutanoyl chloride provided compound **8**. Then intermediate **8** was reduced with triethylsilane and trifluoroacetic acid to yield compound **9**. The compound **10** was obtained by substitution of intermediate **9** with 3-(piperazin-1-yl)benzo[d]isothiazole. The deprotection of intermediate **10** with 4N aqueous NaOH solution gave the desired compound **11**.

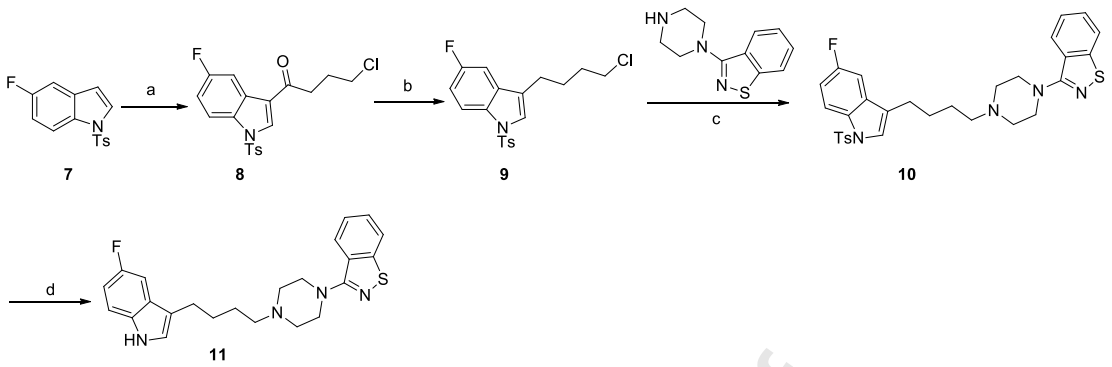
According to Scheme **3**, the 5-fluoro-1H-indole piperazine analogs **15** were prepared. 1-(4-fluorophenyl)piperazine **12** with *N*-bromosuccinimide in the presence of pyridine by electrophilic substitution reaction afforded intermediate **13**. The resulting compound **13** was then treated with 3-(5-fluoro-1H-indol-3-yl)propyl 4-methylbenzenesulfonate **4b** via S_N2 mechanism to obtain intermediate **14**. The target compounds **15** were obtained via the Suzuki cross coupling reaction of intermediate **14** with arylboronic acids.

The preparation of the desired 5-fluoro-1H-indole piperidine derivatives **19** was depicted in Scheme **4**. 4-(2-bromo-4-fluorophenyl)pyridine **16** was treated with arylboronic acids to yield intermediate **17** by Suzuki cross coupling reaction. The target compounds **19** was obtained by hydrogenation and substitution.

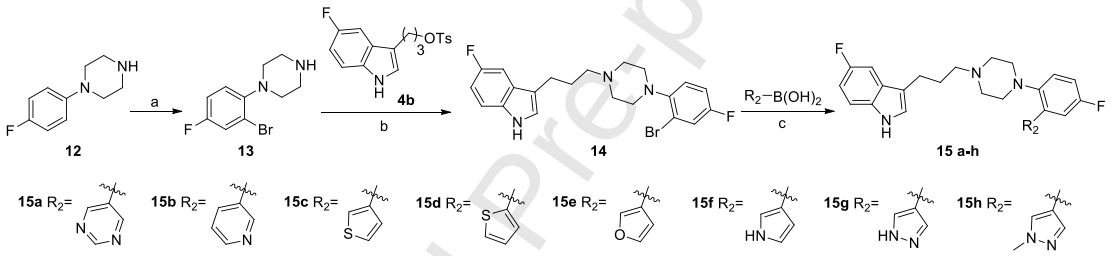


Scheme 1. Reagents and conditions: (a) H_2SO_4 (4%), DMAC, 100 °C; (b) for **4a**: CBr_4, PPh_3,

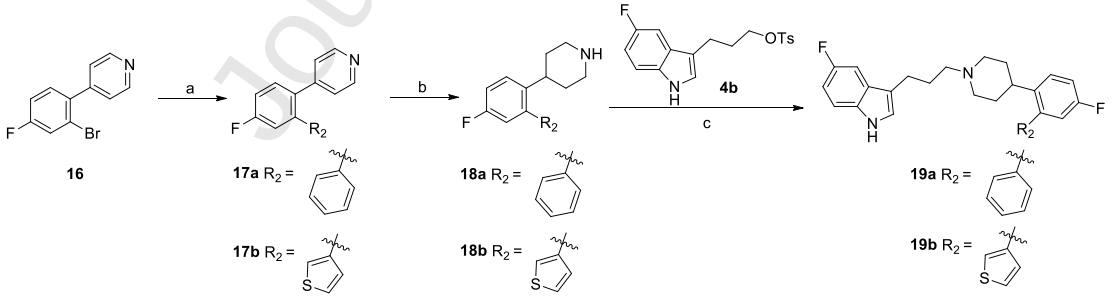
DCM, r.t.; for **4b**: TsCl, Et_3N, DMAP, DCM, r.t.; (c) for **6a**: K_2CO_3, KI, DMF, 100 °C; for **6b** and **6c** : K_2CO_3, CH_3CN, 80 °C.



Scheme 2. Reagents and conditions: (a) 4-chlorobutanoyl chloride, $AlCl_3$, DCM, 0 °C→r.t.; (b) $HSiEt_3$, TFA, 50 °C; (c) K_2CO_3, KI, DMF, 100 °C; (d) 4N NaOH, EtOH/THF, 80 °C.



Scheme 3. Reagents and conditions: (a) NBS, Pyridine, DCM, 0°C→r.t.; (b) K_2CO_3, CH_3CN, 80 °C; (c) $Pd(dppf)Cl_2$, Na_2CO_3, Dioxane/H_2O (3:1), 80 °C.



Scheme 4. Reagents and conditions: (a) $Pd(dppf)Cl_2$, Na_2CO_3, Dioxane/H_2O (3:1), 80 °C; (b) PtO_2, H_2, AcOH, r.t.; (c) K_2CO_3, CH_3CN, 80 °C.

All 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,[11] 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.[12] The inhibition of uptake of [^{3}H]-5-HT into rat brain synaptosomes through the serotonin transporter was also evaluated.[13] All compounds were initially screened at 10μM concentration, and then potent compounds (inhibition >90%) were assayed to obtain their IC_{50} values. The detail results were summarized in Table **1**. The metabolic stability of the test compounds was measured *in vitro* using rat and human liver microsomes, a system widely used to evaluate the susceptibility to first-pass oxidative metabolism (Table **2-3**).[14] The agonist property of preferred compound on 5-HT_{1A}R was evaluated in the [^{35}S]guanosine 5'-O-(3-thio)- triphosphate ([^{35}S]GTPγS) binding assay.[15] Additionally, the antagonist property of preferred compound against 5-HT_7R was tested in the cyclic adenosine 3', 5'-monophosphate (cAMP) functional cellular assays (Table **4**).[16]

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 firstly explored in series **I** (Chart **2**). The three-carbon chain analog **6b** displayed outstanding affinity for 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, K_i = 1.6 nM; 5-HT_7, K_i = 2.2 nM) and better 5-HT reuptake inhibitory activity (IC_{50} = 1.1 nM) (**6b** *vs* **6a**). Similarly, three-carbon chain analog **6c** exhibited higher inhibitory for 5-HT reuptake (IC_{50} = 0.92 nM) and showed more than 90% inhibition for 5-HT_{1A}/5-HT_7 receptors at a concentration of 10 μM (**6c** *vs* **6a**). However, the compound **11** showed dramatically decreased inhibition of 5-HT reuptake when the linker was elongated to four carbons (IC_{50} = 7.08 nM) (**11** *v* **6b**).

In our next series, we investigated the effects of replacing the biphenyl moiety with other aromatic substituents (Chart **2**). As shown in Table **1**, the six-membered aromatic heterocycle derivatives **15a-15b** showed more than 90% inhibition for 5-HT_{1A}/5-HT_7 receptors and 5-HT reuptake at 10μM. Meanwhile, the role of various substituted five-membered aromatic heterocycle groups was also explored. Surprisingly, the thiophen-3-yl analog **15c** displayed higher potency for the 5-HT_{1A}/5-HT_7 receptors and the 5-HT reuptake inhibition compared to **2** [RUI, IC_{50} = 4.0 nM; 5-HT_{1A}, K_i = 8.4 nM; 5-HT_7, K_i = 2.0 nM]. However, the thiophen-2-yl

analog **15d** showed dramatically decreased inhibition of 5-HT reuptake and reduced affinities for the 5-HT_{1A} and 5-HT_7 receptors (**15d** *vs* **15c**). Those results suggested that the position of aromatic heterocycle groups had a great influence on 5-HT reuptake/5-HT_{1A}/5-HT_7 activities. Similarly, the furan-3-yl analog **15e** and pyrazol-4-yl analog **15g-15h** also exhibited high inhibition for 5-HT reuptake and displayed excellent affinities for 5-HT_{1A}/5-HT_7 receptors, whereas pyrrol-3-yl analog **15f** showed selective inhibition for 5-HT_{1A}/5-HT_7 receptors (inhibition ratio > 90%).

Finally, the effect of the piperazine ring was explored. When the biphenyl feature remained, the replacement of the piperazine moiety with the piperidine moiety increased inhibition of 5-HT reuptake (IC_{50} = 14 nM) and binding affinities for 5-HT_{1A} (K_i = 12 nM) (**19a** vs **2**). Meanwhile, the thiophen-3-yl analog **19b** showed a increase in inhibition of 5-HT_{1A} (K_i = 2.6 nM) and a reduction in affinities for 5-HT_7 (K_i = 11 nM) (**19b** vs **19a**).

With the above information in hand, the potent compounds **6a-6c**, **11**, **15a-15c**, **15e**, **15g-15h** and **19a-19b** were selected for rat liver microsomal metabolic stability evaluation *in vitro*. The results are shown in Table **2**. The data indicated that compound **19a** displayed a better metabolic stability ($t_{1/2}$ values was 48.6 min). Moreover, compound **19a** was tested for human liver microsomal metabolic stability and the results indicated that **19a** showed an acceptable profile ($t_{1/2}$ values was 49.93 min) (Table **3**).

The functional properties of compound **19a** on 5-HT_{1A}R and 5-HT_7R were also evaluated. As shown in Table **4,** compound **19a** showed agonist property (EC_{50}=1180 nM) at 5-HT_{1A}R and antagonist effect (IC_{50}=650 nM) at 5-HT_7R *in vitro* functional activity assays.

Compounds **19a** was further selected for profiling in the mouse forced swim test (FST).[17] The compound was administered orally once daily to ICR male mice 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 (40 mg/kg/day, PO). The results are shown in Figure **1**. Compared with vehicle, the selected compound **19a** reduced immobility times in the FST in a dose-dependent manner that was statistically

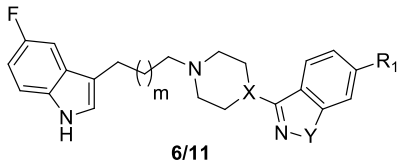
significant at 20 and 40 mg/kg (PO). The positive control, Vortioxetine, also produced a statistically significant reduction of immobility time at 40 mg/kg dose.

Compound **19a** was further characterized in the mouse tail suspension test (TST).[18] The results are shown in Figure **2**. Vilazodone was used as a reference compound (40 mg/kg/day, PO). The compound **19a** was administered orally once daily to ICR male mice for 7 days at doses of 10, 20 and 40 mg/kg/day (PO). Compared to the control group, compound **19a** was dose dependently reduced the immobility time in the TST, which was statistically significant at 20 and 40 mg/kg doses.

In summary, a novel series of aralkyl piperazine and piperidine derivatives were designed and synthesized. Several compounds showed outstanding binding affinities at the 5-HT_{1A}/5-HT_7 receptors and excellent inhibition of 5-HT reuptake. Especially, compound **19a** exhibited a good metabolic stability and showed potent efficacy *in vivo* in the animal test. In addition, compound **19a** was found to be an agonist at 5-HT_{1A} receptor and antagonist at 5-HT_7 receptor. It was suggested that compound **19a** could act as a potential candidate for a promising antidepressant. The preliminary results lay a foundation for the future development of novel derivatives targeting SSRI/5-HT_{1A}/5-HT_7 as potent multi-model antidepressant agents.

Table 1

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



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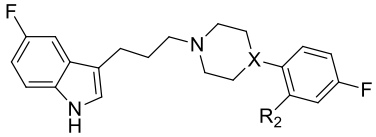


15/19

compd	RUI (IC_{50}, nM)	5-HT_{1A}(K_i, nM)	5-HT_7 (K_i, nM)
6a	23 [b]	1.6 [b]	0.99 [b]
6b	1.1 [b]	1.6 [b]	2.2 [b]
6c	0.92 [b]	100.7% [a]	99.8% [a]
11	7.08 [b]	100.7% [a]	98.5% [a]
15a	102.0% [a]	98.8% [a]	90.9% [a]
15b	104.5% [a]	99.1% [a]	100.2% [a]
15c	4.0 [b]	8.4 [b]	2.0 [b]
15d	21 [b]	38 [b]	11 [b]
15e	3.0 [b]	15 [b]	11 [b]
15f	78.9% [a]	97.4% [a]	99.1% [a]
15g	1.3 [b]	6.3 [b]	0.9 [b]
15h	2.7 [b]	8.4 [b]	1.0 [b]
19a	14 [b]	12 [b]	3.2 [b]
19b	14 [b]	2.6 [b]	11 [b]
Vortioxetine	2.9 [b]	9.5 [b]	26 [b]
Vilazodone	0.5[c]	0.2[c]	3900[c]

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

[b] 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).

[c] Data for Vilazodone was reported by Heinrich et al. [19]

Table 2

Rat Liver Microsomal Metabolic Stability Assay

Compd.	$T_{1/2}$ (min)	CL (μL/min/mg)
6a	3.1	452.1
6b	3.5	396.9
6c	4.6	300.9
11	3.4	402.8
15a	6.6	209.3
15b	5.8	239.7
15c	24.5	56.5
15e	10.3	134.0

15g	7.2	191.6
15h	9.5	146.4
19a	48.6	28.5
19b	18.33	75.6
Vortioxetine	9	153.2
Vilazodone	28.52	48.59
Omeprazole	8.5	162.6

Table 3

Human Liver Microsomal Metabolic Stability Assay

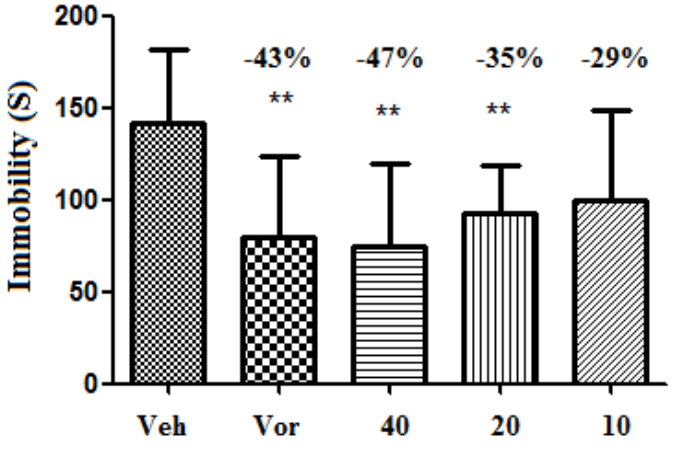
Compd.	$t_{1/2}$ (min)	CL (µL/min/mg)
19a	49.93	27.76
Vortioxetine	214.32	6.47
Vilazodone	53.32	26.00
Dextromethorphan	44.67	31.03

Table 4

5-HT_{1A} and 5-HT_7 Receptor functional activity assays

Compd.	5-HT_{1A}		5-HT_7	
	EC_{50}, nM	IC_{50}, nM	EC_{50}, nM	IC_{50}, nM
19a	1180	-	-	650
Serotonin	18	-	-	-
Methiothepin	-	-	-	0.82

(Functional assays were conducted by Eurofins Cerep SA, Celle L'Evescault, France).



compound **19a** (mg/kg, PO)

Fig. 1. Effect of treatment of mice with compound **19a** at graded doses on the immobility time in

the mouse 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.

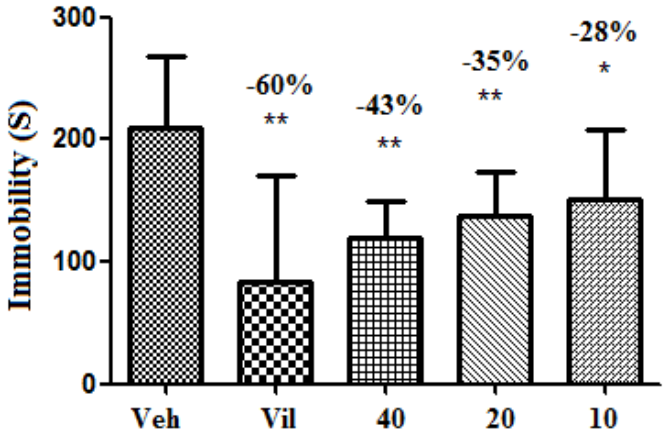


Fig. 2. Effect of treatment of mice with compound **19a** at graded doses on the immobility time in the mouse tail suspension test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

Acknowledgements

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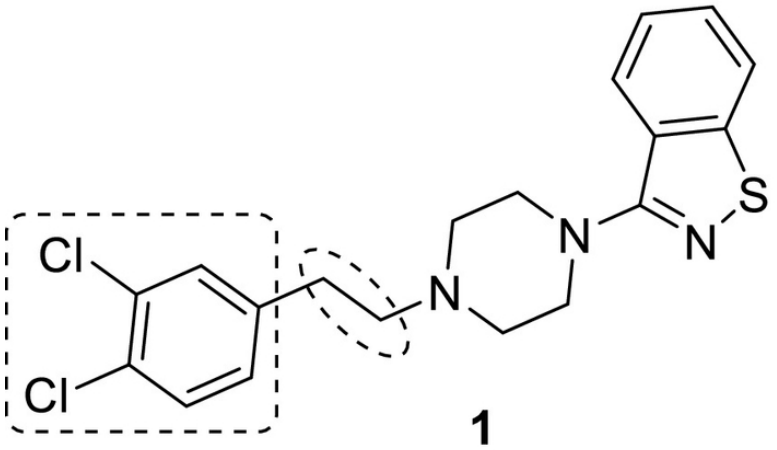
Highlights

- A series of aralkyl piperazine and piperidine derivatives have been synthesized.
- All compounds were evaluated for SSRI/5-HT_{1A}/5-HT_7 activities *in vitro.*
- The potent compounds were screened using the forced swimming test and the tail suspension test *in vivo*.
- Compound **19a** displayed a good metabolic stability and potent antidepressant-like profiles *in vitro* and *in vivo*.



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IC_{50} (nM)	K_i (nM)	
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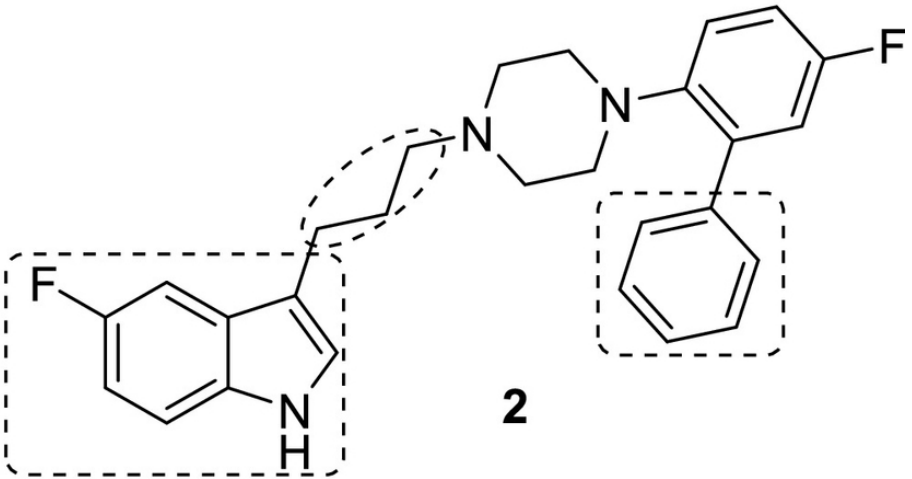


2

IC_{50} (nM)	K_i (nM)	
RUI	5-HT_{1A}	5-HT_7
25	28	3.3

Figure 1

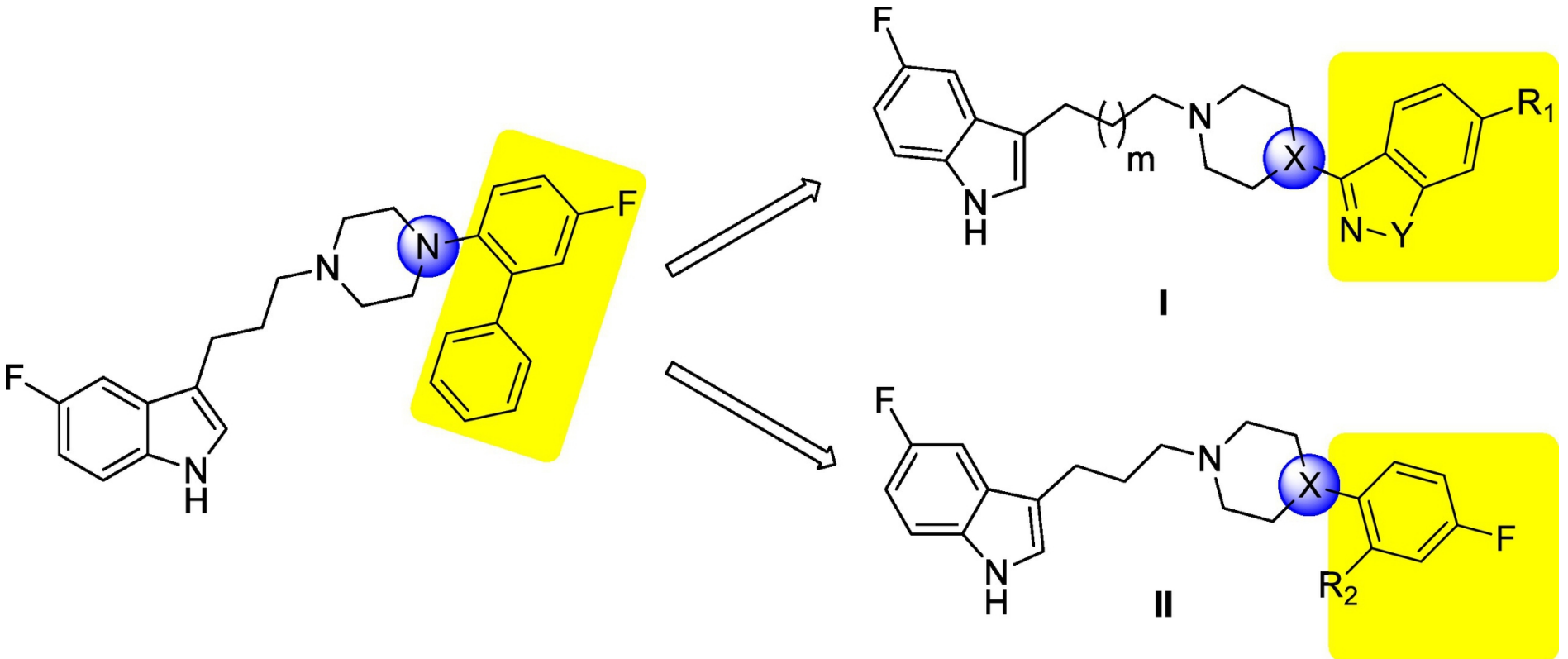
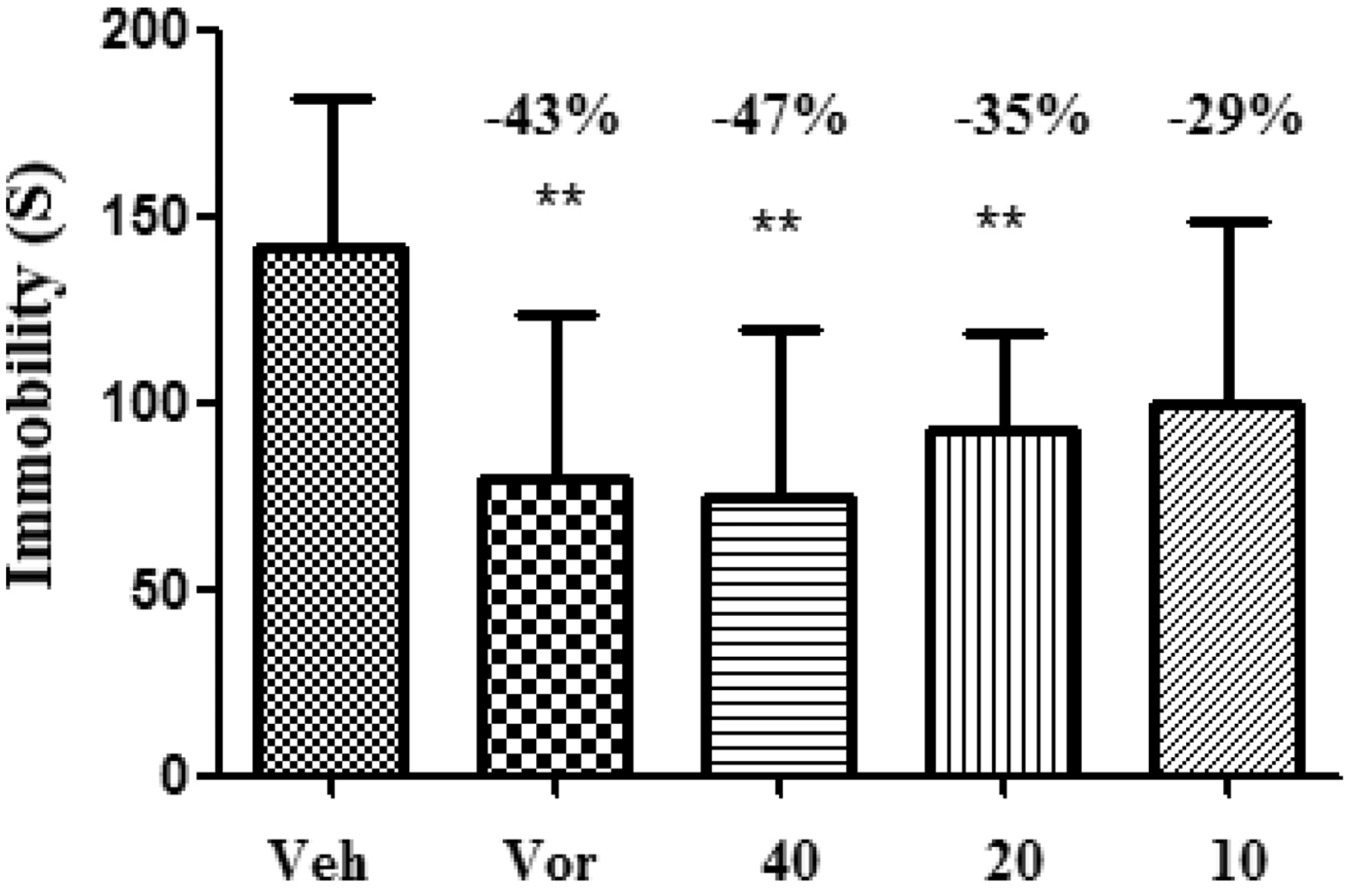
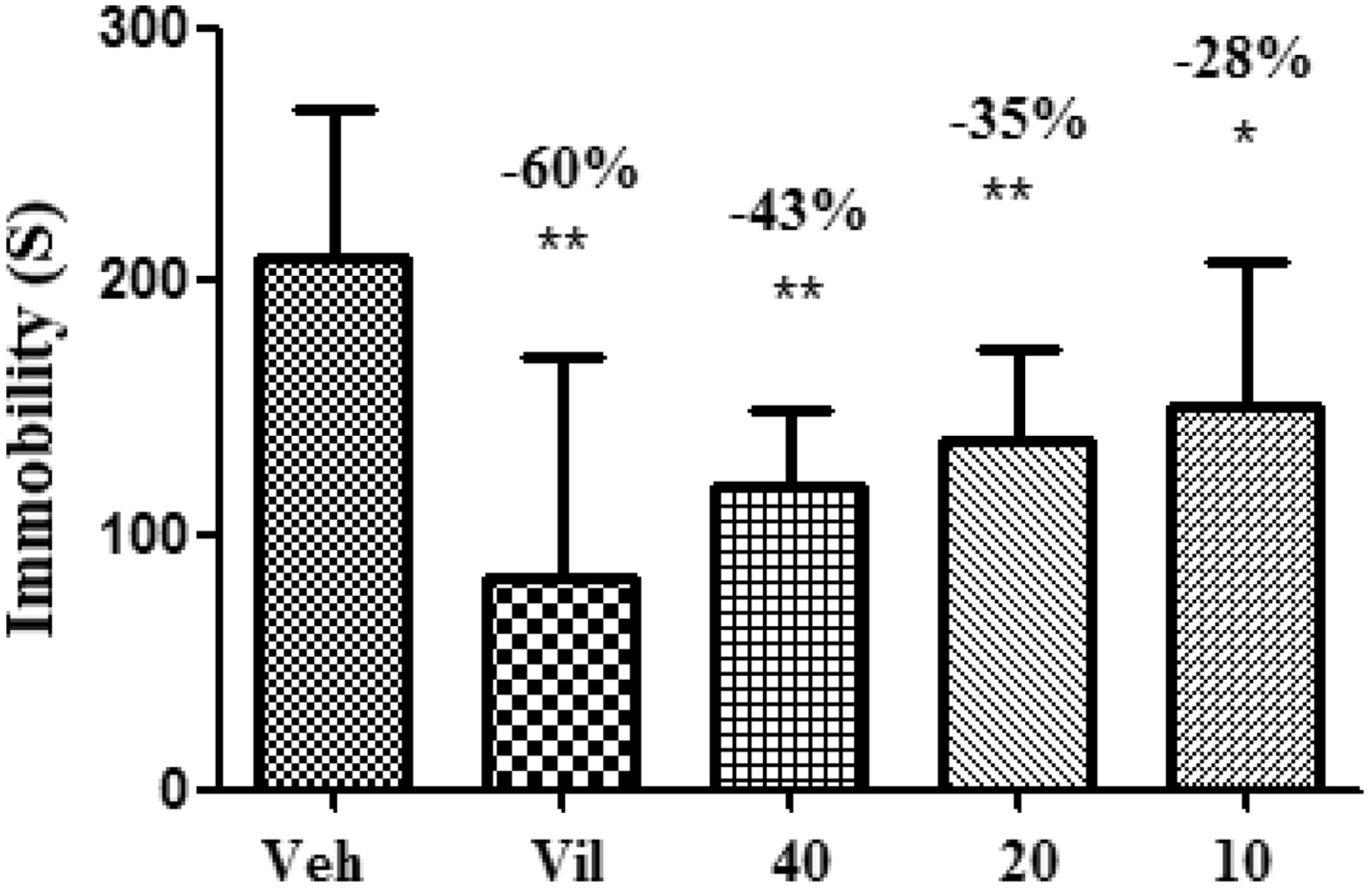


Figure 2



compound **19a** (mg/kg, PO)

Figure 3



compound **19a** (mg/kg, PO)

Figure 4