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Synthesis and antidepressant-like activity of novel alkoxy-piperidine derivatives targeting SSRI/5-HT_{1A}/5-HT_7

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Abstract

A series of novel alkoxy-piperidine derivatives were synthesized and evaluated for their serotonin reuptake inhibitory and binding affinities for 5-HT_{1A}/5-HT_7 receptors. *In vivo* antidepressant activities of the selective compounds were explored using the forced swimming test (FST) and tail suspension test (TST) in mice. The results showed that compounds **7a** (reuptake inhibition (RUI), IC_{50} = 177 nM; 5-HT_{1A}, K_i = 12 nM; 5-HT_7, K_i = 25 nM**)** and **15g** (RUI, IC_{50} = 85 nM; 5-HT_{1A}, K_i = 17 nM; 5-HT_7, K_i = 35 nM) were potential antidepressant agents in animal behavioral models with high 5-HT_{1A}/5-HT_7 receptor affinities and moderate serotonin reuptake inhibition, and good metabolic stability *in vitro*.

Keywords

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

Depression is a chronic and progressive mental disorder with heterogeneous etiology and symptoms and one of the major risk factors for suicide.[1] It is estimated that depression will become the second-largest global health burden and the main cause of disability among all diseases by 2030.[2,3] Although the treatment of depression has been advanced by traditional antidepressants, these drugs still highly unmet the clinical demands with limited efficacy including a long onset of action, moderate patient response, and numerous adverse effects such as weight gain, insomnia and sexual dysfunction.[4-7]

It was suggested that one of the most valuable approaches to enhance the therapeutic efficacy of antidepressants was a combination of SSRIs (selective serotonin reuptake inhibitors) with multiple actions on various 5-HT receptor subtypes.[8,9] SSRIs are believed to act by inhibiting serotonin reuptake into the presynaptic nerve terminals, thereby enhancing the synaptic concentration of serotonin (5-HT) and promoting neurotransmission. The effect of increasing neurotransmission and elevating 5-HT synaptic levels relieved depressive symptoms.[10] Furthermore, the agonism of 5-HT_{1A} receptor has been shown to accelerate or enhance the clinical efficacy of antidepressants represented by vilazodone.[11] The agonistic action of the post-synaptic 5-HT_{1A} receptor also enhances serotonergic neurotransmission, which facilitates the rapid onset of antidepressants.[12,13] Moreover, the 5-HT_7 receptor might be a significant target for the treatment of depression.[14] The blockade of 5-HT_7 receptor has been shown to be effective in the mouse forced swimming test, which has great predictive value for antidepressant-like activity.[15] The combined administration of an SSRI and 5-HT_7 receptor antagonist (SB-269970) also represents antidepressant-like responses in animal behavioral models.[16] The recently approved antidepressant vortioxetine exhibits potent serotonin reuptake inhibition and high 5-HT_{1A}/5-HT_7 receptor affinities associated with long-term safety and tolerability.[17,18] Therefore, compounds targeting on SSRI/5-HT_{1A}/5-HT_7 may act as novel potential antidepressants.

Results from earlier studies by Serafinowska et al. demonstrated that the central piperazine segment in derivative **Ⅰ** could be replaced by the 2-ethoxypiperdine linker in derivative **Ⅱ** to maintain or enhance serotonin transporter (SERT) blockade and high affinity for 5-HT_{1A} receptor.[19] Zajde et al. disclosed that arylsulfonamide derivatives of aryloxyethyl-piperidine **Ⅲ** bearing 2-ethoxypiperdine linker exhibited high affinity and moderate to good selectivity for the 5-HT_7 receptor (Chart.**1**).[20] In our previous studies on SSRI/5-HT_{1A}/5-HT_7, we reported a series of arylalkanol and aralkyl piperazine compounds (Chart.**1**, compound **Ⅳ**) with strong serotonin reuptake inhibition and nanomolar 5-HT_{1A}/5-HT_7 receptor affinities.[21] Since then we have been extending our research to alternatives by using the alkoxyl-piperdine segments as linker replacement (Chart.**2**).

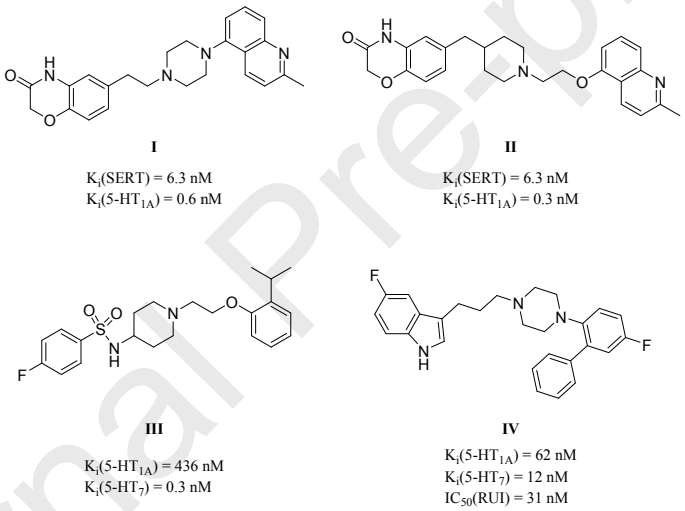
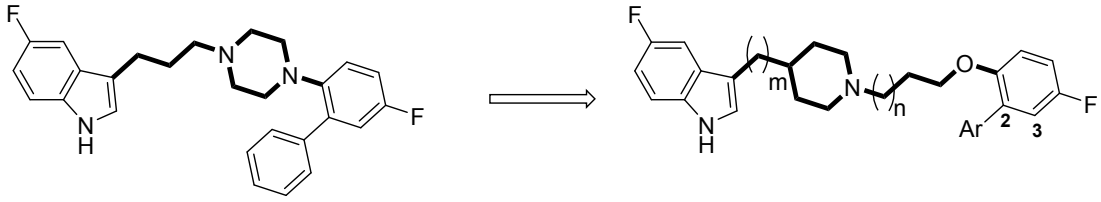


Chart 1. Structures of SSRI/5-HT_{1A} agents and 5-HT_{1A}/5-HT_7 agent

Herein we reported the synthesis and pharmaceutical evaluation of a series of alkoxy-piperidine derivatives (Chart.**2**). Compounds were evaluated for binding affinities for 5-HT_{1A}/5-HT_7 receptors and serotonin reuptake inhibition *in vitro.* The selected compounds were tested for microsomal stabilities *in vitro* and antidepressant-like activities *in vivo*. Preliminary results indicated that the target compounds exhibited high affinities for 5-HT_{1A}/5-HT_7 receptors coupled with moderate serotonin reuptake inhibition and marked antidepressant-like effect *in vivo*.



IV V

Chart 2. The design of alkoxy-piperidine derivatives

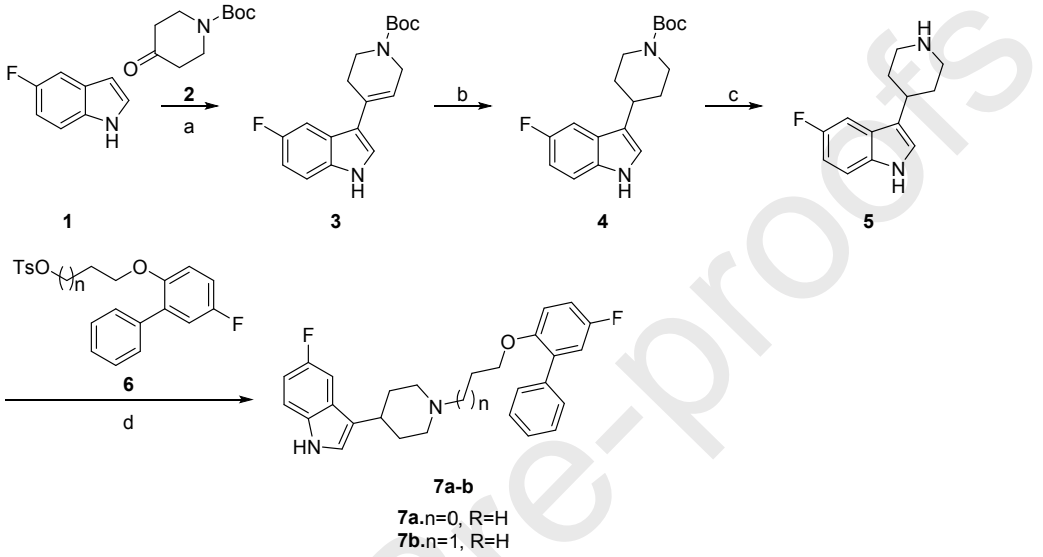
The synthetic routes of all target compounds were outlined in Scheme **1-5**. In Scheme **1**, a series of 3-(piperidin-4-yl)-1*H*-indole derivatives **7** were prepared. Intermediate **3** was obtained by addition of 5-fluoro-1*H*-indole **1** to *tert*-butyl 4-oxopiperidine-1-carboxylate **2** under basic conditions. Then **3** was hydrogenated to provide intermediate **4**. The deprotection of the Boc group of intermediate **4** with 4N HCl/MeOH solution gave intermediate **5**. Then **5** was treated with ([1,1'-biphenyl]-2-yloxy)alkyl 4-methylbenzenesulfonate **6** via SN2 mechanism to yield target compounds **7a-b**.

Shown in Scheme **2** was the preparation of the 3-(piperidin-4-ylmethyl)-1*H*-indole derivatives **12a-b**. Reaction of 5-fluoro-1*H*-indole **1** with 4-pyridinecarboxaldehyde **8** afforded intermediate **9**. Then **9** was reduced under triethylsilane and trifluoroacetic acid conditions to yield intermediate **10**. The target compounds **12a-b** were prepared by the followed hydrogenation and substitution steps.

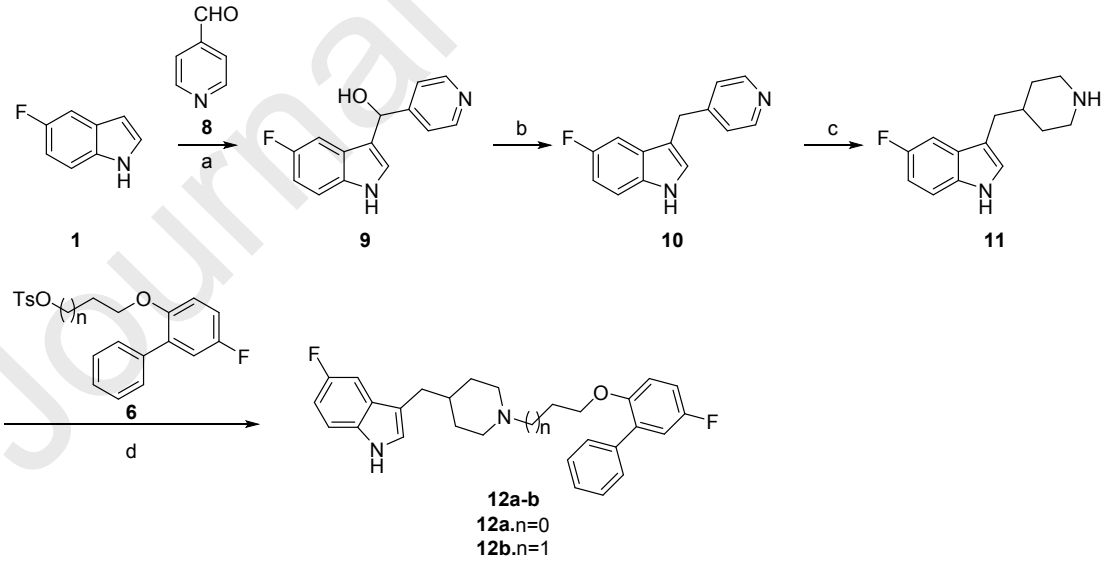
The synthesis of compounds **15a-g** was carried out by a procedure according to Scheme **3**. Substitution of 5-fluoro-3-(piperidin-4-yl)-1*H*-indole **5** with 2-bromo-1-(2-bromoethoxy)-4-fluorobenzene **13** provided intermediate **14.** Suzuki cross-coupling reaction of **14** with arylboronic acid obtained the target compounds **15a-g**.

Scheme **4** showed the preparation of compound **19**. Intermediate **17** was obtained by reaction of 5-fluoro-1*H*-indole **1** with 4-vinylpyridine **16** under refluxing acetic acid conditions. Then hydrogenation of **17** using platinum oxide as a catalyst provided

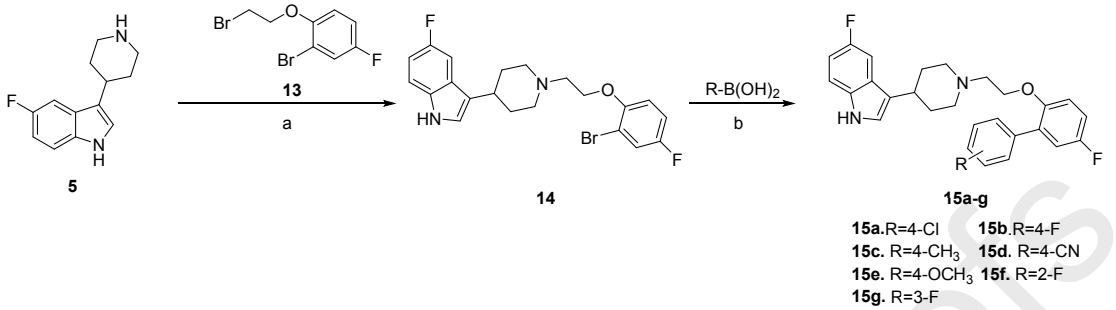
intermediate **18**. The target compound **19** was obtained by substitution via SN2 mechanism. Alternatively, the substitution of intermediate **5** with **20** provided the target compound **21** (Scheme **5**).



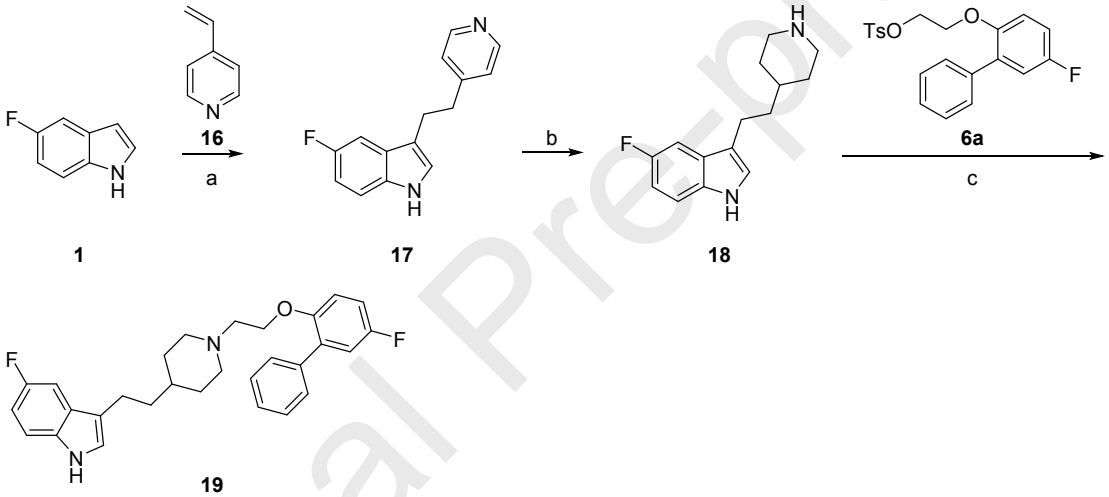
Scheme 1. Reagents and conditions: (a) KOH, CH_3OH, 70 °C; (b)10% Pd/C, H_2, MeOH/THF (1:1), r.t.; (c) 4N HCl/MeOH, MeOH, r.t.; (d) K_2CO_3, KI, CH_3CN, 80 °C.



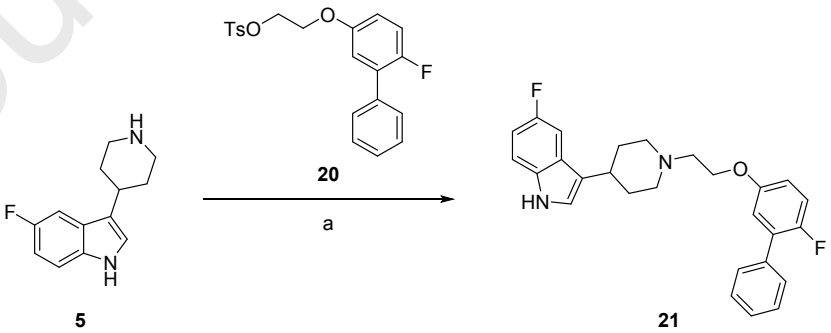
Scheme 2. Reagents and conditions: (a) 50% NaOH, CH_3OH, 0 °C→r.t.; (b) $HSiEt_3$, TFA, DCM, r.t.; (c) PtO_2, H_2, AcOH, r.t.; (d) K_2CO_3, CH_3CN, 80 °C.



Scheme 3. Reagents and conditions: (a) K_2CO_3, KI, CH_3CN, 80 °C; (b) $Pd(dppf)Cl_2$, Na_2CO_3, Dioxane/H_2O (3:1), 80 °C.



Scheme 4. Reagents and conditions: (a) AcOH, 120 °C; (b) PtO_2, H_2, AcOH, r.t.; (c) K_2CO_3, CH_3CN, 80 °C.



Scheme 5. Reagents and conditions: (a) K_2CO_3, CH_3CN, 80 °C.

All synthesized compounds were tested for their inhibition of serotonin reuptake

and binding affinities for 5-HT_{1A}/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[22], and the affinity for the 5-HT_7 receptor was determined by displacement binding to CHO cell membrane homogenates using [^{3}H]-LSD to previous procedures reported.[23] The inhibition of [^{3}H]-5-HT incorporation into rat brain synaptosomes through the serotonin uptake transporter was also evaluated[24]. All compounds were initially screened at 1μM concentration, and potent compounds, which inhibition of serotonin reuptake and binding affinities at 5-HT_{1A}/5-HT_7 receptors all over 90%, were then assayed to obtain their IC_{50} and K_i values. The 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**)[25].

Firstly, we investigated the effects of linker lengths on serotonin reuptake inhibitory and binding affinities for 5-HT_{1A}/5-HT_7 receptors. The length of the chain linking the indole ring to the piperidine was first explored. 5-fluoro-3-(piperidin-4-yl)-1*H*-indole analog **7a** displayed more than 90% inhibition for serotonin reuptake and 5-HT_{1A}/5-HT_7 receptor at a concentration of 1μM, whereas **12a** and **19** exhibited selective inhibition for serotonin reuptake and 5-HT_7 receptor (inhibition ratio > 90%). The result indicated that the indole ring directly linking to piperidine ring was beneficial to increase the affinity for 5-HT_{1A} receptor. The influence of the chain linking the of piperidine ring to the 2-alkoxy-1,1'-biphenyl moiety was also explored. The three-carbon chain analog compound **7b** displayed selective inhibition for the 5-HT_{1A} receptor (**7b** *vs* **7a**). Similarly, compound **12b** exhibited potential high inhibition for 5-HT_7 receptor (inhibition ratio > 85%) merely (**12b** *vs* **12a**). These results suggested that the length of the linker had a great influence on the affinities for 5-HT_{1A}/5-HT_7 receptors.

Next, we focused on the substitution position of the phenyl group at the 4-fluorophenoxyl fragment. Interestingly, compound **21** showed over 90% inhibition for 5-HT_{1A} receptor and potential moderate serotonin reuptake and 5-HT_7 receptor

inhibition (Inhibition ratio > 50%) after moving the phenyl group with one position, whereas 2-ethoxy-5-fluoro-1,1'-biphenyl derivative **7a** exhibited moderate inhibition for serotonin reuptake (IC_{50} = 177 nM) and high affinities for the 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, K_i = 12 nM; 5-HT_7, K_i = 25 nM) as shown in **Table 1** and **Table 2**.

Finally, the effect of different aromatic ring substituents at the C2 position was explored. We first introduced various R substitutes into the para-position of the phenyl group in **15**. Compounds **15a**, **15c-15e** all showed selective inhibition for 5-HT_{1A} receptors (inhibition ratio > 90%) but expressed less than 90% inhibition for dual serotonin reuptake and 5-HT_7 receptor at a concentration of 1μM. Interestingly, the 4-fluorophenyl derivative **15b** exhibited high inhibition for dual serotonin reuptake and 5-HT_{1A} receptor and potential high inhibition for 5-HT_7 receptor (inhibition ratio > 85%). These results indicated that the introduction of fluorophenyl group substituent led to maintain or enhance the multiple actions on SSRI/5-HT_{1A}/5-HT_7 possibly. Then we introduced a fluorophenyl into the ortho-, meta-position of the phenyl group subsequently. The 2-fluorophenyl derivative **15f** also showed high inhibition for dual serotonin reuptake and 5-HT_{1A} receptor and potential high inhibition for 5-HT_7 receptor (inhibition ratio > 85%). Especially, the 3-fluorophenyl derivative **15g** displayed excellent inhibition for the 5-HT_{1A}/5-HT_7 receptors (5-HT_{1A}, K_i = 17 nM; 5-HT_7, K_i = 35 nM) and exhibited higher inhibition for serotonin reuptake inhibitory (RUI, IC_{50} = 85 nM).

With the above information in hand, compounds **7a** and **15g** were selected for metabolic stability evaluation *in vitro*. The results indicated that the two compounds displayed a suitable half-life ($t_{1/2}$ values were 34.5 min and 22.9 min, respectively), which was better than vortioxetine in rat liver microsomal stability assay ($t_{1/2}$ value was 9.0 min). Additionally, **7a** and **15g** were also tested for human liver metabolic stability and showed acceptable profiles ($t_{1/2}$ values were 61.5 min and 61.4 min, respectively) (**Table 3**).

On the basis of *in vitro* studies, compounds **7a** and **15g** were further characterized in the mouse forced swimming test (FST)[26]. **7a** and **15g** were tested at

the dose range of 10, 20 and 40 mg/kg/day (PO) by repeated administration in male mice for 7 days, respectively. As shown in **Fig.1-2**, **7a** and **15g** both produced a reduction effect at different doses in the FST and values were statistically significant at 40 mg/kg (PO). The effect of positive control, vortioxetine, was also statistically significant at 40 mg/kg (PO).

Compounds **7a** and **15g** were also selected for further evaluation in the mouse tail suspension test (TST)[27]. The results are shown in **Fig.3-4**. The compounds were dosed at 10, 20 and 40 mg/kg/day (PO) by repeated administration in male mice for 7 days. **7a** and **15g** both produced a reduction effect at different doses in the TST. Especially, compared with vehicle, **7a** reduced immobility time in the TST statistically significant at 40 mg/kg (PO).

In summary, a series of novel alkoxy-piperidine derivatives were synthesized as our continuing effort to identify potential antidepressant agents targeting SSRI/5-HT_{1A}/5-HT_7. The *in vitro* studies revealed that the length of linker had an important effect on the affinities for 5-HT_{1A}/5-HT_7 receptors. Several compounds displayed outstanding binding affinities for 5-HT_{1A}/5-HT_7 receptors and strong inhibition of serotonin reuptake. Notably, compounds **7a** and **15g** exhibited marked antidepressant-like activity in animal behavioral models. Further studies are ongoing to fully appreciate the therapeutic potential of novel alkoxy-piperidine 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.[a]

Compd.	RUI (Inhibition ratio)	5-HT_{1A} (Inhibition ratio)	5-HT_7 (Inhibition ratio)
7a	96.3%	94.2%	90.4%
7b	87.0%	97.7%	76.1%
12a	100.9%	72.3%	97.2%
12b	101.5%	81.9%	86.4%
15a	27.3%	95.0%	80.3%
15b	90.6%	97.4%	86.5%
15c	38.9%	94.5%	86.2%
15d	63.0%	97.5%	95.8%
15e	12.3%	98.7%	95.9%

15f	97.1%	97.8%	88.7%
15g	94.3%	95.9%	91.0%
19	99.8%	54.3%	94.3%
21	66.6%	96.9%	54.2%
Vortioxetine	101.1%	95.1%	92.5%

[a] Percent inhibition measured at a concentration of 1μM.

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)
7a	177	12	25
15g	85	17	35
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

Microsomal Metabolic Stability Assay

Compd.	Species	$t_{1/2}$ (min)	CL (μL/min/mg)
7a	Rat	34.5	40.2
15g		22.9	60.4
Vortioxetine		9.0	153.2
Omeprazole		8.5	162.6
7a	Human	61.5	22.5
15g		61.4	22.6
Vortioxetine		214.3	6.5
Dextromethorphan		44.7	31.0

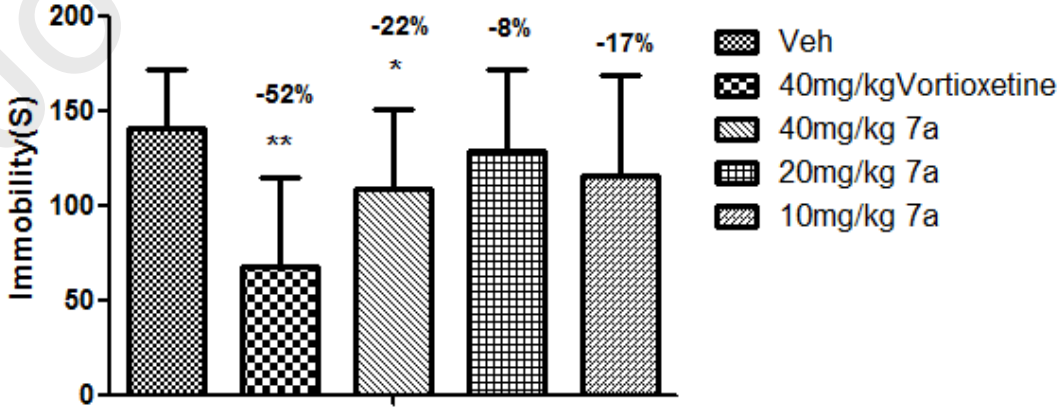


Fig. 1. Effect of treatment of mice with compound **7a** at graded doses on the immobility time in the forced swim test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

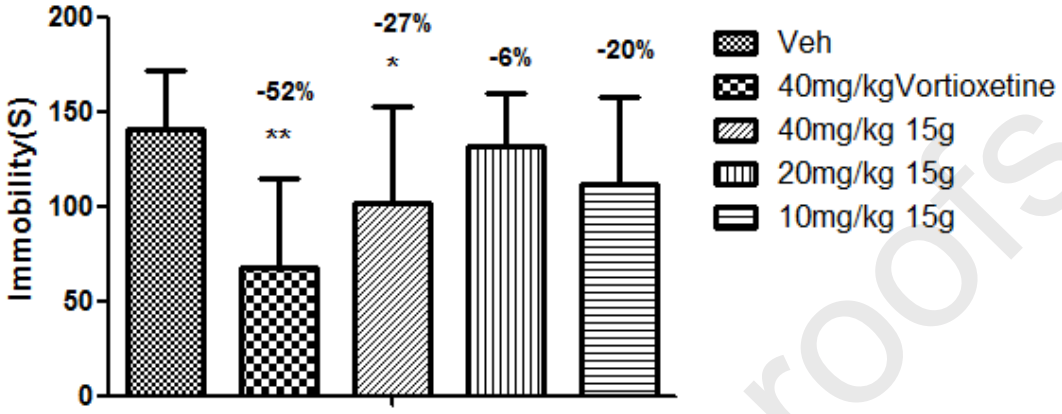


Fig. 2. Effect of treatment of mice with compound **15g** at graded doses on the immobility time in the forced swim test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

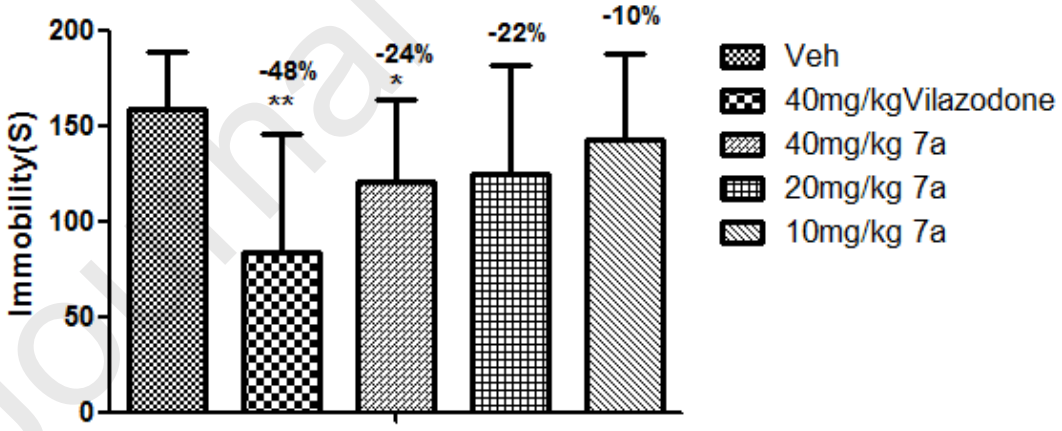


Fig. 3. Effect of treatment of mice with compound **7a** at graded doses on the immobility time in the tail suspension test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

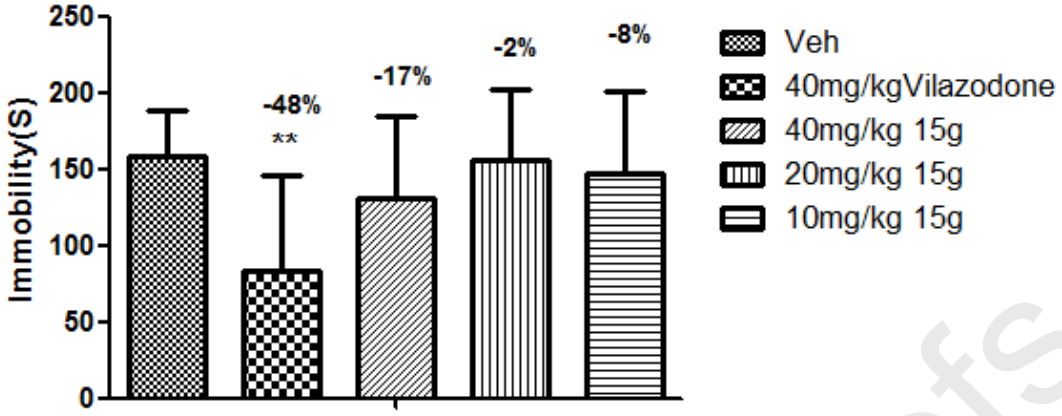


Fig. 4. Effect of treatment of mice with compound **15g** at graded doses on the immobility time in the tail suspension test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

Acknowledgments

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Synthesis and antidepressant-like activity of novel alkoxy-piperidine derivatives targeting SSRI/5-HT_{1A}/5-HT_7

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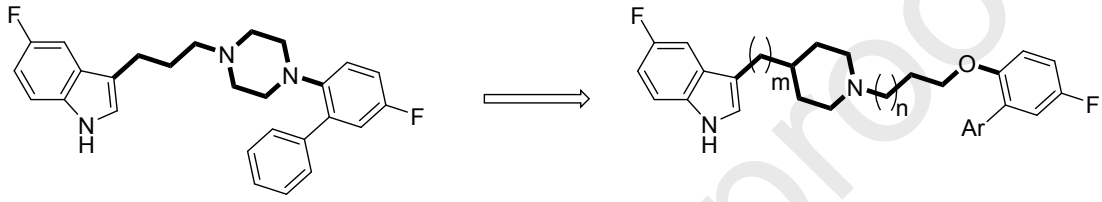
Graphical abstract



IV
Lead Compound

V
Target Compound

Highlights

- A series of novel alkoxy-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*.
- Compounds **7a** and **15g** were potential antidepressant agents in animal behavioral models, and good metabolic stability *in vitro*.