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# Synthesis and biological evaluation of a new class of multi-target heterocycle piperazine derivatives as potential antipsychotics†

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In this study, we designed and synthesized a novel series of multi-receptor ligands as polypharmacological antipsychotic agents by using a multi-receptor affinity strategy. Among them, **3w** combines a multi-receptor mechanism with high mixed affinities for D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and H<sub>3</sub> receptors, and low efficacy at the off-target receptors (5-HT<sub>2C</sub>, H<sub>1</sub> and α<sub>1</sub> receptor) and human ether-à-go-go-related gene (hERG) channel. In addition, compound **3w** exhibits favorable antipsychotic drug-like activities in *in vivo* assessment. An animal behavioral study revealed that compound **3w** significantly reverses apomorphine-induced climbing and MK-801-induced hyperactivity, and avoidance behavior in the CAR test, with a high threshold for catalepsy. Moreover, compound **3w** demonstrates memory enhancement in a novel object recognition task and low liabilities for weight gain and hyperprolactinemia in a long-term metabolic adverse effects model. Thus, **3w** was selected as an antipsychotic candidate for further development.

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## 1. Introduction

Schizophrenia is a chronic and complex psychotic mental disorder that affects around 1% of people. It is characterized by a combination of positive, negative and cognitive impairment.<sup>1</sup> Although current antipsychotics on the market have brought about great progress in the treatment of schizophrenia, such as typical antipsychotics (chlorpromazine and haloperidol, Fig. 1) that have been proven to be effective treatments in controlling positive symptoms, their strong and nonselective blockade of dopaminergic transmission causes numerous side effects, such as tardive dyskinesia (TD), extrapyramidal symptoms (EPS) and hyperprolactinemia, and even exacerbates negative and cognitive symptoms.<sup>2,3</sup> Atypical antipsychotics, such as clozapine and risperidone, are less tightly bound to the dopamine D<sub>2</sub> receptor. Besides this, their affinities for various 5-hydroxytryptamine (5-HT) receptors means that they display more clinical advantages over typical antipsychotics in the treatment of positive symptoms, as well as having small effect size advantages in the

improvement of negative cognitive symptoms and in promoting relapse prevention and cognitive impairment. However, patients who take antipsychotic drugs still suffer from long-term side effects, such as QT (Q wave and the end of the T wave on electrocardiograms) interval prolongation, hyperprolactinemia and weight gain.<sup>4</sup> Therefore, there is still a great clinical need for the development of safer, more effective novel antipsychotics.

From the perspective of targets, activation of the 5-HT<sub>1A</sub> receptor in the frontal cortex will enhance the functions of the mesocortical dopamine pathway, which may improve the negative symptoms and cognitive deficits in patients who have schizophrenia.<sup>5,6</sup> An inverse agonist of 5-HT<sub>2A</sub> could counteract excessive D<sub>2</sub> receptor blockade, which is not only conducive to alleviating extrapyramidal effects but also enhances the efficacy against negative symptoms.<sup>7–9</sup> Also, there is plenty of evidence that strongly supports that combined effects on D<sub>2</sub> and 5-HT<sub>2A</sub> receptors are beneficial to the improvement of both negative symptoms and symptoms positive of schizophrenia.<sup>10,11</sup> Currently, a large number of literature studies have detailed the design of multi-target ligands that can simultaneously modulate and balance their activities at several specific targets to overcome the shortcomings of conventional antipsychotic drugs, such as the novel antipsychotics aripiprazole,<sup>12</sup> brexpiprazole and cariprazine,<sup>13,14</sup> which differ from previous antipsychotics in that they are more inclined to achieve balance and coordinate multiple biological targets (Fig. 1). For example, both brexpiprazole and cariprazine modulate and stabilize DA (dopamine) neurotransmission *via* synergistic effects rather

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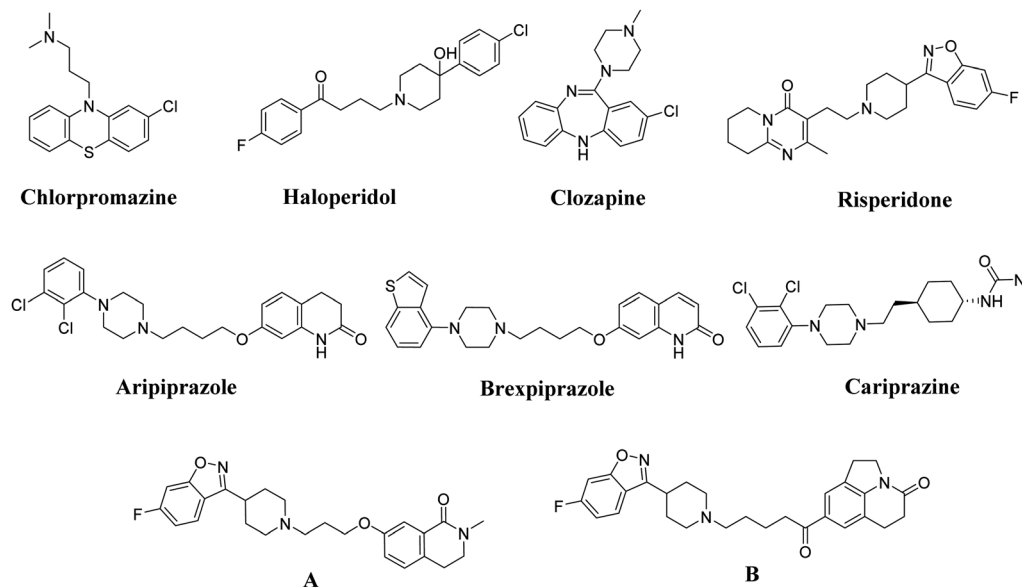


Fig. 1 The structure of representative antipsychotic drugs and representative compounds from our previous study.

than by complete antagonism of  $D_2$  alone.<sup>15,16</sup> In addition, these drugs are partial agonists of  $D_2$ ,  $5-HT_{1A}$ ,  $5-HT_{2A}$ , and  $5-HT_{2C}$  (particularly of  $5-HT_{1A}$  and  $5-HT_{2A}$ ) and exhibit higher levels of intrinsic activity at  $5-HT_{1A}$ .<sup>17,18</sup> These combined actions effectively relieve the behavioral and psychological symptoms of schizophrenia, significantly reduce severe side effects, are associated with a favorable safety profile, and improve cognitive symptoms to some extent.<sup>19</sup>

As schizophrenia has varied and numerous different symptoms, developing multi-target ligands with a poly-pharmacological profile has become a widely used therapeutic approach.<sup>20</sup> Also, exploring novel multi-target ligands that can accurately modulate and balance activities at dopaminergic and serotonergic receptors, especially the  $D_2$ ,  $5-HT_{1A}$  and  $5-HT_{2A}$  receptors, and simultaneously decrease the affinity for other off-target receptors associated with side effects has been our long-standing research interest.<sup>21,22</sup> In our previous study, we explored a series of multi-target ligands, as shown in Fig. 1, where compounds **A** and **B** exhibited a high affinity for dopamine  $D_2$  and serotonin  $5-HT_{1A}$ ,  $5-HT_{2A}$ ,  $5-HT_6$  receptors, and low affinity for  $5-HT_{2C}$ , histamine  $H_1$ , and adrenergic  $\alpha_1$ , which can be regarded as related to side effects. Both compound **A** and **B** showed negligible effects on the human ether-à-go-go-related gene channel (hERG; associated with QT interval prolongation). In the study of animal models, compounds **A** and **B** show favorable anti-schizophrenic activity, such as markedly inhibited APO (apomorphine)-induced hyperlocomotion, and MK-801-induced hyperactivity. In addition, compounds **A** and **B** display a high threshold for acute toxicity, a low tendency to induce catalepsy, as well as negligible side effects (hyperprolactinemia and weight gain) when compared to risperidone. To our satisfaction, compounds **A** and **B** display precognition properties in the novel object recognition task (NOR) in rats,

giving them an advantage over most of the conventional anti-schizophrenic drugs.<sup>23,24</sup>

Inspired by these findings, here we describe a series of new compounds prepared using a molecular hybridization method, the design concept of which is shown in Fig. 2. Their general structure consists of benzothiophenylpiperazine, which is a crucial DA and 5-HT pharmacophore of the novel antipsychotic brexpiprazole, and a special pharmacophore

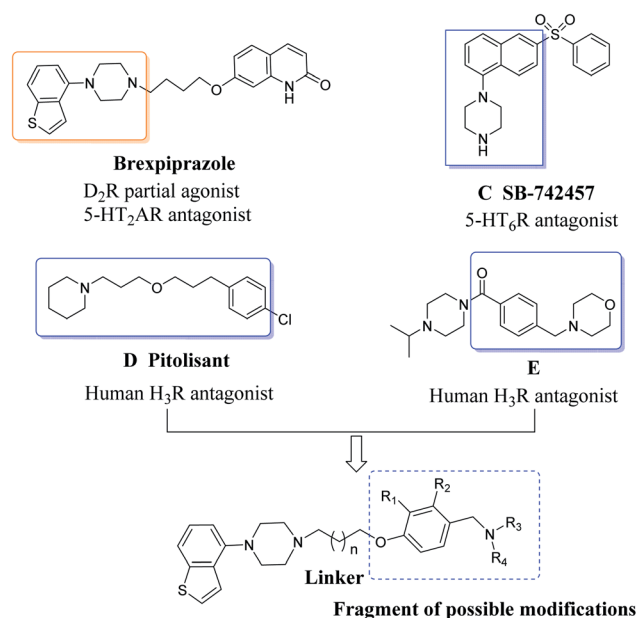
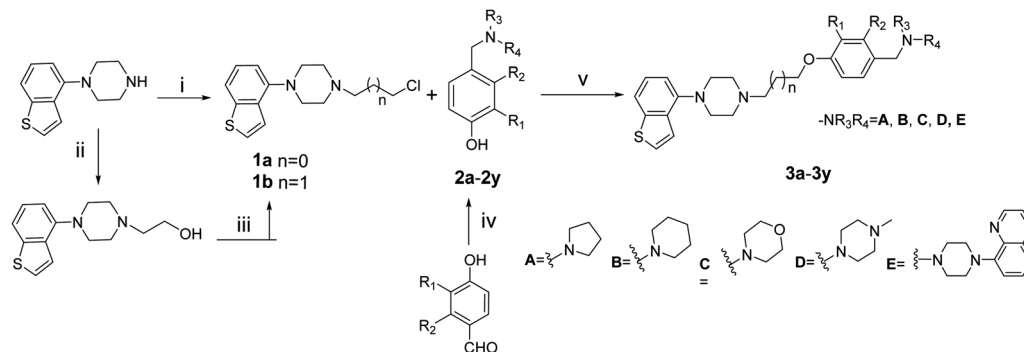


Fig. 2 The design of new benzothiophenylpiperazine derivatives. The privileged structure benzothiophenylpiperazine acts as a base moiety, and aralkylpyrrolidine and piperidine or piperazine fragments that connect the benzene rings *via* a methylene group act as variable modifiers. The two parts are connected *via* a flexible chain to form new derivatives.





**Scheme 1** Reagents and conditions: (i)  $K_2CO_3$ , 1-bromo-3-chloropropane, acetone, rt, 24 h; (ii) acetone,  $K_2CO_3$ , 2-bromoethanol, reflux, 10 h; (iii)  $SOCl_2$ , DCM, reflux, 3 h; (iv) pyrrolidine/piperidine/morpholine/*N*-methylpiperazine/4-piperazinyl quinoline,  $(CH_3COO)_3BHNa$ , DCM, 0 °C–rt, 24 h or MeOH, 0 °C–rt, 24 h; (v)  $K_2CO_3$ , KI,  $CH_3CN$ , 80 °C, 12 h.

(pyrrolidine, piperidine or morpholine connected to a benzene ring *via* a methylene group), derived from reported  $H_3$  and 5-HT<sub>6</sub> receptor antagonists (pitolisant, compounds C and E SB-7424) that showed potential to improve cognitive deficits in clinical trials,<sup>25–27</sup> expect that a mixed DA/5-HT/ $H_3$  receptor affinity profile is beneficial for the cognitive function of schizophrenia. Finally, the two central pharmacophores are connected *via* an appropriate linker, according to the literature, as well as our own experience. Besides this, the effects that different substituents on the benzene ring of the new compounds have on the receptor affinity were investigated. Subsequently, these new derivatives were subject to *in vitro* evaluation and *in vivo* behavioral studies. Among these new derivatives, compound **3w** demonstrates a high level of multi-target activities at  $D_2$ , 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>,  $H_3$  and lacks the receptors associated with side effects (5-HT<sub>2C</sub>,  $\alpha_1$ , and  $H_1$ ).<sup>28–30</sup> In behavioral studies, compound **3w** was found to significantly attenuate MK-801-induced hyperlocomotion as well as apomorphine-induced climbing, exhibiting negligible liability in terms of inducing weight gain, and resulted in no significant hyperprolactinemia compared to risperidone. In addition, NOR testing demonstrated that compound **3w** shows pro-cognitive properties in rats, which conform to the original expectations. Thus, the present study identifies compound **3w** as a potential antipsychotic candidate for the treatment of schizophrenia.

## 2. Results and discussion

### 2.1. Chemistry

The general procedures for the synthesis of the intermediates (**1a**, **1b**, **2a–2y**) and novel compounds **3a–3y** are illustrated in Scheme 1. The substitution of alkyl groups into the aryl-piperazines was achieved through the reaction of benzothio-phenylpiperazine with 1-bromo-3-chloropropane in the presence of anhydrous potassium carbonate to afford **1a**. The benzothio-phenylpiperazine reacts with 2-bromoethanol following reflux in a mixture of thionyl chloride and dichloromethane to give intermediate **1b**. The intermediates **2a–2y** were obtained using *p*-hydroxybenzaldehyde and its derivatives to react with pyrrolidine, piperidine, morpholine and their

derivatives in the presence of sodium triacetoxyborohydride. The desired compounds **3a–3y** were synthesized *via* the reaction of **1a** or **1b** and **2a–2y** using anhydrous potassium carbonate as a base and a trace of potassium iodide as a catalyst.

### 2.2. Biological studies

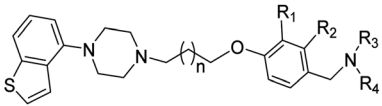
**2.2.1. Ethics statement.** Chinese Kun Ming (KM) mice (20 ± 2.0 g) and Sprague-Dawley (SD) rats (250 ± 5.0 g) were used as experimental animals in this study. The animals were housed under standardized light and temperature conditions and received standard rat chow and tap water *ad libitum*. Animals were randomly assigned to different experimental groups and each group was kept in a separate cage. All the research involving animals in this study follows the guidelines of the bylaws on experiments on animals, and has been approved by the Ethics and Experimental Animal Committee of Jiangsu Ocean University (Project identification code: 2020002, date of approval: 8 January 2020). For the procedural details of the biological studies see the ESI.†

### 2.3. *In vitro* evaluation of new compounds

**2.3.1. Structure–affinity relationships studies.** As mentioned above, the novel compounds in the present study were rationally designed based on available multi-target anti-psychotic drugs and the privileged structures of the reported  $H_3$  and 5-HT<sub>6</sub> receptor antagonists, and the SARs of the new derivatives were preliminarily evaluated *in vitro* (the affinities for  $D_2$ , 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub>,  $H_3$  receptors). The compounds with the best activities were selected and subjected to safety and behavioral studies.

The effect of modifications on the functional activities of the five target receptors was preliminarily evaluated at the beginning of this study. As the results in Table 1 show, all of the compounds in the present study (**3a–3y**) exhibit affinities for  $D_2$ , 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> to some extent, especially compounds **3t** and **3w**, which exhibit high affinity for the  $D_2$ , 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, attributed to the privileged structure of benzothio-phenylpiperazine.

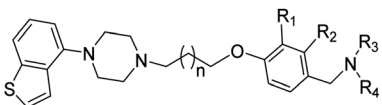





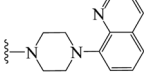
Table 1 Binding affinities for the D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub> and H<sub>3</sub> receptors of the compounds 3a–3y


Cmpd	R <sub>1</sub>	n	R <sub>2</sub>	NR <sub>3</sub> R <sub>4</sub>	Receptor affinity K <sub>i</sub> ± SEM <sup>a</sup> (nM)				
					D <sub>2</sub>	5-HT <sub>1A</sub>	5-HT <sub>2A</sub>	5-HT <sub>6</sub>	H <sub>3</sub>
3a	CH <sub>3</sub>	1	H		94.1 ± 5.6	154.7 ± 10.5	205.6 ± 23.0	463.5 ± 43.0	61.3 ± 3.9
3b	H	1	CH <sub>3</sub>		95.6 ± 11.2	163.8 ± 13.0	276.4 ± 24.2	481.9 ± 52.2	66.9 ± 3.0
3c	F	1	H		100.1 ± 7.0	154.6 ± 15.2	258.6 ± 14.0	382.8 ± 34.6	92.9 ± 3.6
3d	H	1	F		98.7 ± 6.8	144.1 ± 10.4	262.4 ± 17.5	354.2 ± 65.8	54.4 ± 2.0
3e	Cl	1	H		101.2 ± 10.1	157.3 ± 9.8	248.6 ± 22.9	418.5 ± 85.4	58.9 ± 5.5
3f	H	1	Cl		120.1 ± 15.0	184.6 ± 16.3	268.5 ± 20.2	375.6 ± 58.3	75.30 ± 8.5
3g	OCH <sub>3</sub>	1	H		99.5 ± 8.4	149.0 ± 11.7	128.7 ± 15.1	462.4 ± 55.7	62.5 ± 5.0
3h	OCH <sub>3</sub>	0	H		82.5 ± 5.8	354.6 ± 19.3	156.9 ± 12.6	764.3 ± 94.6	164.3 ± 14.5
3i	H	1	H		91.1 ± 4.5	134.7 ± 10.5	185.6 ± 20.1	384.0 ± 51.3	53.0 ± 6.3
3j	CH <sub>3</sub>	1	H		138.2 ± 30.1	305.4 ± 28.2	324.4 ± 27.5	385.4 ± 51.0	85.0 ± 9.8
3k	H	1	CH <sub>3</sub>		249.6 ± 37.5	394.5 ± 38.2	286.1 ± 19.3	403.7 ± 87.8	103.2 ± 24.6
3l	F	1	H		197.4 ± 15.5	285.4 ± 15.7	175.4 ± 25.3	389.5 ± 63.2	88.4 ± 10.1
3m	H	1	F		110.2 ± 8.9	213.2 ± 24.3	145.9 ± 11.5	364.5 ± 55.6	81.5 ± 6.5
3n	Cl	1	H		125.2 ± 10.2	253.1 ± 23.1	152.71 ± 15.6	375.4 ± 42.2	89.4 ± 10.6
3o	H	1	Cl		155.3 ± 13.0	200.4 ± 22.1	176.5 ± 14.5	395.7 ± 48.8	95.7 ± 8.2
3p	OCH <sub>3</sub>	1	H		144.2 ± 15.0	228.5 ± 25.8	197.6 ± 16.9	415.5 ± 81.3	49.3 ± 36.5
3q	H	1	H		138.2 ± 20.7	210.3 ± 31.6	205.5 ± 24.7	438.3 ± 58.1	47.2 ± 6.4
3r	CH <sub>3</sub>	1	H		90.2 ± 12.0	14.6 ± 2.0	12.4 ± 1.3	295.9 ± 42.6	56.2 ± 5.1
3s	F	1	H		78.5 ± 16.5	14.8 ± 28.6	11.5 ± 1.5	304.0 ± 38.6	61.0 ± 5.8
3t	H	1	F		24.6 ± 8.7	11.7 ± 1.9	12.0 ± 2.4	322.2 ± 53.9	54.2 ± 7.3
3u	H	1	Cl		85.6 ± 9.6	145.9 ± 15.3	156.7 ± 19.1	356.3 ± 67.2	43.1 ± 3.5



Table 1 (Contd.)



Cmpd	R <sub>1</sub>	n	R <sub>2</sub>	NR <sub>3</sub> R <sub>4</sub>	Receptor affinity $K_i \pm \text{SEM}^a$ (nM)				
					D <sub>2</sub>	5-HT <sub>1A</sub>	5-HT <sub>2A</sub>	5-HT <sub>6</sub>	H <sub>3</sub>
3v	OCH <sub>3</sub>	1	H		73.0 ± 6.4	128.4 ± 12.6	118.4 ± 10.4	341.7 ± 64.5	48.2 ± 4.2
3w	H	1	H		17.5 ± 1.9	16.7 ± 1.8	5.6 ± 6.5	336.4 ± 65.3	12.1 ± 1.5
3x	H	1	H		86.2 ± 15.0	109.2 ± 12.4	119.5 ± 15.4	313.6 ± 61.0	39.4 ± 26.0
3y	H	1	H		30.8 ± 5.6	58.5 ± 4.2	83.4 ± 9.7	33.1 ± 5.8	753.1 ± 98.5

<sup>a</sup>  $K_i$  values obtained from three experiments, recorded as means ± SEM.

The effects of N-heterocycles (aralkyl pyrrolidine, piperidine, morpholine and piperazine fragments) and 8-(piperazin-1-yl)quinoline connected to a benzene ring were assessed, and the results suggest that the N-heterocycles play a significant role in the SARs. According to the results, compounds (3a–3h) with pyrrolidineyl disubstitution show higher affinity for the D<sub>2</sub> receptor than compounds with piperidyl substitution (3j–3q), but show lower affinity for the D<sub>2</sub> receptor than compounds with morpholinyl, piperazinyl and 8-(piperazin-1-yl)quinolinyl substitution. Similar results were also observed for the 5-HT<sub>1A</sub> receptor. Except for the compounds with piperazinyl (3x) and 8-(piperazin-1-yl)quinolinyl substitution (3y), most of the compounds show moderate to low affinity for the 5-HT<sub>2A</sub> receptor. Compounds 3a–3x show moderate to high affinity for the H<sub>3</sub> receptor, but only 3y exhibits high affinity for the 5-HT<sub>6</sub> receptor, which may be due to the introduction of the privileged structures in the H<sub>3</sub> and 5-HT<sub>6</sub> receptor antagonists.

In the present study, the effects that different electron-withdrawing group of the substituents of the benzene ring, such as –F, –Cl, –CH<sub>3</sub>, and –OCH<sub>3</sub> groups, have on the various receptors were also evaluated. When electron-donating (–CH<sub>3</sub>)

and electron-withdrawing (–F, –Cl, –OCH<sub>3</sub>) groups were introduced at different positions of the benzene ring, of the compounds 3a–3i that bear N-methyl pyrrolidinyl substituents, the substituted compounds 3a–3f display decreased affinities for three receptors (D<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>) compared with 3i, with only the –OCH<sub>3</sub>-substituted derivative 3g showing increased affinity for the 5-HT<sub>1A</sub> receptor. However, the presence of different substituents on the benzene ring does not have any obvious impact on the affinity for the 5-HT<sub>6</sub> and H<sub>3</sub> receptors. In terms of the compounds bearing N-methyl piperidyl substituents (3j–3q), meta and ortho positioned –CH<sub>3</sub> substituted 3j and 3k exhibit diminished affinities for the 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors. However, when –F, –Cl, –CH<sub>3</sub>, and –OCH<sub>3</sub> groups are introduced to the N-methyl morpholinyl substituted derivatives, the formed compounds 3r–3v show a decline in their affinities for the D<sub>2</sub> receptor. Compared to compound 3w, –F and –CH<sub>3</sub>-substituted 3r–3t show improved affinities for 5-HT<sub>1A</sub>, but a negative effect for 5-HT<sub>2A</sub> receptors. Disappointingly, –Cl and –OCH<sub>3</sub> group substituted 3u and 3v exhibit a dramatic decline in affinities for the D<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, and compounds with –F, –Cl, –CH<sub>3</sub>, and –OCH<sub>3</sub>

Table 2 Activities on the 5-HT<sub>2C</sub>, H<sub>1</sub> and  $\alpha_1$  receptors ( $K_i$  nM) of the candidate and reference antipsychotics

Cmpd	Receptor affinity $K_i \pm \text{SEM}^a$ (nM)			hERG IC <sub>50</sub> (nM)
	5-HT <sub>2C</sub>	$\alpha_1$	H <sub>1</sub>	
3t	430.4 ± 52.3	398.2 ± 49.5	668.3 ± 32.5	1158
3w	572.2 ± 91.5	405.5 ± 60.8	819.6 ± 48.7	1765
Risperidone	30.1 ± 2.8	51.4 ± 3.6	23.8 ± 1.5	1480

<sup>a</sup>  $K_i$  values obtained from three experiments, recorded as means ± SEM.



substituents (**3s–3v**) show slight differences in affinity for the 5-HT<sub>6</sub> receptor and less H<sub>3</sub> affinity.

Based on the literature and our previous experience, the space between benzothienopyrrolidine and the phenoxy structure features three carbons. On replacement of the chain lengths of **3g** from three carbons to two carbons, the generated compound **3h** displays diminished affinities for the 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub> and H<sub>3</sub> receptors, but slightly enhanced affinity for D<sub>2</sub> receptor.

In order to obtain compounds that show balance and high affinity, as well as high selectivity for further biological evaluation, herein, we set up three primary selection filter conditions: (a) high potency for the D<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (D<sub>2</sub>, K<sub>i</sub> ≤ 30 nM; 5-HT<sub>1A</sub>, K<sub>i</sub> ≤ 20 nM; and 5-HT<sub>2A</sub>, K<sub>i</sub> ≤ 20 nM); (b) an affinity for H<sub>3</sub> ≤ 15 nM and 5-HT<sub>6</sub> ≤ 350 nM (c) with balanced activities at the D<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, with a potency ratio for any two receptors of ≤2. As a result, compounds **3t** and **3w** were selected for further biological evaluation, including safety and behavioral studies.

### 2.3.2. The receptor selectivity of the selected compounds.

Compared to conventional antipsychotics, atypical antipsychotics significantly reduce the possibilities of unwanted effects such as EPS and tardive dyskinesia (TD; 47). However, these drugs still have pharmacological effects on off-target receptors, such as 5-HT<sub>2C</sub>, H<sub>1</sub>, and α<sub>1</sub>, which are related to many medication-related adverse events.<sup>31</sup> For example, antipsychotic-induced weight gain is probably caused by a synergistic effect on the 5-HT<sub>2C</sub> and H<sub>1</sub> receptors<sup>32</sup> and the inhibition of the α<sub>1</sub> receptor is presumed to be associated with orthostatic hypotension and rhythm problems.<sup>33</sup> Thus, assays of the 5-HT<sub>2C</sub>, H<sub>1</sub> and α<sub>1</sub> receptors were also conducted in this study. As the results in Table 2 show, the affinities of compounds **3t** and **3w** for the 5-HT<sub>2C</sub>, H<sub>1</sub>, and α<sub>1</sub> receptors are far lower than those of risperidone and haloperidol, and **3w** (5-HT<sub>2C</sub>, K<sub>i</sub> = 572.2 nM; α<sub>1</sub>, K<sub>i</sub> = 450.5 nM; H<sub>1</sub>, K<sub>i</sub> = 819.6 nM) exhibits lower affinities for the three receptors than **3t** (5-HT<sub>2C</sub>, K<sub>i</sub> = 430.4 nM; α<sub>1</sub>, K<sub>i</sub> = 398.2 nM; H<sub>1</sub>, K<sub>i</sub> = 668.3 nM), which suggests that **3w** is less likely to be associated with side effects such as weight gain, as well as with a lower incidence of orthostatic hypotension.

### 2.4. hERG channel blockade

Cardiotoxicity is a serious side effect that usually occurs due to the interactions between drugs and various voltage-gated ion channels in the heart, particularly the hERG channel. The hERG

potassium channel mediates the delayed rectified potassium current and drugs that inhibit the hERG channel may cause prolonged QT intervals<sup>34</sup> and increase the occurrence of potentially lethal Torsades de Pointes arrhythmia.<sup>35</sup> Thus, the inhibition of hERG is an effective and widely used indicator for the prediction of the cardiotoxicity of candidate drugs. To predict the cardiotoxicity of compounds **3t** and **3w**, their inhibitory actions on the hERG channel were evaluated in a patch-clamp assay *in vitro*. Compound **3w** (IC<sub>50</sub> = 1765 nM) displays a lower hERG channel inhibition than compound **3t** (IC<sub>50</sub> = 1158 nM) (Table 2), which suggests that compound **3w** has a low propensity to elicit treatment-induced QT interval prolongation than **3t** and risperidone (IC<sub>50</sub> = 1480 nM).

To sum up, compound **3w** displays excellent *in vitro* profiles with favorable affinity for the desired target receptors (D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, H<sub>3</sub> and 5-HT<sub>6</sub>) and lower affinity for the off-target receptors (5-HT<sub>2C</sub>, H<sub>1</sub> and α<sub>1</sub>) and hERG channel. Therefore, compound **3w** was subjected to intrinsic activity, safety assessment and animal behavioral studies to verify its effect on schizophrenia.

### 2.5. Intrinsic activity

Compound **3w** was chosen for further functional characterization because of its excellent *in vitro* activity and favorable safety profile. The results (ESI† Table 1) show that compound **3w** displays feeble agonist activity against the D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub> and H<sub>3</sub> receptors. In the antagonist assays, compound **3w** shows potent antagonism of the five receptors, higher than 85% efficiency. Compound **3w** shows potent D<sub>2</sub> (IC<sub>50</sub> = 11.6 nM) antagonism, and moderate 5-HT<sub>1A</sub> (IC<sub>50</sub> = 218.5 nM), 5-HT<sub>2A</sub> (IC<sub>50</sub> = 141.8 nM), 5-HT<sub>6</sub> (IC<sub>50</sub> = 413.5 nM) and H<sub>3</sub> (IC<sub>50</sub> = 232.3 nM) antagonism.

### 2.6. Acute toxicity

The acute toxicities of **3w** were assessed based on lethal dose, 50% (LD<sub>50</sub>) analyses (Table 3). The LD<sub>50</sub> value of **3w** is over 1000 mg kg<sup>-1</sup>, whereas those of haloperidol and risperidone are 21.0 and 82.0 mg kg<sup>-1</sup>, respectively. Thus, **3w** has a better safety profile than haloperidol and risperidone.

### 2.7. Evaluations of antipsychotic drug-like activities in animal models

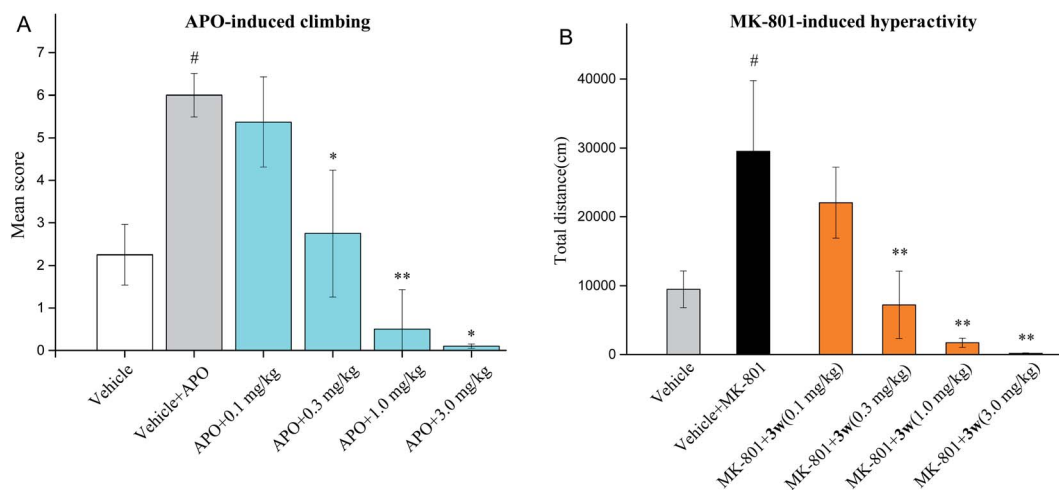
Based on *in vivo* and acute oral toxicity evaluation, compound **3w** was chosen as a candidate for *in vivo* behavioral study and

Table 3 *In vivo* pharmacological profile of the compounds

Cmpd	LD <sub>50</sub>	APO <sup>a</sup>	MK-801 <sup>b</sup>	CAT <sup>c</sup>	CAR <sup>d</sup>	CAT/APO	CAT/MK-801
<b>3w</b>	>1000	0.28	0.17	27.5	2.1	91.07	161.76
Haloperidol	21.0	0.09	0.11	0.12	—	5.67	4.63
Risperidone	82.0	0.05	0.02	0.51	0.65	10.4	32.5

<sup>a</sup> APO: apomorphine-induced climbing (ED<sub>50</sub>, mg kg<sup>-1</sup>, po (per os)). <sup>b</sup> MK-801: MK-801-induced hyperactivity (ED<sub>50</sub>, mg kg<sup>-1</sup>, po). <sup>c</sup> CAT: catalepsy (ED<sub>50</sub>, mg kg<sup>-1</sup>, po). <sup>d</sup> CAR: conditioned avoidance response (ED<sub>50</sub>, mg kg<sup>-1</sup>, po).





**Fig. 3** (A) Effect of compound **3w** on APO-induced climbing in mice (10 per group). The scores are shown as means  $\pm$  SEM. Statistical significances were performed using a nonparametric two-tailed Mann–Whitney U-test: \* $p < 0.05$  and \*\* $p < 0.01$  versus apomorphine treatment; # $p < 0.05$  versus vehicle treatment. (B) Effect of compound **3w** on MK-801-induced hyperactivity in mice (10 per group). The distance expressed as the means  $\pm$  SEM. Statistical evaluation was performed via two-way ANOVA followed by Tukey's test for multiple comparisons. \* $p < 0.05$  and \*\* $p < 0.01$  vs. MK-801 treatment; # $p < 0.05$  vs. vehicle treatment.

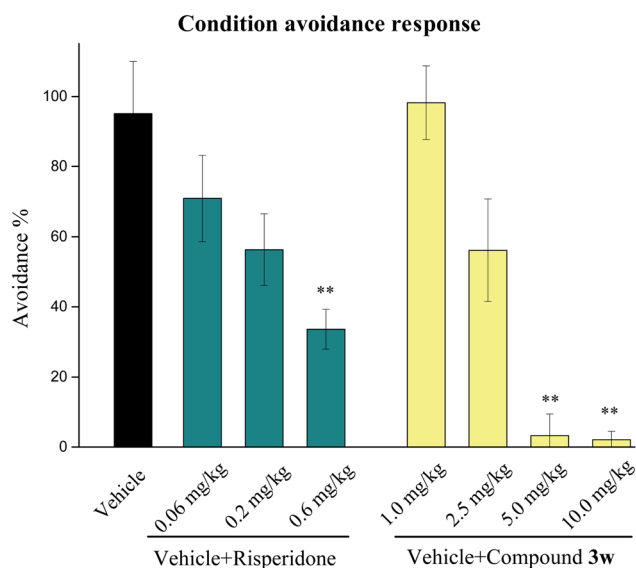
long-term side effects evaluation to verify its antipsychotic-like activities.

**2.7.1. Apomorphine-induced climbing.** The climbing behavior induced by  $D_2$  receptor agonists, such as apomorphine is commonly used as a rodent model of psychoses.<sup>36</sup> Therefore, compounds normalizing climbing behavior demonstrate antipsychotic-like properties. According to the results of this model (Table 3 and Fig. 3A), compound **3w** reverses APO-induced climbing, with an  $ED_{50}$  (50% effective dose) value of  $0.28 \text{ mg kg}^{-1}$ . As positive control drugs, haloperidol and risperidone display significant inhibitory effect on the climbing behavior, with  $ED_{50}$  values of  $0.09$  and  $0.05 \text{ mg kg}^{-1}$ , respectively. This indicates that compound **3w** is a potent antagonist of the  $D_2$  receptor *in vitro*, which would likely alleviate or eliminate the positive symptoms of schizophrenia.

**2.7.2. MK-801-induced hyperlocomotion.** *N*-Methyl-D-aspartate (NMDA) receptors are involved in many important physiological functions in the central nervous system (CNS) and are also important targets in the therapy of several neuropsychiatric diseases. Moreover, abnormalities in this receptor system may cause CNS dysfunction and many non-competitive NMDA receptor antagonists (such as PCP, MK-801 and ketamine) produce schizophrenia-like symptoms (negative and cognitive symptoms) in healthy animals. However, compounds with antipsychotic-like efficacies significantly reverse these symptoms.<sup>37</sup> Therefore, the NMDA receptor antagonist MK-801-induced hyperactivity model is often used to assess the potential antipsychotic-like efficacy of new compounds. In this assay, compound **3w** attenuates the MK-801-induced hyperactivity significantly in mice, with an  $ED_{50}$  value of  $0.17 \text{ mg kg}^{-1}$ . In the control group, mice that receive oral administrations of haloperidol and risperidone show decreased locomotor activities, with  $ED_{50}$  values of  $0.11$  and  $0.02 \text{ mg kg}^{-1}$ , respectively (Table 3 and Fig. 3B). To sum up, these results indicate that compound

**3w** has the antipsychotic-like efficacy of attenuating the negative and cognitive symptoms of schizophrenia.

**2.7.3. Potential for catalepsy.** A significant risk factor associated with schizophrenia treatment is that antipsychotics have the tendency to trigger EPS side effects in humans. To predict the likelihood that a novel agent will produce EPS-like side effects, the rodent catalepsy test has become a commonly used model in antipsychotic drug discovery studies.<sup>38</sup> Thus, compound **3w** was subject to the horizontal bar test in mice to predict its tendency for striatal-mediated side effects and



**Fig. 4** Effect of compound **3w** on the conditioned avoidance response in rats (10 per group). The percentage of avoidance is shown as the mean  $\pm$  SEM, and the level of significance is \* $p < 0.05$  and \*\* $p < 0.01$  vs. the vehicle group. Statistical analysis was performed using the SPSS software.



cataleptogenic potential. In this test, haloperidol ( $ED_{50}$ :  $0.51 \text{ mg kg}^{-1}$ ) and risperidone ( $ED_{50}$ :  $1.15 \text{ mg kg}^{-1}$ ) induce significant cataleptic effects (Table 3), which is in line with their strong antagonistic effect on the  $D_2$  receptor *in vitro*. In comparison, compound **3w** exhibits a low potential cataleptogenic effect ( $ED_{50}$ :  $27.5 \text{ mg kg}^{-1}$ ), which infers that **3w** has a higher threshold for catalepsy and lower incidence of EPS. In addition, compound **3w** exhibits a broad therapeutic index that ranges from 91.07 to 161.76, based on its efficacy (apomorphine and MK-801 models) and its adverse effects (catalepsy). In the positive control groups, the therapeutic index of haloperidol is between 4.63 and 5.67, while the therapeutic index of risperidone is in the range of 10.4–32.5.

**2.7.4. Conditioned avoidance response (CAR) tests.** The conditioned avoidance response (CAR) test is a sensitive method for evaluating antipsychotics that have a high affinity for DA receptors and it can also be used to screen for antipsychotics that primarily affect neurotransmitter receptors.<sup>39,40</sup> In this model, the results show that risperidone and compound **3w** effectively suppress the CAR in rats (Table 3 and Fig. 4), with an

$ED_{50}$  of 2.1 and  $0.65 \text{ mg kg}^{-1}$ . This indicates that compound **3w** shows high efficacy towards DA receptors *in vitro*, which may bring about therapeutic effects in the treatment of schizophrenia.

**2.7.5. Memory study.** The NOR task is based on an animal's innate preference for novelty, and this model is often used to predict the potential of a novel drug for cognitive enhancement.<sup>41,42</sup> In this study, oral administration of compound **3w** ( $1.0\text{--}10.0 \text{ mg kg}^{-1}$ ) has no obvious effect on the total exploration time during the acquisition trial (Fig. 5A) compared with the blank and control groups (risperidone and rivastigmine). In the retention trial (Fig. 5B), the rats that received rivastigmine exhibit a longer exploration ( $0.1$  to  $3.0 \text{ mg kg}^{-1}$ ) time for novel things compared with the vehicle conditions, suggesting that the memory acquired from the acquisition trial is preserved. However, there is no significant difference in the exploration times for the novel and familiar objects when the rats are given risperidone ( $0.02$  to  $0.2 \text{ mg kg}^{-1}$ ) and a low dose of compound **3w** ( $0.1 \text{ mg kg}^{-1}$ ), indicating that risperidone and a low dose of compound **3w** have little or no effect on memory enhancement.

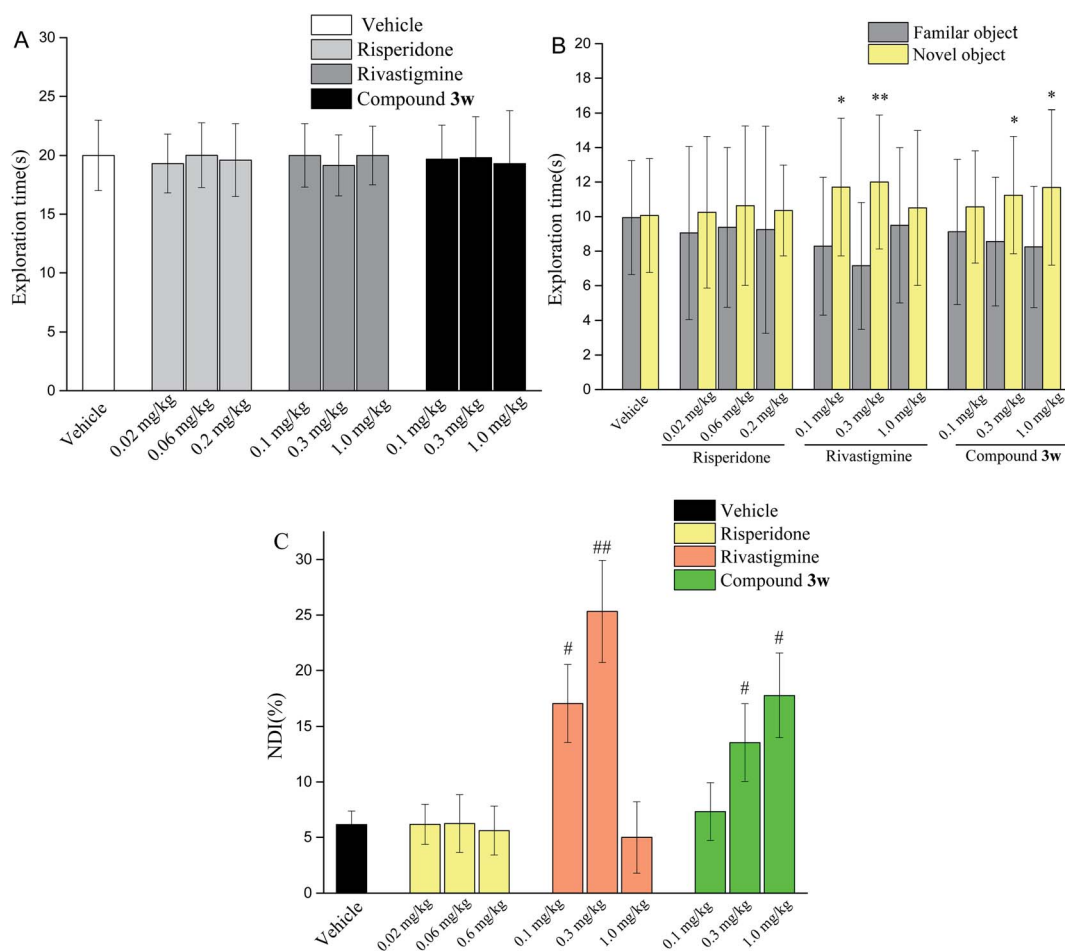


Fig. 5 Effects of compound **3w** on the NOR testing in rats (10 per group). Experimental results acquired 60 min after oral administration of vehicle risperidone ( $0.02$ ,  $0.06$ ,  $0.2 \text{ mg kg}^{-1}$ ), rivastigmine ( $0.1$ ,  $0.3$ ,  $1.0 \text{ mg kg}^{-1}$ ), and **3w** ( $0.1$ ,  $0.3$ ,  $1.0 \text{ mg kg}^{-1}$ ). (A) Exploration times in the acquisition trial; (B) exploration times for the familiar and a novel object during acquisition trials; and (C) novelty discrimination index (NDI) in the retention trial. Data are presented as the mean  $\pm$  SEM ( $n = 10$ ). \*\* $p < 0.01$  and \* $p < 0.05$  compared with a familiar object *via* a paired test; # $p < 0.05$  and ## $p < 0.01$  compared with the vehicle group.



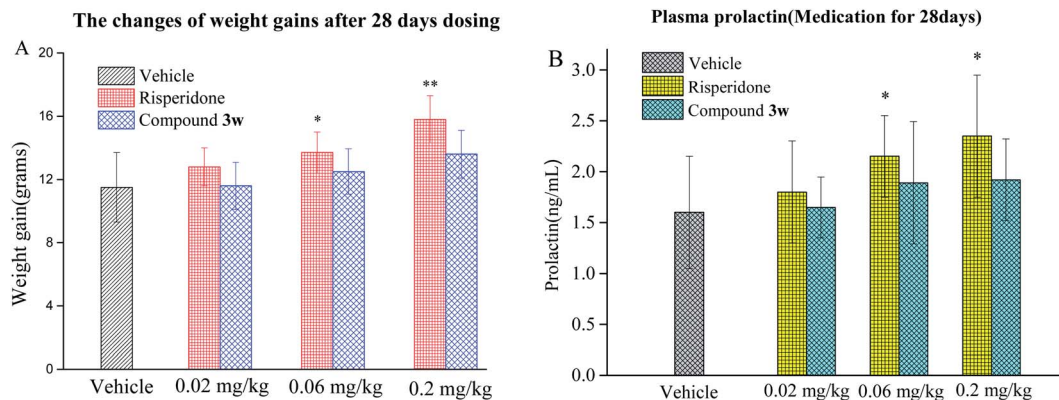


Fig. 6 Effects of 3w and risperidone on body weight (A) and serum prolactin (PRL) (B) in mice after 28 days of administration (10 per group). The weight gain and prolactin are presented as the means  $\pm$  SEM. Statistical evaluation was performed via a student's *t* test. \**p* < 0.05 and \*\**p* < 0.01 versus the vehicle group.

Additionally, the rats receiving 0.3 and 1.0 mg kg<sup>-1</sup> oral doses of compound 3w exhibit an obvious increase in the novelty discrimination index (NDI) as well as those that received 0.1 and 0.3 mg kg<sup>-1</sup> of rivastigmine, whereas under the vehicle conditions or dosed with 0.02 to 0.2 mg kg<sup>-1</sup> of risperidone the rats do not show increased NDI (Fig. 5C). To sum up, these results demonstrate that compound 3w improves recognition memory in this model.

Taken together, compound 3w exhibits good anti-schizophrenic activity in terms of the above-mentioned behavioral testing, especially in terms of memory enhancement in the NOR testing, which is probably due to the addition of H<sub>3</sub> receptor antagonism to its mixed dopamine and 5-hydroxytryptamine receptor antagonist profile.

### 2.8. Weight gain and serum prolactin levels

To determine whether compound 3w has adverse long-term metabolic adverse, it was assessed in terms of its ability to induce weight gain and hyperprolactinemia in an animal model of chronic administration. After 28 days, the mice given compound 3w exhibit negligible weight gain, while the mice given risperidone show significant weight gain (Fig. 6A and B). Besides this, risperidone induces a significant increase in serum prolactin levels, but compound 3w does not, which means that 3w shows a lower incidence of treatment-related side effects than the representative atypical antipsychotic drug risperidone, which might result from its desirable selectivity profile against the 5-HT<sub>2C</sub> and H<sub>1</sub> receptors that are related to the adverse effects of marketed antipsychotics.

## 3. Conclusions

We are engaged in the synthesis and biological evaluation of new arylpiperazine derivatives that have facilitated the discovery of compound 3w with a favorable antipsychotic profile, combining affinities for the D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub> and H<sub>3</sub> receptors. Besides this, compound 3w also exhibits low affinities for the 5-HT<sub>2C</sub>, H<sub>1</sub> and  $\alpha_1$  receptors and hERG

inhibitory activity. The multi-receptor antagonist profile of 3w means that it has a significant effect in the inhibition of schizophrenia-like symptoms, including reversing APO-induced hyperlocomotion and MK-801-induced hyperactivity in mice and restraining the CAR in rats, and has a high threshold in terms of acute toxicity and a lower tendency to induce catalepsy. Moreover, compared to the representative conventional antipsychotic risperidone and the memory-enhancing rivastigmine, compound 3w demonstrates superior effectiveness in cognitive enhancement in NOR tests. Taken together, compound 3w is similar to the compounds A and B that were reported previously by our research group, and is in alignment with our original expectations. Thus, compound 3w was selected for the treatment of schizophrenia and deserves further development.

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## Conflicts of interest

The authors declare no conflict of interest.

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## References

- R. S. Kahn, I. E. Sommer, R. M. Murray, A. Meyer-Lindenberg, D. R. Weinberger, T. D. Cannon, M. O'Donovan, C. U. Correll, J. M. Kane, J. van Os and T. R. Insel, Schizophrenia, *Nat. Rev. Dis. Primers*, 2015, **1**, 15067.
- J. R. Dequardo and R. Tandon, Review Do atypical antipsychotic medications favorably alter the long-term



- course of schizophrenia?, *J. Psychiatr. Res.*, 1998, **32**, 229–242.
- 3 G. W. Arana, An overview of side effects caused by typical antipsychotics, *J. Clin. Psychiatry*, 2000, **61**(suppl. 8), 5–11.
- 4 A. Serretti, D. De Ronchi, C. Lorenzi and D. Berardi, New antipsychotics and schizophrenia: a review on efficacy and side effects, *Curr. Med. Chem.*, 2004, **11**, 343–358.
- 5 S. O. Ogren, The role of 5-HT<sub>1A</sub> receptors in learning and memory, *Behav. Brain Res.*, 2008, **195**, 54–77.
- 6 M. J. Millan, Improving the treatment of schizophrenia: focus on serotonin (5HT)<sub>1A</sub> receptors, *J. Pharmacol. Exp. Ther.*, 2001, **295**, 853–861.
- 7 C. J. Schmidt, S. M. Sorensen, J. H. Kehne, A. A. Carr and M. G. Palfreyman, The role of 5-HT<sub>2A</sub> receptors in antipsychotic activity, *Life Sci.*, 1995, **56**, 2209–2222.
- 8 G. Zhang and R. W. Stackman, The role of serotonin 5-HT<sub>2A</sub> receptors in memory and cognition, *Front. Pharmacol.*, 2015, **6**, 225.
- 9 H. Y. Meltzer and B. W. Massey, The role of serotonin receptors in the action of atypical antipsychotic drugs, *Curr. Opin. Pharmacol.*, 2011, **11**, 59–67.
- 10 S. Miyamoto, G. E. Duncan, C. E. Marx and J. A. Lieberman, Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs, *Mol. Psychiatry*, 2005, **10**, 79–104.
- 11 M. C. Mauri, S. Paletta, M. Maffini, A. Colasanti, F. Dragogna, C. D. Pace and A. Altamura, Clinical pharmacology of atypical antipsychotics: an update, *Clin. Pharmacokinet.*, 2014, **13**, 1163–1191.
- 12 A. B. Casey and C. E. Canal, Classics in chemical neuroscience: aripiprazole, *ACS Chem. Neurosci.*, 2017, **8**, 1135–1146.
- 13 J. E. Frampton, Brexpiprazole: a review in schizophrenia, *Drugs*, 2019, **79**, 189–200.
- 14 S. Caccia, R. W. Invernizzi, A. Nobili and L. Pasina, A new generation of antipsychotics: pharmacology and clinical utility of cariprazine in schizophrenia, *Ther. Clin. Risk Manage.*, 2013, **9**, 319–328.
- 15 A. Yee, Brexpiprazole for the treatment of schizophrenia, *Expert Rev. Neurother.*, 2016, **16**, 109–122.
- 16 D. Mamo, A. Graff, R. Mizrahi, C. M. Shammi and S. Kapur, Differential effects of aripiprazole on D<sub>2</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>1A</sub> receptor occupancy in patients with schizophrenia: a triple tracer PET study, *Am. J. Psychiatry*, 2007, **164**, 1411–1417.
- 17 S. Natesan, G. E. Reckless, J. N. Nobrega, P. J. Fletcher and S. Kapur, Dissociation between in vivo occupancy and functional antagonism of dopamine D<sub>2</sub> receptors: comparing aripiprazole to other antipsychotics in animal models, *Neuropsychopharmacol.*, 2006, **31**(9), 1854–1863.
- 18 R. Mailman and V. Murthy, Third generation antipsychotic drugs: partial agonism or receptor functional selectivity?, *Curr. Pharm. Des.*, 2010, **16**, 488–501.
- 19 R. H. Campbell, M. Diduch, K. N. Gardner and C. Thomas, Review of cariprazine in management of psychiatric illness, *Ment. Health Clin.*, 2018, **7**, 221–229.
- 20 M. Kondej, P. Stępnicki and A. A. Kaczor, Multi-target approach for drug discovery against schizophrenia, *Int. J. Mol. Sci.*, 2018, **19**, 3105.
- 21 Y. Chen, S. Wang, X. Xu, X. Liu, M. Yu, S. Zhao, S. Liu, Y. Qiu, T. Zhang, B. F. Liu and G. Zhang, Synthesis and biological investigation of coumarin piperazine (piperidine) derivatives as potential multireceptor atypical antipsychotics, *J. Med. Chem.*, 2013, **56**, 4671–4690.
- 22 Y. Chen, Y. Lan, S. Wang, H. Zhang, X. Xu, X. Liu, M. Yu, B. F. Liu and G. Zhang, Synthesis and evaluation of new coumarin derivatives as potential atypical antipsychotics, *Eur. J. Med. Chem.*, 2014, **74**, 427–439.
- 23 J. Jin, K. Zhang, F. Dou, C. Hao, Y. Zhang, X. Cao, L. Gao, J. Xiong, X. Liu, B. F. Liu, G. Zhang and Y. Chen, Isoquinolinone derivatives as potent CNS multi-receptor D<sub>2</sub>/5-HT<sub>1A</sub>/5-HT<sub>2A</sub>/5-HT<sub>6</sub>/5-HT<sub>7</sub> agents: synthesis and pharmacological evaluation, *Eur. J. Med. Chem.*, 2020, 112709.
- 24 X. Cao, Y. Zhang, Y. Chen, Y. Qiu, M. Yu, X. Xu, X. Liu, B. F. Liu and G. Zhang, Synthesis and biological evaluation of fused tricyclic heterocycle piperazine (piperidine) derivatives as potential multireceptor atypical antipsychotics, *J. Med. Chem.*, 2018, **61**, 10017–10039.
- 25 J. C. Schwartz, The histamine H<sub>3</sub> receptor: from discovery to clinical trials with pitolisant, *Br. J. Pharmacol.*, 2011, **163**, 713–721.
- 26 N. Upton, T. T. Chuang, A. J. Hunter and D. J. Virley, 5-HT<sub>6</sub> receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease, *Neurotherapeutics*, 2008, **5**, 458–469.
- 27 M. A. Letavic, L. Aluisio, R. Apodaca, M. Bajpai, A. J. Barbier, A. Bonneville, P. Bonaventure, N. I. Carruthers, C. Dugovic, I. C. Fraser, M. L. Kramer, B. Lord, T. W. Lovenberg, L. Y. Li, K. S. Ly, H. Mcallister, N. S. Mani, K. L. Morton, A. Ndifor, S. D. Nepomuceno, C. R. Pandit, S. B. Sands, C. R. Shah, J. E. Shelton, S. S. Snook, D. M. Swanson and W. Xiao, Novel benzamide-based histamine H<sub>3</sub> receptor antagonists: the identification of two candidates for clinical development, *ACS Med. Chem. Lett.*, 2015, **6**, 450–454.
- 28 M. D. Wood, C. Heidebreder, C. Reavill, C. R. Ashby Jr and D. N. Middlemiss, 5-HT<sub>2C</sub> receptor antagonists: potential in schizophrenia, *Drug Dev. Res.*, 2001, **54**, 88–94.
- 29 M. He, C. Deng and X. F. Huang, The role of hypothalamic H<sub>1</sub> receptor antagonism in antipsychotic-induced weight gain, *CNS Drugs*, 2013, **27**(6), 423–434.
- 30 P. A. Gwartz and H. L. Stone, Coronary blood flow and myocardial oxygen consumption after alpha adrenergic blockade during submaximal exercise, *J. Pharmacol. Exp. Ther.*, 1981, **217**, 92–98.
- 31 S. C. Chang and M. L. Lu, Metabolic and Cardiovascular Adverse Effects Associated with Treatment with Antipsychotic Drugs, *J. Exp. Clin. Med.*, 2012, **4**, 103–107.
- 32 H. A. Nasrallah, Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles, *Mol. Psychiatry*, 2008, **13**, 27–35.
- 33 J. Leung, A. M. Barr, R. M. Procyshyn, W. G. Honer and C. Pang, Cardiovascular side-effects of antipsychotic drugs:



- the role of the autonomic nervous system, *Pharmacol. Ther.*, 2012, **135**, 113–122.
- 34 M. Recanatini, E. Poluzzi, M. Masetti, A. Cavalli and F. De Ponti, QT prolongation through hERG K<sup>+</sup> channel blockade: current knowledge and strategies for the early prediction during drug development, *Med. Res. Rev.*, 2005, **25**, 133–166.
- 35 E. Raschi, L. Ceccarini, F. D. Ponti and M. Recanatini, hERG-related drug toxicity and models for predicting hERG liability and QT prolongation, *Expert Opin. Drug Metab. Toxicol.*, 2009, **5**, 1005–1021.
- 36 C. A. Jones, D. Watson and K. Fone, Animal models of schizophrenia, *Br. J. Pharmacol.*, 2011, **164**, 1162–1194.
- 37 E. W. Tuplin, M. R. Stocco and M. R. Holahan, Attenuation of MK-801-induced behavioral perseveration by typical and atypical antipsychotic pretreatment in rats, *Behav. Neurosci.*, 2015, **129**, 399–411.
- 38 D. A. Sykes, H. Moore, L. Stott, N. Holliday, J. A. Javitch, J. R. Lane and S. J. Charlton, Extrapyramidal side effects of antipsychotics are linked to their association kinetics at dopamine D<sub>2</sub> receptors, *Nat. Commun.*, 2017, **8**, 763.
- 39 M.-L. Wadenberg, Conditioned avoidance response in the development of new antipsychotics, *Curr. Pharm. Des.*, 2010, **16**, 358–370.
- 40 M. Feng, J. Gao, N. Sui and M. Li, Effects of central activation of serotonin 5-HT<sub>2A/2C</sub> or dopamine D<sub>2/3</sub> receptors on the acute and repeated effects of clozapine in the conditioned avoidance response test, *Psychopharmacology*, 2015, **232**, 1219–1230.
- 41 L. Rajagopal, B. W. Massey, M. Huang, Y. Oyamada and H. Y. Meltzer, The Novel Object Recognition Test in Rodents in Relation to Cognitive Impairment in Schizophrenia, *Curr. Pharm. Des.*, 2014, **20**(31), 5104–5114.
- 42 M. Horiguchi, K. E. Hannaway, A. E. Adekun, M. Huang, K. Jayathilake and H. Y. Meltzer, D-1 receptor agonists reverse the subchronic phencyclidine (PCP)-induced novel object recognition (NOR) deficit in female rats, *Behav. Brain Res.*, 2013, **238**, 36–43.

