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Discovery of a new class of multi-target heterocycle piperidine derivatives as potential antipsychotics with pro-cognitive effect

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ABSTRACT

A series of benzoisoxazoleylpiperidine derivatives were synthesized by using the multi-target strategies and their potent affinities for dopamine (DA), serotonin (5-HT) and human histamine H_3 receptors have been evaluated. Of these compounds, the promising candidate **4w** displayed high affinities for D_2, D_3, 5-HT_{1A}, 5-HT_{2A} and H_3, a moderate affinity for 5-HT_6, negligible effects on the human ether-a-go-go-related gene (hERG) channel, low affinities for off-target receptors (5-HT_{2C}, adrenergic α_1 and H_1). In addition, the animal behavioral study revealed that, compared to risperidone, compound **4w** significantly inhibited apomorphine-induced climbing and MK-801-induced movement behaviors with a high threshold for catalepsy and low liabilities for weight gain and hyperprolactinemia. Results from the conditioned avoidance response test and novel object recognition task demonstrated that **4w** had pro-cognitive effects. Thus, the antipsychotic drug-like activities of **4w** indicate that it may be a potential polypharmacological antipsychotic candidate drug.

Schizophrenia is a severe neuropsychiatric disorder that affects approximately 1% of the population[1]. Currently, the primary therapeutic avenues for schizophrenia include pharmaceutical treatments, such as the first-generation antipsychotics (FGAs), includes thioridazine, chlorpromazine and haloperidol[2]. Second-generation antipsychotics (SGAs) includes clozapine[3] and risperidone[4], and the novel antipsychotics brexpiprazole and cariprazine[5]. Although significant progress has been made in the treatment of schizophrenia and current medication regimens have favorable levels of effectiveness, there was no antipsychotic drug can effectively eliminate all symptoms and none can fully address the cognitive deficits caused by schizophrenia[6]. An ideal antipsychotic medication should not only possess therapeutic efficacy but also markedly alleviate or eliminate all symptoms, be free of side effects and/or minimize possible negative consequences (e.g., reduce the inhibition of hERG channels, weight gain, hyperglycemia), and improve memory and cognition. Therefore, research and development of more safer and effective antipsychotic medicines still in great need.

Viewed from the target's perspective, the dopamine D_2 receptors and serotonin receptors (especially the 5-HT_{1A} and 5-HT_{2A} receptors) are crucial targets for schizophrenia[7]. Most of the available antipsychotic drugs exert their effect by inhibiting the activity of D_2 receptor, and it is the direct and effective way to treat schizophrenia. Several recent studies found the partial dopamine agonism can reduce the risk for hyperprolactinaemia, even decrease the risk of sexual dysfunction, which beneficial to improve the safety and tolerance in the clinical treatment[8,9]. The functioning of 5-HT_{1A} receptor may be described as antagonistic to the serotonin 5-HT_{2A} receptor. Activation of the 5-HT_{1A} receptor will be conducive to alleviating the negative symptoms and cognitive disorders in patients with schizophrenia[10]. 5-HT_{2A} receptor antagonism has been implicated in the enhanced efficacy against negative schizophrenic symptoms, and counteracts excessive D_2 receptor blockade and which significantly reduce extrapyramidal effects[11]. There is plenty of evidence strongly supports that combined effects on D_2 and 5-HT_{2A} receptors is beneficial to the improvement of both negative symptoms and symptoms positive of schizophrenia[12]. Thus, developing novel multi-target ligands that can accurately modulate and balance activities at dopaminergic and serotoninergic receptors, especially the D_2, 5-HT_{1A} and 5-HT_{2A} receptors, and simultaneously decrease the affinity for other off-target receptors is an interesting and potentially useful pursuit[4].

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Using this strategy, previous studies from our research group have developed a series of novel compounds based on benzoisoxazoleylpiperidine. Several of these compounds significantly alleviate schizophrenia-like symptoms (inhibit the apomorphine-induced climbing behavior and MK-801-induced hyperactivity), are associated with lower weight gain and lower prolactin levels, displayed procognition properties in a novel object recognition task in rats, and have obvious therapeutic effects.[13–16] And their binding affinities for the various receptors that associated with schizophrenia were summarized in the Table 1. Compound **S1**, **S2** and **S3** all showed high affinity for D_2, 5-HT_{1A} and 5-HT_{2A} receptors. In addition, compounds **S1** and **S3** exhibited good affinity for D_3 receptors, **S2** and **S3** showed high strong 5-HT_6 receptor potency. Moreover, all of them showed lower affinities for the off-target receptors (5-HT_{2c}, α_1 and H_1). Based on this research, we're trying to endow the new compounds with additional H_3 receptor potency, as a large amount of scientific data have demonstrated that actions at the histamine H_3 and 5-HT_6 receptor may constitute a promising approach to treat the cognitive symptoms in schizophrenia[17,18]. The histamine H_3 receptor is a member of the G protein-coupled receptor (GPCR) family[19]. It is an important regulator of several neurotransmitters, including DA, 5-HT, acetylcholine, and norepinephrine[20]. Pharmacological studies have shown that H_3 receptor antagonists and inverse agonists have potential applications in the therapy of various CNS disorders, including cognitive deficits, depression, Alzheimer's disease, Parkinson's disease, epilepsy, and schizophrenia[21]. For example, the H_3 antagonist Bavisant currently on the market has been used as a therapeutic intervention for the promotion of wakefulness, and animal studies have already demonstrated that the H_3 receptor antagonists ABT-239 and A-431404 have the potential to ameliorate cognitive deficits relevant to schizophrenia whereas risperidone and olanzapine do not [22,23]. Many antischizophrenia drugs available have no or low H_3R affinity, such as chlorpromazine and risperidone[24,25], even the novel atypical antipsychotics aripiprazole[5]. Therefore, adding on H_3 receptor antagonism to mixed D/5-HT receptors antagonist profile will contribute to the cognitive function for patients with schizophrenia, and with batter therapeutic effects.

To verify this multi-receptor affinity strategy, a series of novel compounds was synthesized (Table 2) by using the molecular hybridization method[26,27]. This strategy for design multi-target ligands as shown in Fig. 1, the benzoisoxazoleylpiperidine groups were reserved as optimal structure to guarantee their effects on D_2, 5-HT_{1A} and 5-HT_{2A} receptors as they are crucial DA and 5-HT pharmacophores of commercial atypical antipsychotics such as risperidone, paliperidone and brexpiprazole. In addition, the privileged fragments of reported H_3 receptor antagonists[13] (**S4** and Pitolisant) were introduced. Furthermore, the molecular structures of the novel compounds were further optimized according to pharmacological actions and pharmacodynamics to maximize the therapeutic benefits. Finally, extensive structure–activity relationship (SAR) studies, including in *vitro* receptor profiles and in *vivo* behavioral studies, were conducted. Among these compounds, **4w** demonstrated best in *vitro* activities, and exhibited potent antipsychotic effect in *vivo* behavioral studies. Especially, compound **4w** exhibited pro-cognitive properties and negligible side effects (weight gain and hyperprolactinemia). As a result, the compound **4w** was developed as an atypical antipsychotic candidate for the treatment of schizophrenia based on the D_2, 5-HT_{1A}, 5-HT_{2A} and H_3 multi-target profile.

The syntheses of intermediates (**1a**, **1b**, **2a-2af**, **3**) and the compounds **4a-4w** are illustrated in Scheme 1. The **1a, 1b** were synthesized through benzoisoxazoleylpiperidine reacted with appropriate haloalkanes in the presence of anhydrous potassium carbonate. The compounds **2a-2ab** were prepared from p-hydroxy benzaldehyde and its derivatives via the Mannich reaction in the presence of pyrrolidine, piperidine, morpholine, or their derivatives and sodium triacetoxyborohydride. **2ac-2af** prepared by reaction of pyrrolidine, piperidine, morpholine, or their derivatives with 4-(2-bromoethyl) phenol in the presence of diisopropylethylamine. There are two methods to obtain the desired **4a-4w**. For Method A, it's suitable for synthesize the important intermediate which without substituent on the benzene ring. For example, intermediate **3** was obtained by reaction of **1a** with p-hydroxybenzaldehyde in the presence of potassium carbonate, furthermore, get the desired product **4a** and **4j**. The method **B** was utilized to prepare the compounds with substituents on benzene ring. By using this method, it's easy to synthesize purity and separate the intermediates **2ac-2af**. The intermediates **1a** and **1b** prepared from method **A** were reacted with the derivatives of **2a-2ab** and **2ac-2af** yielded the target compounds **4a-4w.** Combination of two methods provides an effective way to synthesize the different target compounds, saved lot of tediously separate process.

The SARs of the synthesized compounds were preliminarily evaluated in *vitro*, and the compounds with desired activities were selected and further characterized for safety in terms of pharmacology. Finally, the most promising candidate was subjected to behavioral study and pharmacokinetic evaluation.

The in *vitro* evaluations results (*Ki* values) were shown in Table 2. All of the compounds exhibited affinities for D_2, 5-HT_{1A} and 5-HT_{2A} to some extent. In addition to these three receptors, these compunds also showed affinity for the H_3 receptor; however, this affinity varied between the pharmacophores. From an integral view, most of the compounds displayed higher affinities for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors, which may be attributed to the benzoisoxazoleylpiperidine groups in their structure.

The effects of cyclic *N*-heterocyclic links to phenoxy structures were assessed and the present results showed the cyclic *N*-heterocyclic play an important role in SARs. Most of compounds with a morpholinyl substitution exhibited higher affinity for the desired four receptors than with pyrrolidine and pyridine substitution (e.g. **4q** *vs* **4d** and **4i**. Table 2), but the compound with *N*-methyl piperazinyl and 4-methylpiperidyl substitution displayed higher affinities for H_3 receptor (**4j** *vs* **4r-4u**). When pyrrolidinyl in compound **4e** was replaced by *N*-methyl piperazine, it formed compound **4t**, which showed improved affinities for all the four

Table 1
Binding Affinities for the Receptors Associated with Schizophrenia of Compounds S1-S3.

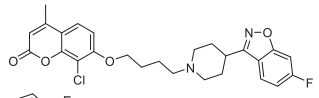
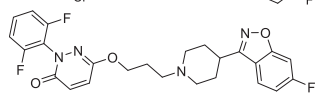
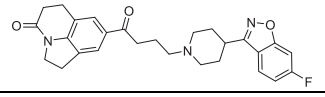
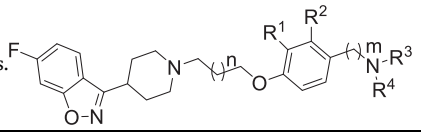
Compound	Receptor affinity K_i (nM)							
	D_2	D_3	5-HT_{1A}	5-HT_{2A}	5-HT_{2c}	5-HT_6	α_1	H_1
S1 	2.6	4.3	3.3	0.3	1700.7	228.4	2569.2	1125.3
S2 	5.0	35.7	5.9	0.3	1584.1	0.5	337.2	1998.0
S3 	2.9	1.7	8.6	0.7	616.0	5.6	43.1	630.1

Table 2

Binding affinities for D_2, 5-HT_{1A}, 5-HT_{2A} and H_3 receptors of compounds **4a**-**4w** and reference antipsychotics.



Cmpd	R_1	n	m	R_2	NR_3R_4	Receptor affinity Ki ± SEM (nM)[a]			
						D_2	5-HT_{1A}	5-HT_{2A}	H_3
4a	H	1	1	H		12.2 ± 1.2	81.6 ± 5.3	8.6 ± 0.5	93.7 ± 13.7
4b	Cl	1	1	H		14.1 ± 0.3	79.2 ± 8.5	10.1 ± 0.8	125.3 ± 9.8
4c	H	1	1	CH_3		12.6 ± 0.7	89.3 ± 11.3	11.5 ± 1.5	112.6 ± 10.5
4d	OCH_3	1	1	H		6.2 ± 0.7	77.9 ± 5.4	10.8 ± 1.9	151.2 ± 13.4
4e	H	1	1	H		10.8 ± 1.9	89.7 ± 6.9	5.1 ± 0.6	269.5 ± 37.5
4f	H	1	2	H		9.7 ± 0.5	7.4 ± 0.8	9.4 ± 0.8	13.4 ± 3.2
4g	F	1	1	H		10.3 ± 1.2	90.6 ± 5.5	6.5 ± 0.5	270.1 ± 32.0
4h	H	1	1	F		11.6 ± 0.9	92.1 ± 8.0	6.2 ± 0.4	312.3 ± 27.5
4i	OCH_3	1	1	H		9.5 ± 1.1	67.7 ± 5.4	9.0 ± 0.8	256.0 ± 27.4
4j	H	1	1	H		1.5 ± 0.1	85.5 ± 7.5	4.4 ± 0.3	166.3 ± 19.2
4k	H	2	1	H		2.0 ± 0.3	95.4 ± 8.6	4.5 ± 0.3	153.2 ± 16.0
4l	F	1	1	H		1.9 ± 0.3	100.1 ± 7.6	5.6 ± 0.1	195.5 ± 15.0
4m	H	1	1	F		2.0 ± 0.5	92.2 ± 8.0	5.2 ± 0.7	187.0 ± 24.0
4n	Cl	1	1	H		2.5 ± 0.1	105.2 ± 7.6	5.7 ± 0.8	148.7 ± 10.2
4o	H	1	1	Cl		5.4 ± 0.4	152.2 ± 19.7	6.3 ± 3.5	163.4 ± 20.2
4p	H	1	1	CH_3		3.1 ± 0.2	100.2 ± 12.2	5.1 ± 0.6	163.7 ± 17.5
4q	OCH_3	1	1	H		2.6 ± 0.1	112.1 ± 9.5	4.9 ± 0.3	147.2 ± 14.5
4r	H	1	2	H		7.4 ± 0.8	9.6 ± 1.2	42.9 ± 5.6	11.9 ± 1.3
4s	H	2	1	H		2.7 ± 0.3	4.4 ± 0.2	5.1 ± 0.3	15.8 ± 2.7
4t	H	F	1	H		1.7 ± 0.3	11.9 ± 1.2	4.7 ± 0.2	8.2 ± 0.7
4u	H	2	1	H		1.9 ± 0.2	12.4 ± 2.1	4.3 ± 0.6	9.2 ± 1.1
4v	H	1	2	H		13.4 ± 1.5	16.4 ± 2.1	8.6 ± 0.8	270.7 ± 25.1
4w	H	1	2	H		10.5 ± 0. 2	5.6 ± 0.35	8.4 ± 0.6	14.0 ± 1.5
risperidone						3.9 ± 0.3	182 ± 15.0	0.19 ± 0.02	1105.5 ± 126.3
aripiprazole						9.7 ± 1.1	19.8 ± 2.6	12.1 ± 1.6	2361.0 ± 357.4

[a] K_i values obtained from three experiments, recorded as means ± SEM.

receptors. The 4-methylpiperidyl substituted derivative **4r** displayed decreased affinities for 5-HT_{1A} and 5-HT_{2A} while increased affinities for D_2 and H_3 receptor (**4r** *vs* **4w**).

When electron-withdrawing (-F, -Cl, –OCH_3) and electron-donating groups(–CH_3) were introduced to the meta and ortho positions in compound **4a** and **4f** which bearing *N*-methyl pyrrolidinyl and *N*-methyl piperidyl substitution, respectively, the afforded compounds **4a-4d** and **4f-4i** (Table 2) exhibited diminished affinities for D_2, 5-HT_{1A}, 5-HT_{2A} and H_3 receptors. Similarly, when an electron acceptor or electron donor group was introduced to benzene ring of the compounds which bearing *N*-methyl morpholinyl (**4k-4q**), the affinities were reduced more than the unsubstituted compound **4j** (Table 2). These results indicating that the benzene ring different substituents have negative effect on the affinities of D_2, 5-HT_{1A} and 5-HT_{2A} receptors. However, there was no obvious variety regulation in the affinity for the H_3 receptor.

We further investigated the effect of spacers between benzoisoxazoleylpiperidine and phenoxy structures. Our previous SAR research has shown that the linker length plays a significant role in the regulation of the receptor potency, and the optimum length of the linker generally considered were three carbons or four carbons.[13–15] Therefore, the structure modification was focused on three carbons and four carbons. As shown in the Table 2, when the chain lengths of compound **4j** were prolonged from three to four carbons, the generated compound **4k** exhibited slightly lower affinity levels at D_2, 5-HT_{1A} and 5-HT_{2A} but increased affinity for H_3. Elongation of the linker from three carbon to four carbon resulted in lower affinity levels at D_2, 5-HT_{1A} and H_3 but decreased affinity for 5-HT_{2A} (**4t** *vs* **4u**). While chain lengths between the phenoxy group and alkyl cyclic *N*-heterocyclic also influenced the activities of these compounds. In the molecular structure of compound **4j**, when the methylene was transformed into ethyl, it produced

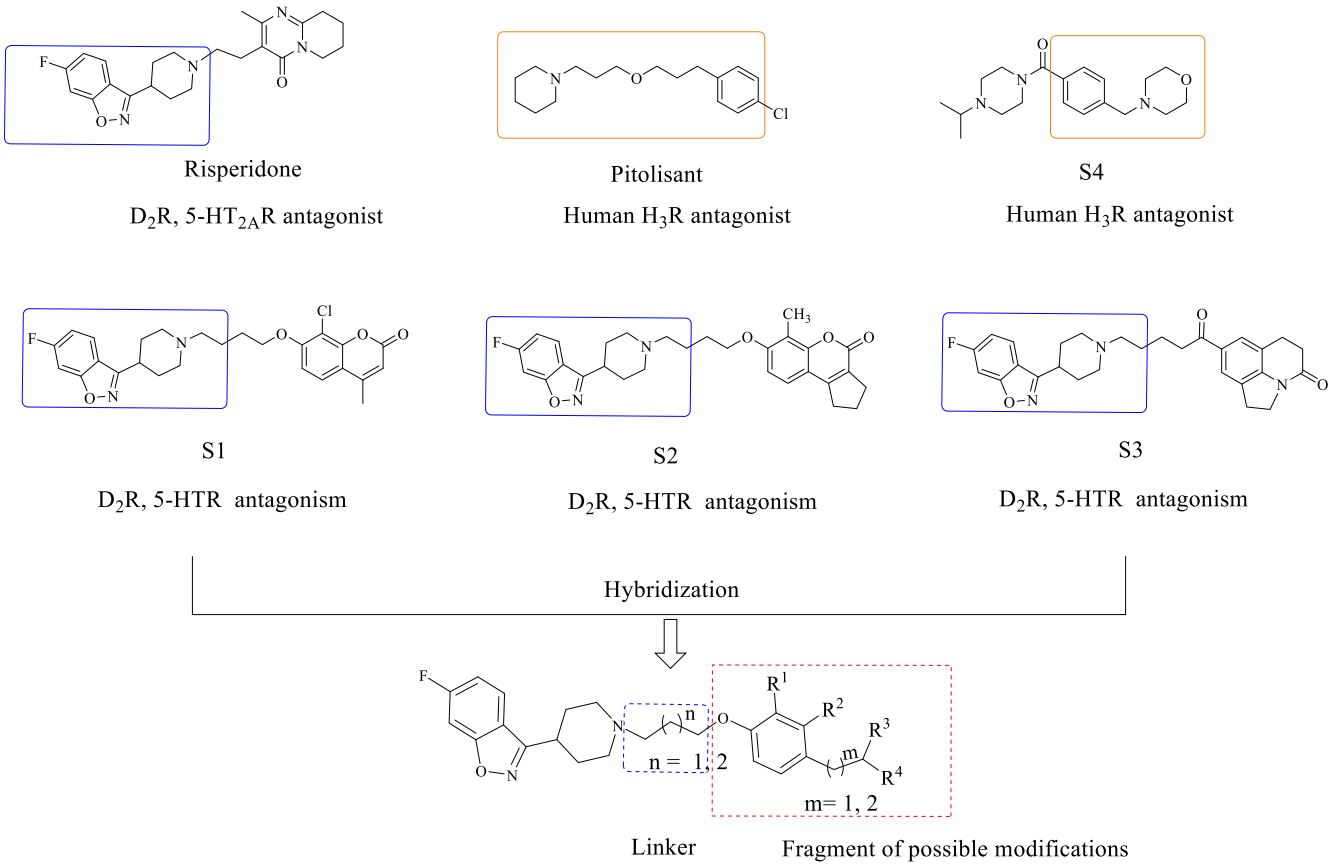


Fig. 1. Strategy for design multi-target ligands. The privileged structures benzoisoxazoleylpiperidine as base moiety, aralkyl pyrrolidine, piperidine and piperazine derivatives were introduced as variable modifiers, the two parts were connected by appropriate linker formed new compounds. By adjusting the substituents of variable modifiers and the length of flexible chain to investigate their structure–activity relationships for the effect to the multi-receptors.

compound **4w**, which had an improved affinity for 5-HT_{1A}. When the alkyl cyclic *N*-heterocyclic was transformed into piperidine ring, replacement of the methylene with ethyl, enhanced its binding abilities at D_2, 5-HT_{1A} and H_3 (**4e** *vs* **4f**). Disappointingly, when the *N*-heterocyclic was changed to *N*-methyl piperazine to prolong the spacer, the affinities for the desired four receptors all decreased (**4t** *vs* **4v**). Thus, the present study found that the appropriate length of the linker between aryl-piperazines and the phenoxy structure should be three carbons and that the spacer length between this structure and cyclic *N*-heterocyclic influenced the comprehensive effects of the pharmacophores.

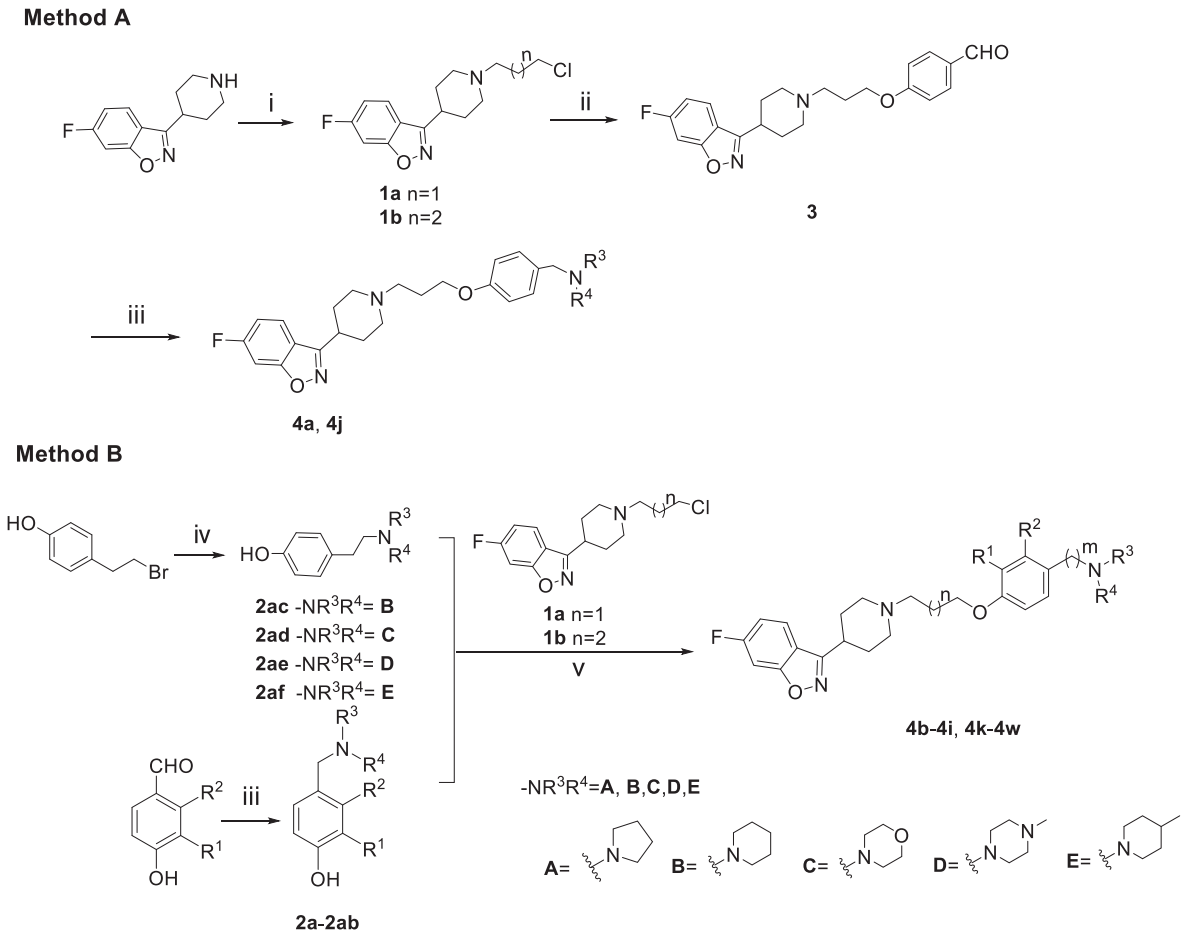
It's clear from the in *vitro* results that most of the compounds displayed high affinities for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors, which was similar to the previously reported compounds S1, S2 and S3[13–16]. In addition, all of the compounds showed additional affinities for the H_3 receptor in varying degrees, but much higher than that of the positive medicine risperidone and aripiprazole.

Based on the in *vitro* evaluations, compounds with outstanding affinities were selected for further pharmacological and behavioral assessments. The selected compounds had to meet the following primary conditions: (1) high affinities for D_2, 5-HT_{1A} and 5-HT_{2A} (D_2, $K_i \leq 10$ nM; 5-HT_{1A}, $K_i \leq 10$ nM; and 5-HT_{2A}, $K_i \leq 20$ nM); (2) the ability to balance activities at multiple receptors with a potency ratio for any two receptors ≤ 10; and (3) an affinity for $H_3 \leq 15$ nM. As a result, compounds **4f**, **4t**, **4u** and **4w** were selected for further biological evaluations.

To determinate the pharmacological effects of the candidates on various off-target receptors (5-HT_{2C}, H_1 and α_1 receptors) which related to many medication-related adverse events[28], for example, the antipsychotic-induced weight gain and hyperglycaemia may induced by the synergistic effects of activations at H_1 and 5-HT_{2C}, and the inhibition of α_1 might lead to orthostatic hypotension and rhythm problems[29]. Therefore, the characterizations of the candidate compounds at these receptors were conducted in the present study. In addition, the pharmacological effects on the desired D_3 and 5-HT_6 receptors also have been tested, as these receptors play an important role in ameliorate the negative and cognitive symptoms of schizophrenia[18,30].

The results revealed that compounds **4f**, **4 t**, **4u** and **4w** had lower affinities for the off-target receptors (5-HT_{2c}, H_1 and α_1) than that of the positive drug risperidone and aripiprazole (Table 2), and similar to the compounds our previous reported[13–16], which suggested that they had low liability to cause side effects. It is worth noting that compounds **4f**, **4 t**, **4u** and **4w** showed higher affinities for D_3 receptor ($K_i < 40$ nM), moderate affinities for 5-HT_6 ($K_i < 150$ nM). However, the affinities for D_3 and 5-HT_6 of risperidone and haloperidol were lower than that of **4w**, which will be beneficial to treat the negative and cognitive symptoms of schizophrenia.

Cardiotoxicity is an important criterion for the safety assessment of a drug[31]. There is plenty of available evidence suggests that the QT interval prolongation related to the blockade of potassium currents, particularly IKr which is mediated by an ion channel encoded by the HERG, and most conventional and atypical antipsychotics can prolongation of the QTc interval as the hERG blockade[32,33]. To assess the cardiotoxicity of compounds **4f**, **4t**, **4u** and **4w,** their inhibitory actions on hERG were assessed in *vitro* (Table 3). Automated patch-clamp analyses revealed that all of the selected compounds had lower levels of inhibition on hERG (**4f**: IC_{50} = 2290.2 nM; **4t**: IC_{50} = 1560.4 nM; **4u**: IC_{50} = 1056.5 nM; **4w**: IC_{50} = 2410.1 nM) compared to risperidone (IC_{50} = 167.0 nM) and aripiprazole (IC_{50} = 896.5 nM). This was particularly evident in the IC_{50} of compound **4w** (IC_{50} = 2410.1 nM), which exceeded that of risperidone and aripiprazole about ten-fold. It



Scheme 1. Reagents and conditions: (i) 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane, Acetone, K_2CO_3, RT, 24 h; (ii) 4-Hydroxybenzaldehyde, K_2CO_3, KI, CH_3CN, 70 °C, 12 h; (iii) pyrrolidine, piperidine, morpholine or piperazine derivatives, DCM/MeOH, $(CH_3COO)_3BHNa$, 0 °C - RT, 24 h; (iv) DIPEA, CH_3CN, 60 °C, 18 h; (v) K_2CO_3, KI, CH_3CN, 80 °C, 12 h.

Table 3
Activities on D_3, 5-HT_6, 5-HT_{2C}, α_1 and H_1 Receptors and hERG Channels of Candidates and Reference Antipsychotics.

Cmpd	Receptor affinity K_i ± SEM (nM)[a]					hERG IC_{50} (nM)
	D_3	5-HT_6	5-HT_{2C}	α_1	H_1	
4f	15.2 ± 2.4	149.2 ± 14.7	752.5 ± 65.2	536.1 ± 39.5	1016.5 ± 82.5	2290.2 ± 246.6
4t	23.2 ± 1.2	93.3 ± 10.8	572.8 ± 60.1	331.5 ± 40.8	719.5 ± 54.2	1560.4 ± 198.3
4u	37.6 ± 2.7	122.7 ± 15.5	425.6 ± 33.2	353.6 ± 37.3	642.4 ± 78.5	1056.5 ± 171.5
4w	9.6 ± 1.5	141.0 ± 15.5	610.4 ± 71.3	438.2 ± 39.8	968.3 ± 98.1	2410.1 ± 327.1
risperidone	14.3 ± 1.8	1329.2 ± 143.0	20.9 ± 2.2	3.2 ± 0.5	32.6 ± 4.7	167.0 ± 23.2
aripiprazole	10.8 ± 1.5	318.0 ± 49.5	18.2 ± 2.9	8.9 ± 1.2	48.9 ± 5.8	896.5 ± 98.4

[a] K_i values obtained from three experiments, recorded as means ± SEM.

may indicate that compound **4w** held low propensity to elicit treatment-induced QT interval prolongation compared to the other compounds.

To sum up, all the results showed that compound **4w** exhibited excellent in *vitro* profiles among the four candidates. Thus, compound **4w** was subjected to intrinsic activity and animal model study to verify its antipsychotic-like behavioral activities. The results of the intrinsic activity of **4w** were shown in Table S1 (Supporting Information), in agonist assay, compound **4w** showed feeble agonist activity for D_2, D_3, 5-HT_{1A}, 5-HT_{2A}, H_3 and 5-HT_6 receptors compared with reference compounds, respectively. In antagonist assays, **4w** showed antagonist for the six receptors, potent D_2 (IC_{50} = 11.3 nM), D_3 (IC_{50} = 63.7 nM) antagonism, accompanied with 5-HT_{1A} (IC_{50} = 246.6 nM), 5-HT_{2A} (IC_{50} = 185.5 nM), H_3 (IC_{50} = 27.6 nM), 5-HT_6 (IC_{50} = 348.4 nM) antagonism.

The apomorphine animal model is widely used to assess compounds in terms of their antagonistic effects on D_2 and/or the potential for reducing schizophrenic symptoms[21]. Mice that received oral administrations of compound **4w** exhibited significant and dose-dependent decreases in apomorphine-induced climbing (Fig. 2**A**); the effective dose, 50% (ED_{50}) of compound **4w** was 0.09 mg/kg (Table 4). In the positive control groups, mice oral administrations of risperidone, haloperidol and aripiprazole exhibited significant reductions in APO-induced climbing with ED_{50} values of 0.02 mg/kg, 0.09 mg/kg and 0.3 mg/kg respectively. The results indicate that compound **4w** has the potential to antagonize the D_2 receptor, which would likely attenuate the positive symptoms of schizophrenia.

The NMDA receptor antagonist MK-801-induced hyperactivity has become a common and important animal model for assessing the potential therapeutic effect in schizophrenia patients[34,35]. The results of this study show that compound **4w** induced a marked dose-dependent

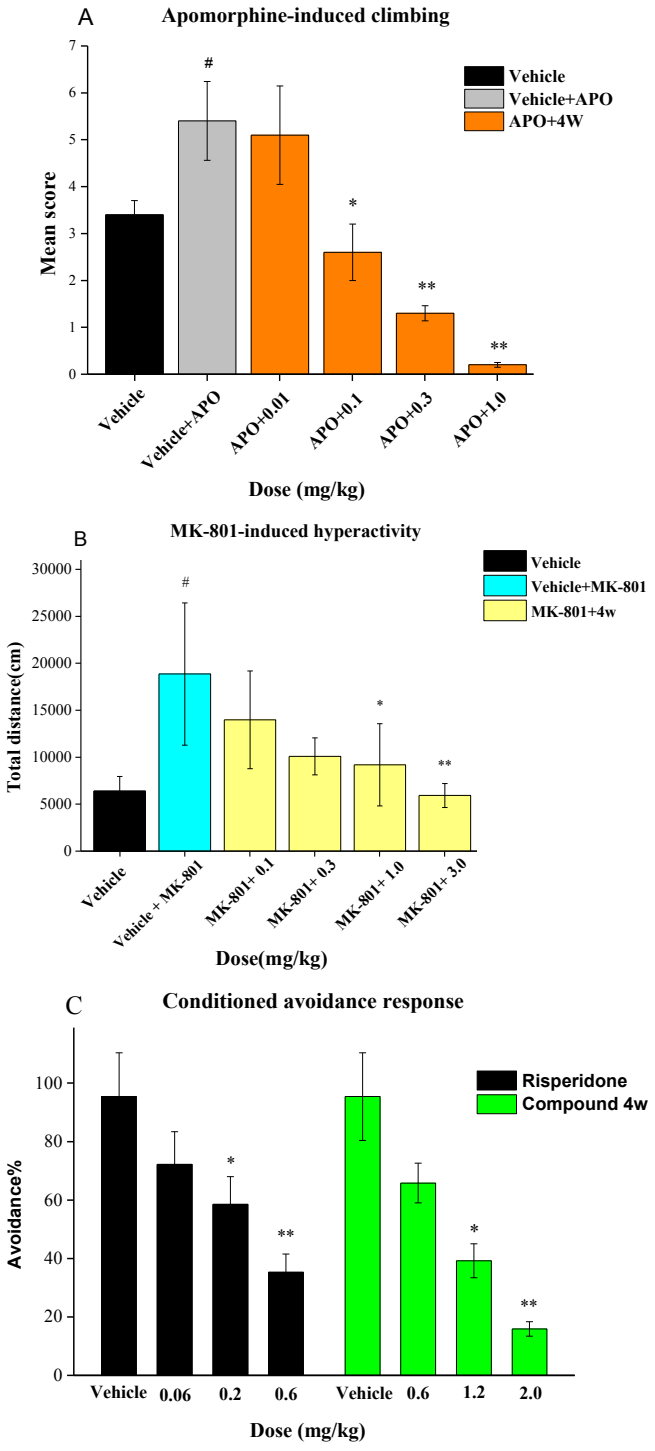


Fig. 2. (**A**) Effect of compound **4w** on APO-induced climbing in mice (10/groups). The score are shown as means ± SEM. Statistical significances were performed by the nonparametric two-tailed Mann-Whitney *U* test: #, $p < 0.05$ versus vehicle treatment; *, $p < 0.05$ and **, $p < 0.01$ versus apomorphine treatment; (**B**) Effect of compound **4w** on MK-801-induced hyperactivity in mice (10/group). The distance are presented as the means ± SEM. Statistical evaluation was conducted by two-way ANOVA followed by Tukey's test for multiple comparisons. #, $p < 0.05$ versus vehicle treatment; **, $p < 0.01$ and *, $p < 0.05$ versus MK-801 treatment; (**C**) The influence of risperidone and compound **4w** on conditioned avoidance response in rats (10/group). The percentage of avoidance is shown as mean ± SEM. And the level of significance indicated *, $p < 0.05$ and **, $p < 0.01$ versus vehicle group. Statistical analysis was performed by using SPSS software.

Table 4
In Vivo Pharmacological Profile of Compound 4w.

Cmpd	APO [a]	MK-801 [b]	CAT [c]	CAR [d]	CAT/APO	CAT/MK-801
4w	0.09	0.2	32.0	1.3	355.6	213.3
haloperidol	0.09	0.1	0.3	–	3.6	2.9
risperidone	0.02	0.04	1.6	0.6	81.0	40.5
aripiprazole	0.25	0.3	11.2	0.2	44.9	35.1

[a] APO: apomorphine-induced climbing (ED_{50}, mg/kg, po).
[b] MK-801: MK-801-induced hyperactivity (ED_{50}, mg/kg, po).
[c] CAT: catalepsy (ED_{50}, mg/kg, po).
[d] CAR: conditioned avoidance response (ED_{50}, mg/kg, po).

suppression of MK-801-induced schizophrenia-like symptoms (Fig. 2**B**) with ED_{50} value of 0.2 mg/kg (Table 4). Contrast with **4w**, mice treated with risperidone, haloperidol and aripiprazole showed decreased locomotor activities with ED_{50} values of 0.04 mg/kg, 0.1 mg/kg and 0.3 mg/kg respectively. These results indicated that compound **4w** has a significant effect in the treatment of symptoms.

To predict the likelihood of compound **4w** would produce EPS-like side effects, the effects of **4w** in mice were evaluated by the horizontal bar test to determine possibility in terms of producing catalepsy. Haloperidol (ED_{50}: 0.3 mg/kg), risperidone (ED_{50}: 1.6 mg/kg) and aripiprazole (ED_{50}: 11.2 mg/kg) all produced significant cataleptic effects (Table 4) that reflected the strong antagonism of the D_2 receptor by these drugs. In comparison, compound **4w** displayed a lower potential for cataleptogenic effects (ED_{50}: 32.0 mg/kg), which infers that **4w** has a higher threshold for catalepsy and less tendency to produce EPS.

In addition, the evaluations predicting efficacy (APO and MK-801 models) and adverse effects (catalepsy) demonstrated that **4w** has a broader therapeutic index (213.3 to 355.6 Table 4) than that of haloperidol (3.6 to 2.9), risperidone (40.5 to 80.0) and aripiprazole (44.9 to 35.1). Thus, compound **4w** has a higher security.

The conditioned avoidance response (CAR) test model has high levels of reliability and validity for predicting the positive symptoms of schizophrenia.[36,37] Therefore, the CAR test was conducted in rats, as the rats has several advantages in behavioral studies and higher nervous activity studies, such as its easily adapted to the environment and easy to train, sensitive to punishment and rewards. The results of present study showed that **4w** markedly suppressed the CAR in rats (Fig. 2**C**) with an ED_{50} of 1.3 mg/kg. Meanwhile, the ED_{50} of the risperidone and aripiprazole control groups 0.6 mg/kg and 0.2 mg/kg respectively, which indicates that compound **4w** had beneficial effects on the positive symptoms of schizophrenia.

The novel object recognition (NOR) task is often used to predict the potential of a novel drug for cognitive enhancement.[15,38] Oral administration of the test compound (0.05–0.3 mg/kg) have no obvious influence on total exploration time (Fig. 3**A**) and similarly the oral administration of risperidone (0.3 mg/kg) did not exhibit induce

cognitive improvements. Rats given oral dose compound **4w** (0.3 mg/kg), rivastigmine (0.3 mg/kg) and aripiprazole (0.3 mg/kg) in the retention trial exhibited a longer exploration time for novel things compared to the vehicle condition (Fig. 3**B**), and also exhibited an obvious increase in the novelty discrimination index (NDI) (Fig. 3**C**). However, rats that received vehicle or a lower dose of the compound **4w** (0.05 mg/kg) did not differ in terms of exploration times. From the comparison of the results (Fig. 3**C**), it has been found that the group given risperidone (0.3 mg/kg) showed lower NDI, which approximates the vehicle group. And the groups given compound **4w** (0.3 mg/kg) showed higher NDI than that given rivastigmine (0.3 mg/kg) but slightly lower than that given aripiprazole (0.3 mg/kg).

The results of the NOR test which relate to the cognitive behavior revealed that compound **4w** potentially plays a role in the promotion of recognition memory in this rat model, indicating that **4w** may improve cognitive function.

Taken together, those behavioral testing demonstrate that **4w** can

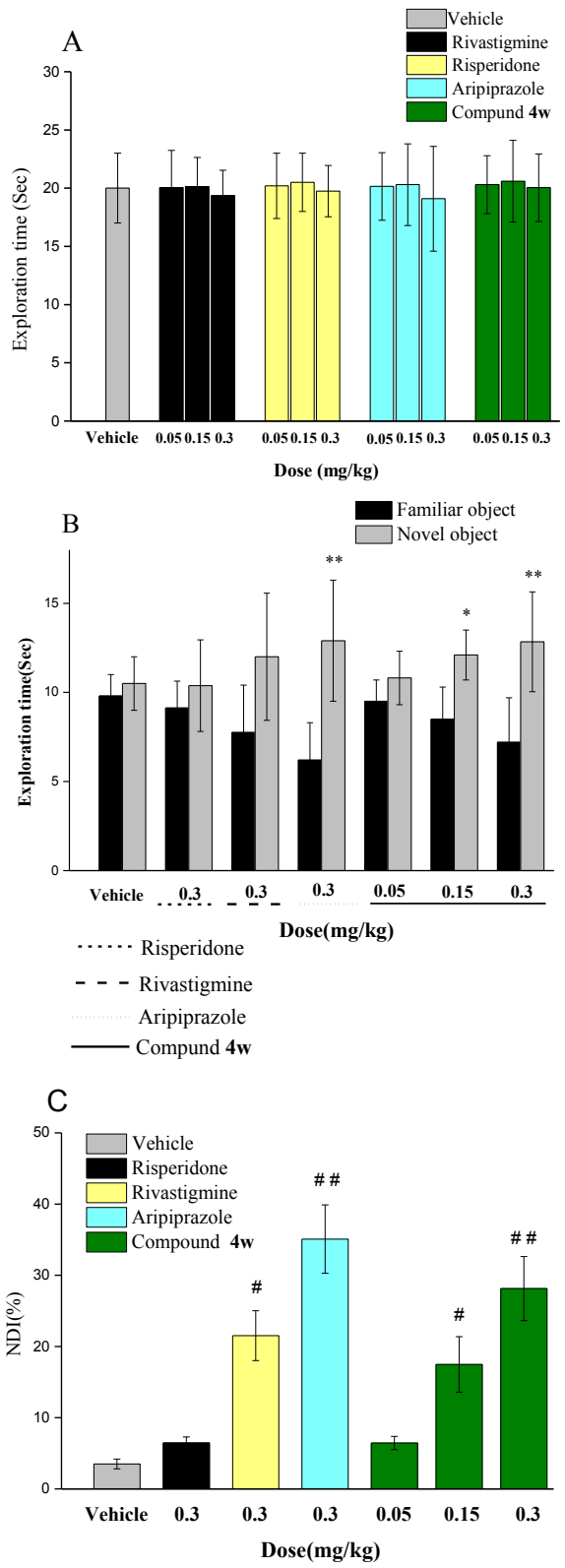


Fig. 3. Effects of compound **4w** on NOR test in rats (10/group). Experimental results acquired 60 min after oral administration of vehicle risperidone (0.05, 0.15, 0.3 mg/kg), rivastigmine (0.05, 0.15, 0.3 mg/kg), aripiprazole (0.05, 0.15, 0.3 mg/kg) and **4w** (0.05, 0.15, and 0.3 mg/kg). (**A**)The time spent in exploring two identical objects during acquisition trials; (**B**) The time spent in exploring familiar and a novel object during acquisition trials. (**C**) Novelty discrimination index (NDI) are shown as the mean ± SEM (n = 10). **, $p < 0.01$ and *, $p < 0.05$ compared with familiar object by paired test; #, $p < 0.05$ and ##, $p < 0.01$ compared with vehicle group.

effectively inhibit the schizophrenia-like symptoms with higher threshold for catalepsy, especially compound **4w** shown the potential improvement in cognitive function, it's probably due to adding on the H_3 receptor antagonism to mixed D/5-HT receptor antagonist profile.

Although the atypical antipsychotics have many advantages than conventional antipsychotics, studies showed that one of the main drawbacks of the atypical antipsychotics is their long-term metabolic adverse effects, which may cause poor treatment compliance and eventually lead to clinical deterioration,[2–6] such as the representative of atypical antischizophrenic risperidone trigger the treatment-related side effect wight gain and hyperglycemia. Thus, it is necessary and important to make an assessment for a compound's side effects as a treatment for schizophrenia.

To determine whether compound **4w** would induce weight gain and hyperprolactinemia, this ligand was assessed in an animal model of chronic administration (28 days). Although mice given compound **4w** for 28 days did not exhibit obvious weight gain, the positive control group (risperidone) did show significant weight gain (Fig. 4**A**). In addition, the serum prolactin levels of compound **4w** group did not exhibit significant increase (Fig. 4**B**) compared with the risperidone and aripiprazole groups. The results indicate that compound **4w** has less side effect, which seldom reported in the literature and the experimental results agree well with our intended purpose.

The pharmacokinetic characteristics are described in Table 5. In rats that received an oral dose (4.0 mg/kg, n = 6), there was a half-life ($t_{1/2}$) of 5.0 h whereas rats that received an intravenous injection (0.5 mg/kg, n = 6) had a $t_{1/2}$ of 1.7 h. The long plasma half-life demonstrated that **4w** probably have good tolerability and longer duration of action. The area under the curve (AUC) values were 347.6 ng·h/mL after oral administration *vs* 179.8 ng·h/mL after intravenous administration. The maximum concentration (Cmax) was 102.1 μg/L and the time of maximum plasma concentration (T_{max}) was 0.5 h after oral administration *vs* the C_{max} was 129.0 μg/L and the T_{max} was 0.08 h after intravenous. The clearance of **4w** were 10.4 L/h/kg oral administration and 2.7 L/h/kg after intravenous, respectively. In addition, oral absolute bioavailability was 24.0%. These favorable drug-like pharmacokinetic properties make compound **4w** a potential clinical atypical antipsychotics candidate.

In summary, a series of heterocycle piperidine derivatives were designed and synthesized by using the multi-receptor affinity strategy and molecular hybridization method, and selected candidates were evaluated as potential new antipsychotic agents. Of these ligands, compound **4w** exhibited favorable binding at D_2, D_3, 5-HT_{1A}, 5-HT_{2A} and H_3, moderate binding at 5-HT_6, and satisfactory selectivity profiles for off-target receptors (5-HT_{2C}, H_1 and α_1) that have been closely linked to the negative side effects of other marketed antipsychotics. In addition, behavioral animal experiments revealed **4w** reversed the APO-induced hyperlocomotion and MK-801-induced hyperactivity in mice, could significantly restrain the CAR in rats, which indicated that compound **4w** has a significant effect in the treatment of the schizophrenia-like symptoms. Furthermore, **4w** also exhibited low levels of inhibition at the hERG channel, and the lack of a tendency to induce catalepsy. Moreover, compared to compounds **S1**, **S2** and other compounds have been reported in literatures,[39] compound **4w** showed positive effect on the cognitive function in the NOR tests in rats. Those characteristics of **4w** may owe to the H_3/D/5-HT receptor antagonist profile. Overall, the **4w** similar to the compound **S3** which has been reported previously by research group, it has been approved for clinical trials in 2018 by the China Food and Drug Administration (CFDA). Finally, pharmacokinetic studies demonstrated that **4w** had a favorable drug-like pharmacokinetic profile. Thus, compound **4w** has the potential to be a developed as a multi-target antipsychotic candidate drug.

Statistics

In the in vitro binding assays, results were transferred to the program

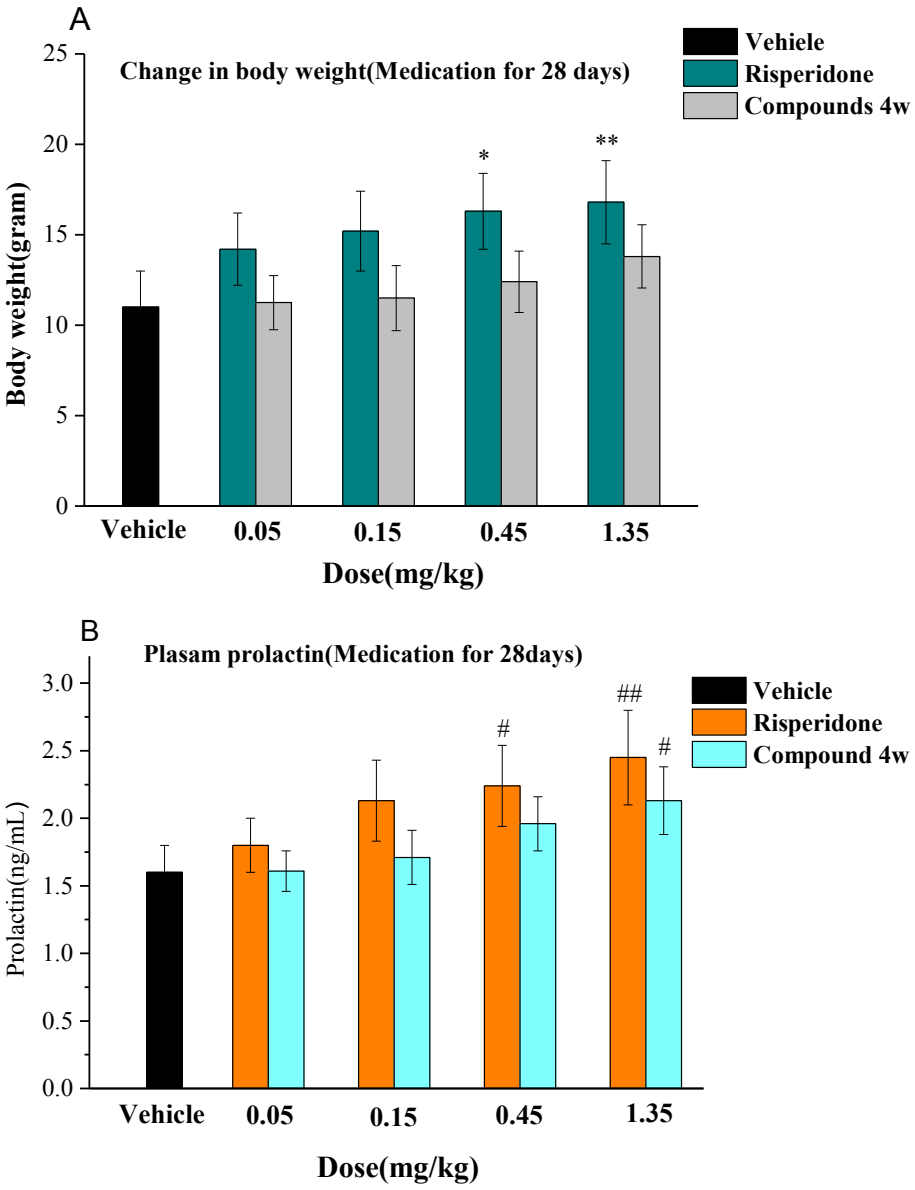


Fig. 4. (**A**) Effects of **4w** and risperidone on body weight in mice after 28 days administration (10/group). The weight gain presented as the means ± SEM. Statistical evaluation was performed by student's *t* test. *, $p < 0.05$ and **, $p < 0.01$ versus vehicle group; (**B**) Effects of **4w** and risperidone on serum prolactin (PRL) in mice after 28 days administration (10/group). The data are presented as the mean ± SEM. Statistical evaluation was conducted by Student's *t* test: #, $p < 0.05$ and ##, $p < 0.01$ versus vehicle.

Table 5
Pharmacokinetic data of Compound **4w** in rats (n = 6/group).

Dose (mg/kg)	C_{max} (ug/L)	$t_{1/2}$ (h)	Tmax (h)	CLz (L/h/kg)	Vz (L/kg)	AUC(0-t) (ug/L × h)	AUC(0-∞) (ug/L × h)	F %
i.v.(0.5 mg)	129.0	1.7	0.1	2.7	6.5	179.8	185.0	
i.g.(4.0 mg)	102.1	5.0	0.5	10.4	89.5	347.7	453.6	24.0

Statistical Package for the program SPSS (Statistical Package for the Social Sciences) for further analysis. In the apomorphine-induced climbing and MK-801-induced hyperactivity test, data were analyzed by the nonparametric two-tailed Mann-Whitney *U* test. In the conditioned avoidance response test, the statistical analysis was performed by using SPSS software. In the weight gain and serum prolactin test, the statistical evaluation was conducted by Student's *t* test.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Experimental procedures for synthetic chemistry and the spectroscopic data of the compounds in the text; Receptor binding studies; hERG affinity; Behavioral studies; Weight Gain and Serum Prolactin; Pharmacokinetics study; Intrinsic activity of compound **4w**; The full statistic data of the figure in the text; ^{1}H-NMR, ^{13}C-NMR, HRMS and HPLC of **4w**. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127909.

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