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Synthesis and evaluation of amide, sulfonamide and urea – benzisoxazole derivatives as potential atypical antipsychotics†

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In this paper, we report the optimization of a series of novel, potential antipsychotic derivatives combining potent dopamine D_2, D_3 and serotonin 5-HT_{1A}, 5-HT_{2A} receptor affinities. The pharmacological features of compound **27** are a high affinity for dopamine D_2, D_3 and serotonin 5-HT_{1A}, 5-HT_{2A} receptors. Moreover it possesses low affinity for 5-HT_{2C} and H_1 receptors (to reduce the risk of obesity associated with chronic treatment) and hERG channels (to reduce the incidence of torsade des pointes). Furthermore, compound **27** inhibited apomorphine-induced climbing, MK-801-induced hyperactivity and DOI-induced head twitch without observable catalepsy at the highest dose tested in mice. Taken together, among the amide derivatives, we identified compound **27** as a potential antipsychotic lead candidate.

Introduction

Schizophrenia, a neuropsychiatric disorder affecting more than 1% of the world's population, is a devastating and costly disease.[1] Typical antipsychotics (*e.g.*, haloperidol, Fig. 1) exert control of positive symptoms of schizophrenia by opposing the excessive stimulation of D_2 receptors resulting from hyperactivity of mesolimbic dopaminergic projections. However, it fails to alleviate the negative symptoms such as social withdrawal, flattened affect, and cognitive deficits.[2] Moreover, typical antipsychotics are associated with extrapyramidal symptoms (EPS).[3–5] The introduction of clozapine as the first atypical antipsychotic (*e.g.*, clozapine, ziprasidone and risperidone, Fig. 1) in 1989 represented a new development in the treatment of schizophrenia. Atypical antipsychotics possess potent antagonism for serotonin 5-HT_{2A} and dopamine D_2 receptors.[6] These drugs are effective in treating a range of psychiatric symptoms. Moreover, they have fewer reported extrapyramidal adverse events compared to those reported with typical antipsychotic medications.[7] However, patients treated with the majority of the atypical antipsychotics have reported clinically significant weight gain, dyslipidemia, diabetes, and QT interval prolongation.[8–11] Therefore, novel drugs that possess improved efficacy and favorable side-effect profiles are needed.

The growing recognition that multi-receptor ligands, which simultaneously act at multiple targets, may yield a more desirable drug profile than selectively targeted drugs has opened a new approach for the development of therapeutics.[12–14] In general, research over the past decade has shown that the clinical efficacy of antipsychotic drugs is linked to a complex binding profile. Moreover, many previous studies have demonstrated the importance of multi-target G-protein-coupled receptors in schizophrenia.[15,16] Blockade of the mesolimbic D_2 receptor increases the efficacy of atypical antipsychotics against positive symptoms associated with schizophrenia.[17] The D_3 receptor seems to play a role in flexible behaviors, a behavioral component that is affected by schizophrenia.[18] D_3 antagonism may improve cognition[19] and reduce the risk of causing extrapyramidal side effects.[20] ABT-925 is a selective dopamine D_3 receptor antagonist that has an approximately 100-fold higher *in vitro* affinity for dopamine D_3 *versus* D_2 receptors.[21] The safety and tolerability of ABT-925 have been examined in phase 1 studies, where ABT-925 was generally well tolerated up to the highest dose levels tested (600 mg single dose, 500 mg once daily [QD] for 7 days).[22] ABT-925 is currently undergoing phase II clinical trials in patients with schizophrenia.[22] The 5-HT_{1A} receptor plays a crucial role in regulating psychoemotional, cognitive and motor functions in the central nervous system.[23,24] Preclinical data indicate that 5-HT_{1A} receptor activation contributes to the improved activity of certain atypical antipsychotic drugs, such as effective treatment of cognitive and negative

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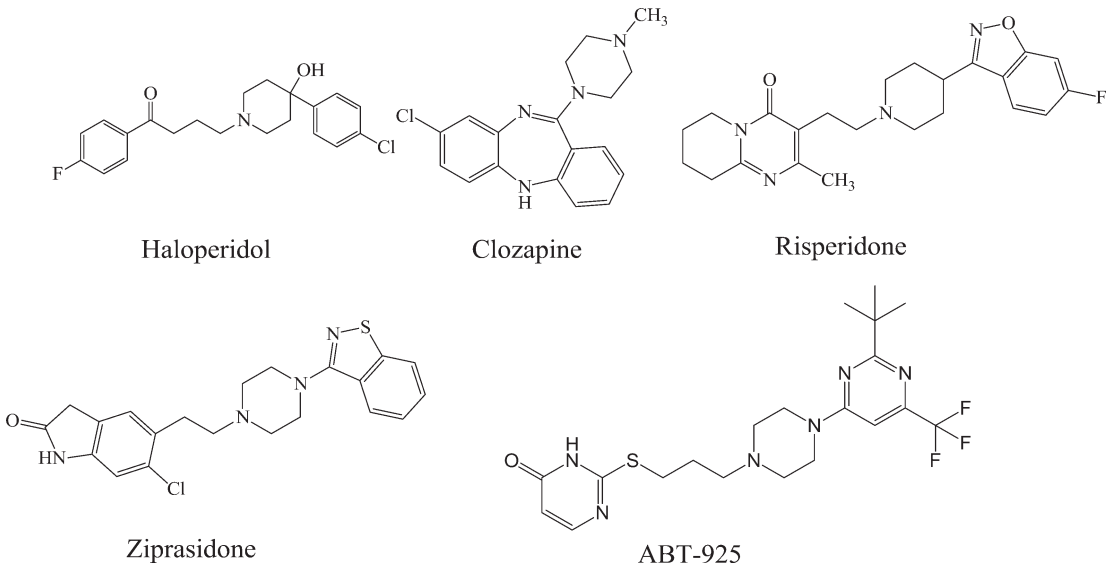


Fig. 1 Structures of a typical (haloperidol) and atypical antipsychotics.

symptoms, and decreased development of EPS in schizophrenia.[25] 5-HT_{2A} receptor antagonism is believed to contribute to the atypical antipsychotic profile.[17] Furthermore, the H_1 and 5-HT_{2C} receptors may be involved in the weight gain associated with the treatment of schizophrenia *via* atypical antipsychotic drugs.[26–29] Thus, the aim of our work was to develop novel antipsychotics that act on dopaminergic and serotonergic receptors with a low affinity for the H_1 and 5-HT_{2C} receptors. Our goal was to develop a pharmacological agent that would effectively treat the positive symptoms, the negative symptoms and the cognitive impairment associated with schizophrenia, without producing weight gain.

In fact, the latest effort was the development of compound **1** (Fig. 2), a 4-isoquinolinesulfonamide with a semi-rigid alkylene spacer, classified as a multi-receptor atypical antipsychotic.[30,31] In our previous studies, compounds **2–5** exhibited obvious antipsychotic properties without observable catalepsy in animal models.[32–35] Compound **4** possesses high affinity for dopamine D_2, D_3 and serotonin 5-HT_{1A}, 5-HT_{2A} receptors, and it possesses low affinity for H_1 and 5-HT_{2C} receptors (to reduce the risk of obesity associated with chronic treatment). Furthermore, fewer preclinical adverse events were noted with compound **4** compared with risperidone in assays that measured prolactin secretion and weight gain. In the present study, we developed an amide, sulfonamide and urea with a semi-rigid alkylene spacer system linked to the 4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl group, which is a key chemical structure found in atypical antipsychotics. This strategy led to the synthesis of compounds **10–33** (Fig. 2), which allowed us to understand the SAR (structure–activity relationship) and evaluate the pharmacological efficacy. We report here the synthesis and behavioral investigation of new compounds as novel and potential antipsychotics, characterized by potent binding affinities for

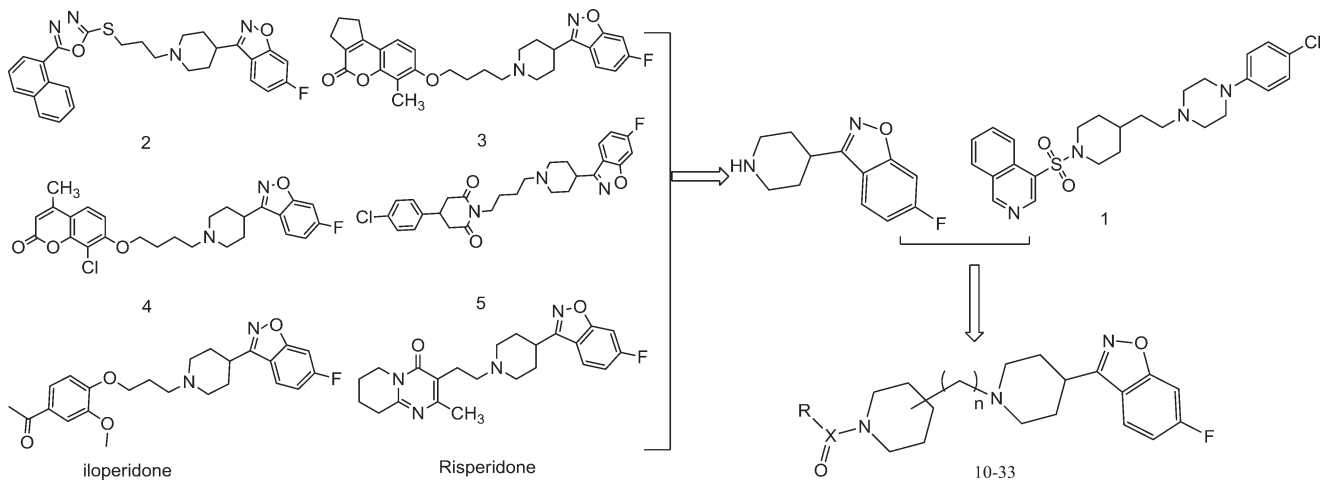


Fig. 2 Design of new amide derivatives.

the D_2, D_3, 5-HT_{1A}, 5-HT_{2A}, H_1, 5-HT_{2C}, α_1 and α_2 receptors. Furthermore, three primary behavioral models were used to evaluate compounds. Inhibition of apomorphine-induced climbing, MK-801-induced hyperactivity and DOI-induced head twitch predicted efficacy for the positive symptoms of schizophrenia and induction of catalepsy benchmarked the liability for extrapyramidal motor side-effects.

Chemistry

The general strategy for the synthesis of compounds **10–31** is summarized in Scheme 1. The *N*-Boc-protected piperidin-4-ylmethanol (**6a**), piperidin-4-ylethanol (**6b**) and piperidin-3-ylmethanol (**6c**) reacted with tosyl chloride to give compounds **7a–c**. Then, the intermediates **7a–c** reacted with 4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidine to give the respective tertiary amines **8a–c**. Boc removal with EA/HCl gave compounds **9a–c**, which reacted with acyl chlorides, sulfonyl chlorides or isocyanate to yield compounds **10–33**.

Pharmacology

All of the new compounds (free bases) were dissolved in 5% DMSO and buffer. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptor, [^{3}H]8-OH-DPAT, rat brain cortex; (b) serotonin 5-HT_{2A} receptor, [^{3}H]ketanserin, rat brain cortex; (c) serotonin 5-HT_{2C} receptor, [^{3}H]mesulergine, rat brain cortex; (d) dopamine D_2 receptor, [^{3}H]spiperone, rat striatum; (e) dopamine D_3 receptor, [^{3}H]7-OH-DPAT, rat olfactory tubercle; (f) histamine H_1 receptor, [^{3}H]mepyramine, guinea pig cerebellum; (g) adrenergic α_1 receptor, [^{3}H]prazosin, rat brain cortex; (h) adrenergic α_2 receptor, [^{3}H]rauwolscine, rat brain cortex.

Total binding was determined in the absence of non-specific binding and test compounds. Specific binding was determined in the presence of test compounds. Non-specific binding was determined as the difference between total and specific binding.

$$\text{Percentage of inhibition (\%)} = (\text{total binding} - \text{specific binding}) \times 100\%/(\text{total binding} - \text{nonspecific binding})$$

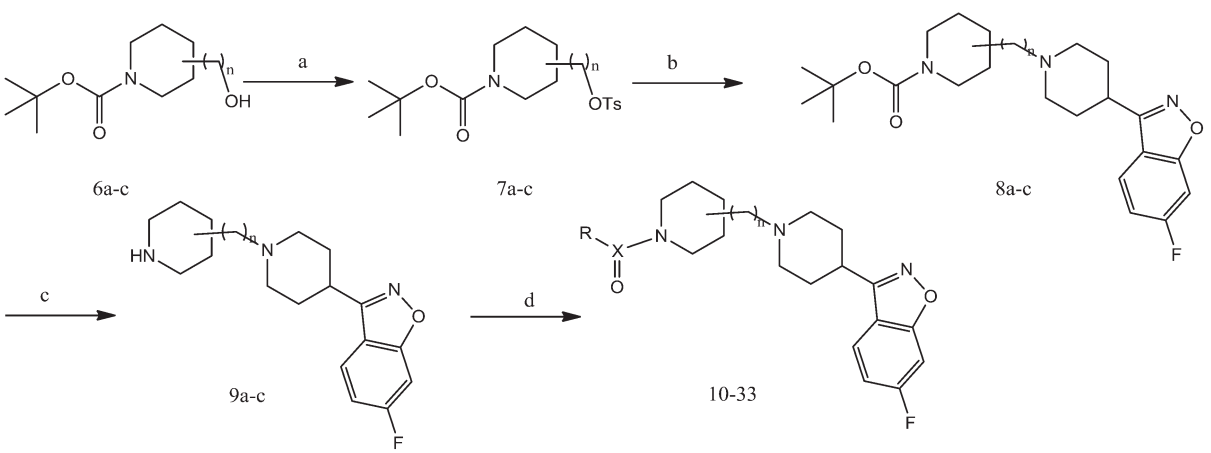
Blank experiments were carried out to determine the effect of 5% DMSO on the binding; no such effects were observed. Compounds were tested at least three times at six concentrations (10^{-5} M to 10^{-10} M), IC_{50} values were determined by nonlinear regression analysis using a Hill equation curve fitting. K_i values were calculated based on the Cheng and Prussoff equation: $K_i = IC_{50}/(1 + C/K_d)$, where C represents the concentration of the radioligands used, and K_d its receptor dissociation constant was calculated for each labeled ligand. Mean K_i values and SEM are reported for at least three independent experiments. Binding affinities were expressed as K_i values in Tables 1–4.

Selected compounds were further evaluated *in vivo* in mice using apomorphine-induced climbing, MK801-induced hyperactivity, DOI-induced head twitch and catalepsy models.

Results and discussion

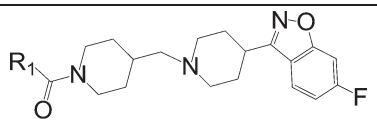
In vitro studies of new compounds

In this work, our initial focus was to investigate the affinities of urea derivative compounds on D_2, 5-HT_{1A} and 5-HT_{2A} receptors (Table 1, compounds **10–14**). As shown in Table 1, compounds **10** and **11** with alkyl substituents demonstrated weak affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors. It is worth mentioning that compounds **12** and **13** with aromatic substituents have improved affinities to D_2, 5-HT_{1A} and 5-HT_{2A} receptors, in contrast to compounds **10** and **11**. Moreover, the compound with a chlorine (Cl) on the phenyl (compound **13**, D_2, K_i = 85.6 nM; 5-HT_{2A}, K_i = 6.8 nM) showed higher affinity than the compound with a CH_3 (compound **12**, D_2, K_i = 178.1 nM; 5-HT_{2A}, K_i = 26.9 nM) to D_2 and 5-HT_{2A} receptors. However, the introduction of a benzyl group (compound **14**) resulted in a dramatic decrease in affinities for the three receptors.

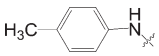
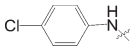


Scheme 1 Reagents and conditions: (a) TsCl, CH_2Cl_2, Et_3N, 0 °C rt; (b) 4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidine, Et_3N, CH_3CN, reflux, 24 h; (c) EA/HCl; (d), acyl chloride or isocyanate, Et_3N, CH_2Cl_2, 0 °C rt.

Table 1 Binding affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **10–14** and reference antipsychotics[a]

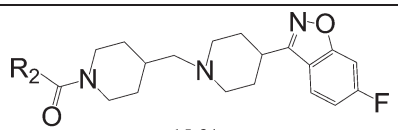


10-14

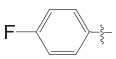
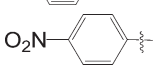
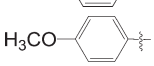
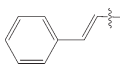
Compound	R_1	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
10		>10 000[b]	>10 000	568.6 ± 68.6
11		>10 000	>10 000	1098.2 ± 156.9
12		178.1 ± 19.2	276.5 ± 29.6	26.9 ± 3.2
13		85.6 ± 9.1	298.9 ± 38.6	6.8 ± 1.5
14		1268.2 ± 168.9	>10 000	265.3 ± 29.8
Clozapine		130.7 ± 15.2	185.6 ± 19.1	12.9 ± 1.3
Risperidone		2.8 ± 0.3	190.2 ± 16.1	0.25 ± 0.03

[a] K_i values are taken from three experiments, expressed as means ± SEM. [b] The K_i values were not calculated because the inhibition percentages at 10 μM were too low.

Table 2 Binding affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **15–21**[a]



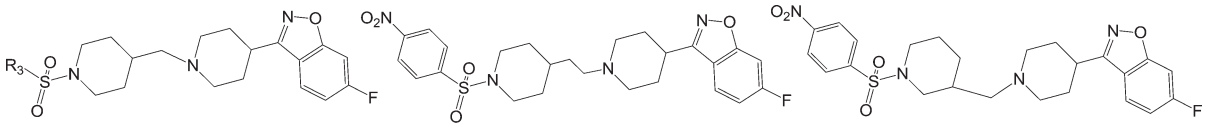
15-21

Compound	R_2	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
15		>10 000[b]	>10 000	215.6 ± 27.8
16		>10 000	>10 000	310.2 ± 45.2
17		1273.8 ± 230.1	598.9 ± 65.7	113.8 ± 21.6
18		86.8 ± 9.1	156.9 ± 16.3	19.0 ± 1.1
19		194.6 ± 23.5	268.7 ± 38.1	71.8 ± 9.2
20		1191.1 ± 230.1	2500	120.8 ± 13.9
21		>10 000	>10 000	2115.0 ± 312.1

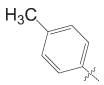
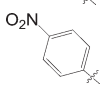
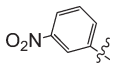
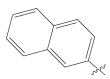
[a] K_i values are taken from three experiments, expressed as means ± SEM. [b] The K_i values were not calculated because the inhibition percentages at 10 μM were too low.

Next, we investigated the effect of replacing the urea derivatives with amide derivatives (Table 2, compounds **15–20**). As shown in Table 2, compounds **15** (cyclopropyl) and **16** (tertiary butyl) with alkyl substituents showed weak affinities for D_2 and 5-HT_{1A}, and moderate affinity to the 5-HT_{2A} receptor. Furthermore, compounds **18–19** with fluorine (F) and nitro (NO_2) on the phenyl group showed higher affinity than substituents with hydrogen (H) and a CH_3O group to the D_2, 5-HT_{1A} and 5-HT_{2A} receptors. However, the introduction of a 3-phenyl-2-propenoyl group (compound **21**) resulted in the

Table 3 Binding affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **22–33**[a]



22-31 32 33

Compound	R_3	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
22		2500	>10 000[b]	>10 000
23		1287.1 ± 156.9	>10 000	1523.5 ± 162.1
24		210.9 ± 25.6	789.2 ± 98.7	367.6 ± 42.1
25		190.4 ± 24.2	478.8 ± 68.9	112.9 ± 18.8
26		32.6 ± 4.5	21.9 ± 3.5	15.7 ± 2.2
27		8.7 ± 1.0	16.9 ± 2.1	0.79 ± 0.1
28		589.7 ± 60.5	1011.2 ± 126.6	26.9 ± 3.6
29		758.8 ± 80.1	917.3 ± 115.1	135.2 ± 16.1
30		1382.5 ± 140.5	1201.8 ± 134.3	32.6 ± 41.9
31		1290.8 ± 136.6	165.1 ± 190.6	454.9 ± 53.2
32	—	>10 000	1366.1 ± 225.3	66.8 ± 71.1
33	—	1099.1 ± 124.5	990.3 ± 113.3	30.1 ± 4.5

[a] K_i values are taken from three experiments, expressed as means ± SEM. [b] The K_i values were not calculated because the inhibition percentages at 10 μM were too low.

Table 4 Binding affinities for the D_3, H_1, 5-HT_{2C}, α_1 and α_2 receptors (K_i nM ± SEM) and hERG of compounds **26**, **27** and reference antipsychotics[a]

Compound	Receptor affinity K_i ± SEM[a] (nM)					IC_{50} ± SD[b] (nM)
	D_3	H_1	5-HT_{2C}	α_1	α_2	hERG
Ripersidone	15.1 ± 2.0	41.2 ± 5.1	25.9 ± 4.1	7.8 ± 1.0	56.8 ± 6.8	778 ± 82.1
Clozapine	340.1 ± 35.6	8.1 ± 0.8	33.1 ± 3.6	47.2 ± 5.4	78.1 ± 9.2	901 ± 12.2
26	236.8 ± 33.1	43.6 ± 5.1	136.8 ± 18.1	1098.2 ± 115.2	568.3 ± 67.3	1390.1 ± 162.3
27	16.6 ± 2.8	2845.2 ± 311.4	998.2 ± 12.31	2356.2 ± 289.2	967.3 ± 99.1	3810.2 ± 415.9

[a] K_i values are taken from three experiments, expressed as means ± SEM. [b] IC_{50} value is the mean of 3 determinations.

loss of affinity to all three receptors. These results suggested that an aromatic group has greater affinity to the three receptors than an alkyl group.

To further explore the SAR of these compounds, we replaced the amide derivatives with sulfonamides (Table 3, compounds **22–33**). According to Table 3, compounds **22** (phenyl) and **23** (substituent with CH_3 on the phenyl) showed weak affinities to all three receptors. Interestingly, the introduction of a withdrawing groups on the phenyl group improved the affinities to all three receptors. Compounds **24** and **25** (substituents containing a fluorine or cyanide on the phenyl) exhibited moderated affinities for all three

receptors. Substituents with trifluoromethyl (CF_3) on the phenyl (compound **26**, D_2, K_i = 32.6 nM; 5-HT_{1A}, K_i = 21.9 nM; 5-HT_{2A}, K_i = 15.7 nM) had good affinities to all three receptors. Substituents with a nitro group (NO_2) on the phenyl (compound **27**, D_2, K_i = 8.7 nM; 5-HT_{1A}, K_i = 16.9 nM; 5-HT_{2A}, K_i = 0.79 nM) exhibited higher affinities to all three receptors than did those with the other withdrawing groups. Moreover, compound **27** displayed a higher affinity for all three receptors (D_2, K_i = 130.7 nM; 5-HT_{1A}, K_i = 185.6 nM; 5-HT_{2A}, K_i = 12.9 nM) compared with clozapine. The phenyl ring substituted with NO_2 in the *ortho*-position (compound **28**, D_2, K_i = 589.7 nM; 5-HT_{1A}, K_i = 1011.2 nM; 5-HT_{2A}, K_i =26.9 nM) and *meta*-position (compound **29**, D_2, K_i = 758.8 nM; 5-HT_{1A}, K_i = 917.3 nM; 5-HT_{2A}, K_i = 135.2 nM) caused a dramatic decrease of affinities to all three receptors in comparison to compound **27**. This indicates that affinities for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors depended on the location of the substituent on the phenyl ring. We also investigated the effect of replacing the phenyl ring with naphthalene and thiophene (Table 3, compounds **30** and **31**). According to these results, compounds **30** and **31** displayed a dramatic decrease in affinity to the D_2 and 5-HT_{1A} receptors. These results also indicate the importance of the phenyl ring for affinity to the D_2 and 5-HT_{1A} receptors. We investigated the effect of the length of the linker between the piperidine ring and the 4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl group. As shown in Table 3, chain lengths of one (compound **27**) to two (compound **32**) carbon atoms resulted in significantly reduced D_2, 5-HT_{1A} and 5-HT_{2A} receptor binding. Therefore, the binding affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors are dependent upon chain length. Finally, we changed the piperidine ring 4-position (compound **27**) to the 3-position (compound **33**), which caused a dramatic decrease in affinity to the D_2 and 5-HT_{1A} receptors.

In line with the multiple receptor targeting approaches for the development of new antipsychotic agents, compounds **26** and **27** were selected for further tests of their binding to the D_3, H_1 and 5-HT_{2C} receptors because they had high affinities for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors. The results showed that compound **27** (K_i = 16.6 nM) displayed an equivalently affinity to D_3 receptors with risperidone (K_i = 15.1 nM). Furthermore, compounds **26** and **27** showed weak affinities to α_1 and α_2 adrenergic receptors (Table 4).

Treatment of schizophrenia with atypical antipsychotic drugs has been associated with weight gain. Two receptors, histamine H_1 and 5-HT_{2C} have been suggested to be involved in this adverse event. Hence, the observation that compound **27** has a much lower affinity (K_i = 2845.2 nM) for H_1 receptors than risperidone (K_i = 41.2 nM). As shown in Table 4, moreover, compound **27** had weak affinity to the 5-HT_{2C} receptor (K_i = 998.2 nM) in comparison to risperidone (K_i = 25.9 nM). Based on the above results, compound **27** exhibited low potential to elicit treatment-associated weight gain.

Recent studies have shown that a wide range of medications can prolong the length of time between the start of the Q wave and the end of the T wave on an electrocardiogram (QT interval). Specific examples include sertindole and grepafloxacin.[36] Indeed, efforts to predict an increased risk for long-QT syndrome have focused on assays that test hERG channel activity. As shown in Table 4, compounds **26** and **27** exhibited weak affinities for hERG compared with risperidone and clozapine. Interestingly, the low affinity of **27** (IC_{50} = 3810.2 nM) for hERG compared with risperidone and clozapine may decrease the propensity of these compounds to elicit treatment-induced QT interval prolongation.

The intrinsic activity of compound **27** was selected for further investigation based on its interaction with multiple receptors. As shown in Table 5, **27** showed stimulatory activity in an agonist assay on D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors displayed minimal agonist activity, in all cases displaying less than 10% of the efficacy of the reference endogenous agonist. In an antagonist assay, **27** inhibited four receptors (D_2, D_3, 5-HT_{1A} and 5-HT_{2A}) by over 95%.

Taken together, these results indicate that compound **27** shows high affinity for D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors, as well as a low affinity for the H_1 and 5-HT_{2C} receptors. Therefore, it was selected as a promising atypical antipsychotic agent and subjected to *in vivo* pharmacological characterization.

Behavioral studies

The apomorphine-induced climbing model has been classically linked to motor agitation, one of the positive symptoms of schizophrenia.[37] As shown in Table 6, compound **27** had an ED_{50} of 0.4 mg kg^{-1}; in comparison, risperidone, clozapine and haloperidol reversed apomorphine-induced climbing with ED_{50} values of 0.012, 4.52 and 0.17 mg kg^{-1}, respectively.

The observation that uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 induce schizophrenic symptoms in healthy subjects and exacerbate existing psychoses in schizophrenic patients has suggested that endogenous

Table 5 The activities of compound **27** and reference compounds to D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors

Receptor	Compound	Activation (10 μM, %) (mean ± SD) (n = 3)	Inhibition (10 μM, %) (mean ± SD) (n = 3)
D_2	Dopamine	100.9 ± 1.9	—
	Perphenazine	—	99.5 ± 10.2
	27	5.9 ± 0.9	100.4 ± 5.8
D_3	Dopamine	99.5 ± 5.8	—
	Spiperone	—	97.9 ± 4.9
	27	3.7 ± 0.7	95.8 ± 6.5
5-HT_{1A}	5-HT	99.1 ± 8.7	—
	WAY-100635	—	100.1 ± 9.8
	27	8.2 ± 0.9	98.7 ± 5.4
5-HT_{2A}	5-HT	98.5 ± 8.6	—
	Ketanserin	—	99.6 ± 3.7
	27	6.8 ± 0.7	100.2 ± 1.9

Table 6 *In vivo* pharmacological profile of compound **27**. Inhibition of different behavioral responses after oral administration of the test and reference compounds in mice

Compound	APO[a]	MK-801[b]	DOI[c]	CAT[d]	CAT/APO	CAT/MK-801
27	0.40 (0.31–0.52)	0.71 (0.23–0.85)	0.57 (0.24–1.36)	43.53	108.8	61.3
Risperidone	0.012 (0.01–0.015)	0.013 (0.01–0.024)	0.038 (0.02–0.09)	0.38	31.7	29.2
Clozapine	4.52 (3.38–6.28)	2.11 (1.75–2.45)	—	55.89	12.4	26.5
Haloperidol	0.17 (0.14–0.20)	0.14 (0.08–0.22)	—	0.47	2.8	3.4

[a] APO: apomorphine-induced climbing (mg kg^{-1}, po). [b] MK-801: MK-801-induced hyperactivity (mg kg^{-1}, po). [c] DOI: DOI-induced head twitch (mg kg^{-1}, po). [d] CAT: catalepsy (mg kg^{-1}, po).

dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathogenesis of schizophrenia.[38] It is well known that systemic administration of MK-801 increases dopamine cell firing rate in the brain.[39] In particular, hyperactivity produced by a low dose of MK-801 is dependent upon D_3 receptor stimulation.[39] Thus, the hyperactivity induced by MK-801 can be reduced *in vivo* by multi-target antipsychotic drugs characterized by high affinity and selectivity degree for D_3 receptor.[40] In this test, compound **27** significantly inhibited MK-801-induced hyperactivity, with an ED_{50} value of 0.71 mg kg^{-1} (Table 6). In comparison, risperidone, clozapine and haloperidol yielded ED_{50} values of 0.013, 2.11 and 0.14 mg kg^{-1}, respectively.

Catalepsy is often used as a measure for predicting the incidence of extrapyramidal motor disorders. In this model, it was clear that haloperidol had the highest propensity to induce catalepsy (ED_{50} 0.47 mg kg^{-1}), in agreement with the high capacity of this drug to block the D_2 receptor.[41] In contrast, compound **27** exhibited a low potential to induce catalepsy (ED_{50}, 45.53 mg kg^{-1}, Table 6). The therapeutic indices of compound **27** based on its efficacy (apomorphine or MK-801 models) and its side effects (catalepsy) were in the range 61–108, while the therapeutic indices of risperidone was roughly 29–31. Thus, in contrast to risperidone and clozapine, compound **27** had a higher threshold for inducing catalepsy, which might, by analogy, translate into lower clinical EPS liability.

The *in vivo* efficacy of compound **27** and selected analogs was evaluated in mice DOI [(±)-2,5-dimethoxy-4-iodoamphetamine-HCl]-induced head twitch model. This model measures blockade of head twitches induced by DOI which is a 5-HT_{2A} agonist.[42] As shown in Table 6, compounds **27** and risperidone have a ED_{50} of 0.57 mg kg^{-1} and 0.038 mg kg^{-1}, respectively.

Overall, compound **27** significantly inhibited apomorphine-induced climbing behavior, MK-801-induced hyperactivity and DOI-induced head twitch. Compound **27** also demonstrated a low propensity to induce unwanted extrapyramidal motor disturbances at therapeutically useful doses.

Conclusion

In summary, we describe the synthesis and pharmacological evaluation of a series of amide derivatives of benzisoxazole as potential multi-target antipsychotics. Among the derivatives synthesized, compound **27** showed high affinity for dopaminergic (D_2 and D_3) and serotonergic (5-HT_{1A} and 5-HT_{2A}) receptors and low affinity for H_1 receptors and hERG channels. Furthermore, animal models showed that compound **27** had high potential for treating symptoms of schizophrenia without causing catalepsy. Taken together, among the amide derivatives, we identified compound **27** as a potential antipsychotic lead candidate. Our studies demonstrate that the novel derivative may serve as a promising antipsychotic lead and hence pave the way for further investigation around this chemical space.

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