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Original article

Synthesis and evaluation of new coumarin derivatives as potential atypical antipsychotics

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ABSTRACT

In this paper, we report the synthesis of novel, potential antipsychotic coumarin derivatives combining potent dopamine D_2, D_3 and serotonin 5-HT_{1A}, 5-HT_{2A} receptors properties. We describe the structure activity relationship that leads us to the promising derivative: 7-(4-(4-(6-fluorobenzo[*d*]isoxazol-3-yl) piperidin-1-yl)butoxy)-6-methyl-2,3-dihydrocyclopenta[*c*]chromen-4(1*H*)-one **27**. The unique pharmacological features of compound **27** are a high affinity for dopamine D_2, D_3 and serotonin 5-HT_{1A}, 5-HT_{2A} receptors, together with a low affinity for H_1 receptor (to reduce the risk of obesity under chronic treatment). In animal models, compound **27** inhibited apomorphine-induced climbing and MK-801-induced hyperactivity without observable catalepsy at the highest dose tested. In particular, compound **27** was more potent than clozapine.

1. Introduction

Schizophrenia is a chronic incapacitating syndrome that affects 1% of the population [1]. First generation anti-psychotic drugs (APs) are dopamine 2 (D_2) receptor antagonists. While effective in reducing positive symptoms, these drugs are ineffective in treating negative symptoms and cognitive dysfunction and commonly cause extrapyramidal syndrome (EPS) [2–4]. Second generation APs (*e.g.*, clozapine, ziprasidone and risperidone, Fig. 1) target the D_2 receptor, as well as other receptors and have a lower incidence of EPS [5]. However, a major issue with many atypical antipsychotics is their association with numerous side effects, including substantial weight gain and QT interval prolongation [6–9]. Therefore, there is a tremendous unmet need for new antipsychotic medications that effectively treat all aspects of the disease, while possessing a side-effect profile that poses little challenge to compliance.

During the past decade, experimental evidence suggested that a complex binding profile is linked to the clinical efficacy of antipsychotic drugs. Indeed, the importance of designing multi-target G-protein-coupled receptors to deal with schizophrenia has been pointed out by many studies [10–12]. The serotoninergic system plays a variety of roles in the regulation of the prefrontal cortex (PFC) and is highly associated with emotional control, sleep, mood, cognitive behavior and memory [13,14]. The pyramidal neurons of the PFC possess numerous serotoninergic receptors, including 5-HT_{1A} and 5-HT_{2A} receptors [15]. Several studies have shown that activation of 5-HT_{1A} receptor increases dopamine release in the frontal cortex, which may improve negative symptoms and cognitive deficits in schizophrenia [16]. Serotonin acting at 5-HT_{2A} receptor, inhibits neuronal activity in the substantia nigra and ventral tegmental areas. A growing number of studies have reported that 5-HT_{2A} receptor antagonists increase the activity of nigrostriatal DA-containing neurons following moderate D_2 receptor blockade associated with antipsychotic drugs [17,18]. The blockade of 5-HT_{2A} receptors has been implicated in both the enhanced efficacy against negative schizophrenic symptoms and improved EPS profile of the atypical antipsychotics [19]. Dopamine plays important roles in behavior and cognition in the central nervous system (CNS) [20]. Blockade of mesolimbic D_2 receptor increases the efficacy of atypical antipsychotics against positive symptoms associated with schizophrenia [21]. The role of D_3 receptor in antipsychotic therapy is currently unknown; however, D_3 antagonists may enhance acetylcholine release in the frontal cortex, thereby improving cognitive deficits. A growing number of preclinical studies suggest

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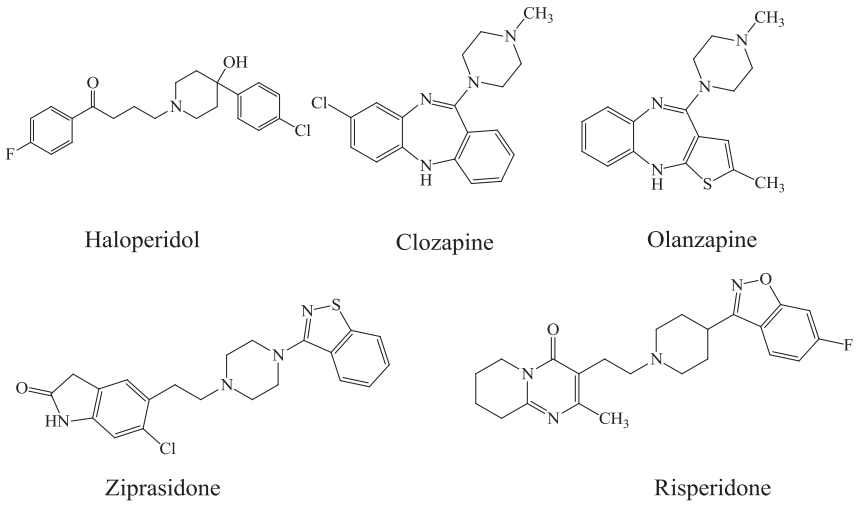


Fig. 1. Structures of a typical (haloperidol) and some atypical antipsychotics.

that the D_3 receptor may be a useful target for amelioration of the negative and cognitive symptoms associated with schizophrenia and substance abuse disorders [22–25]. Moreover, H_1 receptor may be involved in the weight gain associated with the treatment of schizophrenia *via* atypical antipsychotic drugs [26,27]. Thus, the aim of our work is to develop a novel antipsychotics that acts on dopamine D_2 and D_3, serotonin 5-HT_{1A} and 5-HT_{2A} receptors with a low affinity for the H_1 receptor, so that it could effectively cure positive symptoms, negative symptoms and cognitive impairment without the weight gain side-effect.

In our previous study, coumarin derivatives showed obviously antipsychotic activity. Compound **1** 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 receptor (to reduce the risk of obesity associated with chronic treatment) [28]. Furthermore, compound **1** exhibited obviously antipsychotic without observable catalepsy in animal models. In order to expand the structure–activity relationships of coumarin derivatives, the present study focused on the synthesis and pharmacological evaluation of a new class of antipsychotic agents which connect 3-position and 4-position into ring derivatives (Fig. 2). The target compounds were subjected to preliminary pharmacological evaluation to determine their affinity for D_2, D_3, 5-HT_{1A}, 5-HT_{2A} and H_1 receptors. The appropriate compounds have to be chosen here for the basic behavioral screening of their atypical antipsychotic potency.

2. Chemistry

The synthesis of the novel coumarin derivatives was performed according to the reaction pathways illustrated in Schemes 1 and 2. The 2-carboalkoxcyclohexanone derivatives (**4**) were synthesized, exploiting the Dieckmann condensation of the corresponding pimelic esters (**3**) (Scheme 1) by treatment with $AlCl_3$ and triethylamine; this provided a good yield [29]. Subsequently, 2-carboalkoxcyclohexanone derivatives (**4**) reacted with substituted resorcinol *via* the Pechmann reaction to give 7-hydroxycoumarin intermediates (**5**) [30]. The standard alkylation procedure of intermediates **5** with 1,4-dibromobutane, 1,3-dibromopropane or 1,5-dibromopentane led to derivatives **6** which were then condensed with appropriate amines to yield the target compounds **7**–**30** (Scheme 1, Tables 1–3).

Finally, 3-hydroxy-6*H*-benzo[*c*]chromen-6-one (**32**) was conveniently prepared by the condensation of the 2-bromobenzoic acid with resorcinol [31]. Subsequently, 3-hydroxy-6*H*-benzo[*c*]chromen-6-one (**32**) reacted with 1,4-dibromobutane or 1,3-dibromopropane led to derivatives **33**, which were then condensed with appropriate amines to yield compounds **34**, **35** (Scheme 2, Table 3).

3. Pharmacology

3.1. In vitro studies

All the new compounds were dissolved in 5% DMSO. 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;

Total binding was determined in the absence of no-specific binding and compounds. Specific binding was determined in the presence of 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 and no effects were observed. Compounds were tested at least three times over a 6 concentration range (10^{-5} M to 10^{-12} M), IC_{50} values were determined by nonlinear regression analysis using 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 hot ligand used and K_d its receptor dissociation constant were 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.

3.2. In vivo studies

Selected compounds were further evaluated in *vivo* animal models, including the apomorphine-induced climbing, MK801-induced hyperactivity and catalepsy models.

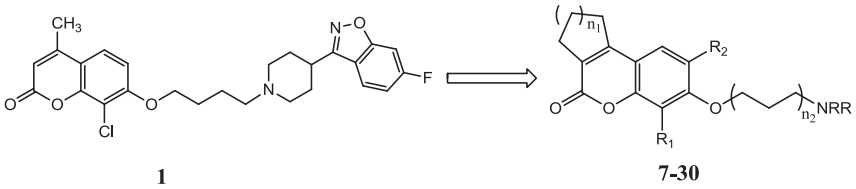
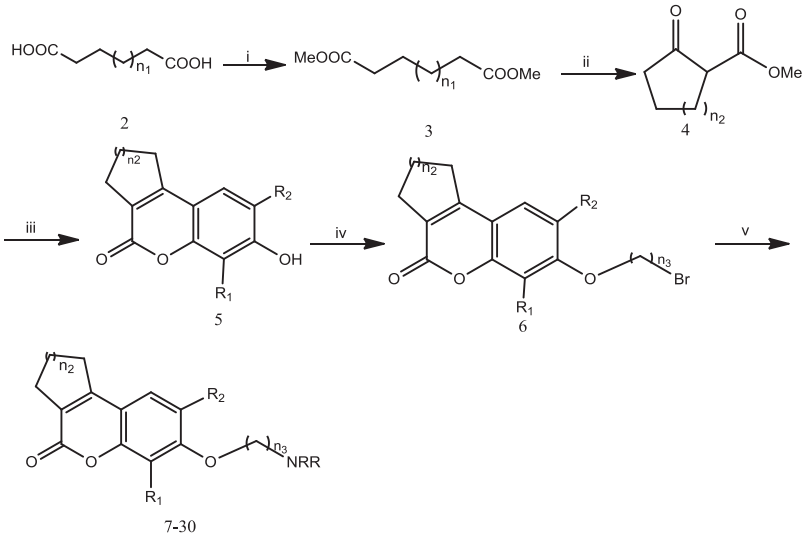
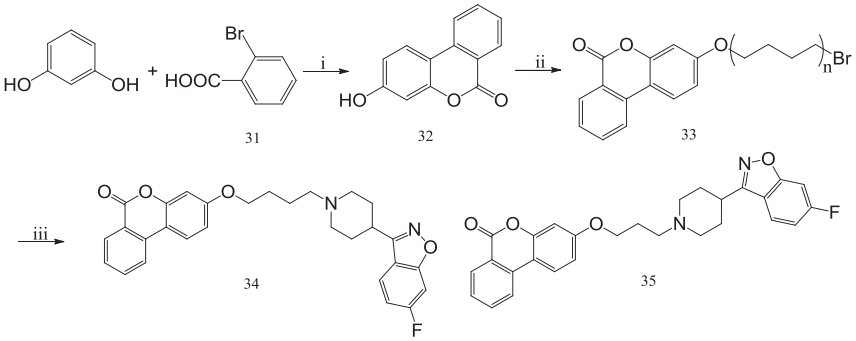


Fig. 2. Design of new coumarin derivatives.



Reagents and conditions: (i) MeOH, conc H_2SO_4; (ii) $AlCl_3$, Et_3N, CH_2Cl_2, rt; (iii) $Bi(NO_3)_3 \cdot 5H_2O$, 80°C; (iv) $Br(CH_2)_{3\text{-}5}Br$, K_2CO_3, Acetone, reflux; (v) CH_3CN, K_2CO_3, KI, reflux.

Scheme 1.



Reagents and conditions: (i) NaOH, $CuSO_4$; (ii) $Br(CH_2)_{3\text{-}4}Br$, K_2CO_3, Acetone, reflux; (iii) CH_3CN, K_2CO_3, KI, reflux.

Scheme 2.

4. Results and discussion

4.1. In vitro studies of new compounds

In this work, our initial design focus was to investigate the effect of different amine moieties for the affinities to D_2, 5-HT_{1A} and 5-HT_{2A} receptors (Table 1, compounds **7**–**19**). As shown in Table 1, when amine moieties were phenylpiperazine, 2,3-dichloro phenylpiperazine, 4-trifluorophenylpiperazine, 2,3-dimethylphen ylpiperazine and 3-trifluoromethyl phenylpiperazine, compounds **7**–**11** exhibited good affinities for the 5-HT_{1A} and 5-HT_{2A} receptors, but showed weak activity for the D_2 receptor. Interestingly, the 2-methoxyphenylpiperazine derivative **12** ($K_i = 0.2$ nM) displayed the strongest affinity for 5-HT_{1A} receptors. Moreover, compound **12** (D_2, $K_i = 5.6$ nM; 5-HT_{2A}, $K_i = 3.9$ nM) displayed the high affinity for D_2 and 5-HT_{2A} receptors. According to the results, compound **12** (5-HT_{1A}, $K_i = 0.2$ nM) displayed higher affinity for the 5-HT_{1A} receptor compared with risperidone (5-HT_{1A}, $K_i = 190.2$ nM). The phenyl ring substituted with methoxy in the ortho-position (compound **12**) increased the activity at all three receptors compared with in the para- and meta-positions (compounds **13** and **14**). Therefore, affinity for all three receptors is

Table 1
Binding Affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **7**–**19** and reference antipsychotics.[a]

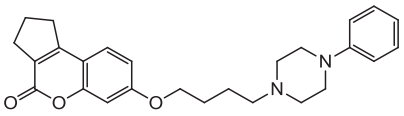
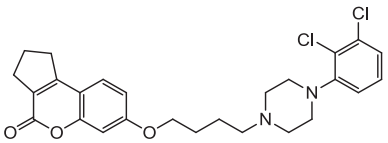
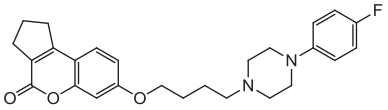
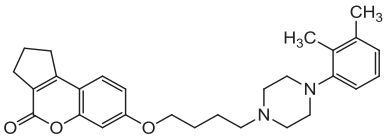
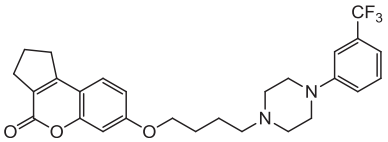
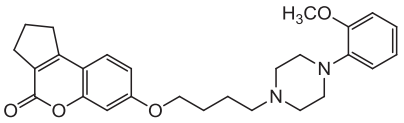
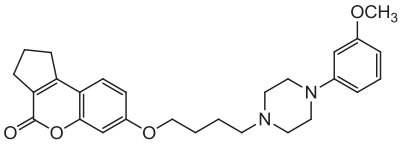
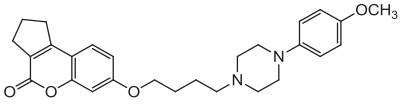
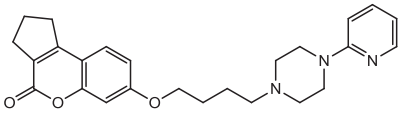
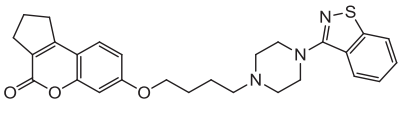
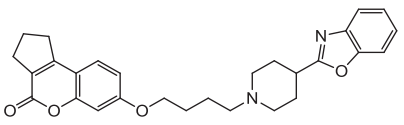
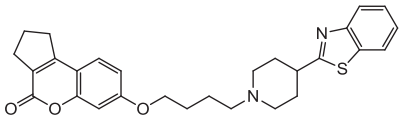
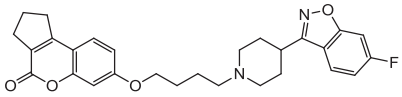
Compound	Structure	Receptor affinity $K_i \pm$ SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
7		>10000[b]	56 ± 7.3	59.2 ± 7.9
8		1895.6 ± 198.2	68 ± 6.5	79.3 ± 9.1
9		>10,000	28.9 ± 4.3	16.5 ± 2.6
10		1598.6 ± 198.6	22.1 ± 3.6	11.7 ± 3.1
11		>10000[b]	19.3 ± 2.9	173.6 ± 19.6
12		5.6 ± 0.6	0.2 ± 0. 01	3.9 ± 0.4
13		1056 ± 136.5	22.4 ± 3.9	895.2 ± 10.2
14		>10000[b]	1235.6 ± 169.8	>10000[b]
15		>10000[b]	1259.6 ± 236.9	1398.7 ± 155.6
16		522.1 ± 62.3	968.6 ± 116.8	653.1 ± 79.3
17		1886.4 ± 195.6	26.8 ± 3.9	16.2 ± 2.8

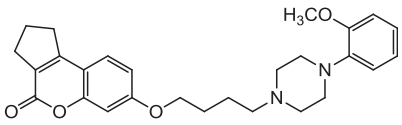
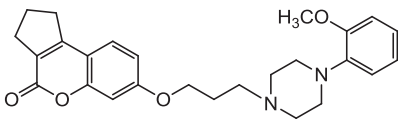
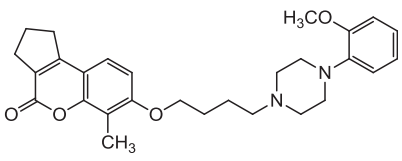
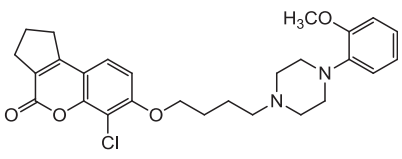
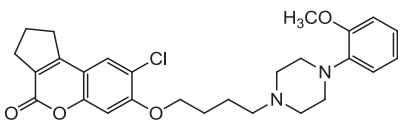
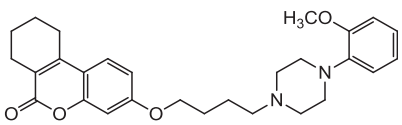
Table 1 (*continued*)

Compound	Structure	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
18		>10000[b]	1596.3 ± 172.5	1865.2 ± 202.5
19		24.4 ± 3.1	47.5 ± 5.8	13.1 ± 1.5
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.

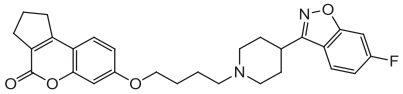
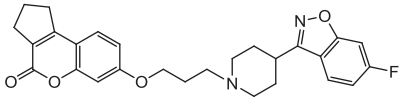
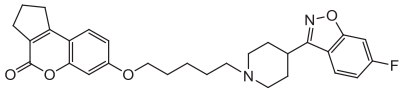
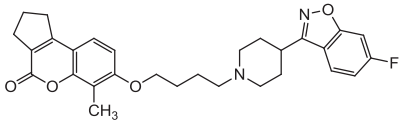
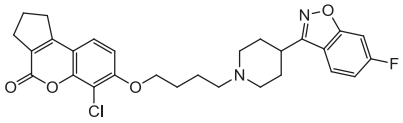
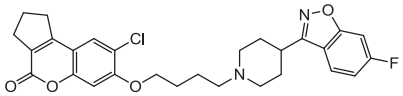
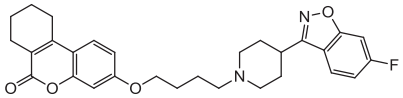
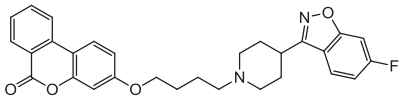
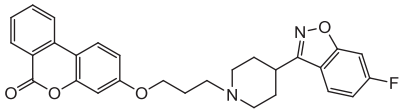
dependent upon the location of the substituent on the phenyl ring. However, substitution of the phenylpiperazine with 1-(pyridin-2-yl)piperazine or (benzo[*d*]isothiazol-3-yl)piperazine groups (compounds **15** and **16**) reduced the affinity for all three receptors. Compound **17** bearing 2-(piperidin-4-yl)benzo[*d*]oxazole showed low affinities for the D_2 receptor (K_i = 1886.4 nM) and high affinities for 5-HT_{1A} (K_i = 26.8 nM) and 5-HT_{2A} (K_i = 16.2 nM). 2-(piperidin-4-yl)benzo[*d*]thiazole derivative **18** showed weak

Table 2
Binding affinities for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **20**–**24**.[a]

Compound	Structure	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
12		5.6 ± 0.6	0.2 ± 0. 01	3.9 ± 0.4
20		>10000[b]	639.6 ± 69.2	618.2 ± 68.5
21		569.5 ± 59.2	349.4 ± 36.9	175.4 ± 18.4
22		159.2 ± 16.2	156.9 ± 17.2	104.6 ± 11.6
23		>10000[b]	84.8 ± 9.1	36.6 ± 3.8
24		756.6 ± 80.1	1124.2 ± 123.5	1031.8 ± 116.1

[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.

Table 3
Binding affinities for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds **25**–**30**, **34** and **35**.[a]

Compound	Structure	Receptor affinity K_i ± SEM (nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
19		24.4 ± 3.1	47.5 ± 5.8	13.1 ± 1.5
25		>10000[b]	>10000[b]	188.2 ± 19.1
26		698.2 ± 82.5	1052.3 ± 115.7	425.6 ± 56.9
27		12.7 ± 1.6	7.8 ± 1.1	2.2 ± 0.3
28		18.9 ± 2.0	11.4 ± 1.6	5.3 ± 0.7
29		145.9 ± 14.9	139.6 ± 15.1	248.9 ± 30.2
30		623.8 ± 64.2	>10000[b]	125.6 ± 11.8
34		>10000[b]	>10000[b]	>10000[b]
35		>10000[b]	>10000[b]	>10000[b]

[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.

affinities for the three receptor. It should be noted that compounds **19** (D_2, K_i = 24.4 nM; 5-HT_{1A}, K_i = 47.5 nM; 5-HT_{2A}, K_i = 13.1 nM) showed high affinities for the three receptors. These results indicate that compounds bearing (6-fluorobenzo[*d*]isoxazol-3-yl) piperidine and 2-methoxyphenylpiperazine moieties (compounds **12** and **19**) possess higher affinity for all three receptors compared with those with other amine moieties.

When amine moiety was 2-methoxyphenylpiperazine moiety, compound **12** (four carbon atoms) exhibited very high affinities for the three receptors, especially had strong affinity for 5-HT_{1A} receptor. In addition, compound **20** with three carbon atoms resulted in reduction for the three receptors. The substituents of CH_3 or Cl at the 8 (compounds **21** and **22**) or 6-position (compound **23**) of coumarin produced obviously decrease in the affinities for the three receptors compare to compound **12**. Furthermore, change the five-membered ring to six-membered ring, compound **24** display low affinities for the three receptors.

When amine moiety was 6-fluorobenzo[*d*]isoxazol-3-yl)piperidine, chain lengths of three (**25**) or five (**26**) carbon atoms resulted in significantly reduced D_2, 5-HT_{1A} and 5-HT_{2A} receptors binding. Taking account of the data presented above, the length of the alkyl chain appeared to have a direct impact on affinity for the three receptors. It was worthy that introducing substituents at 8-position derivatives **27** (CH_3) D_2, K_i = 12.7 nM; 5-HT_{1A}, K_i = 7.8 nM; 5-HT_{2A}, K_i = 2.2 nM) and **28** (Cl) (D_2, K_i = 18.9 nM; 5-HT_{1A}, K_i = 11.4 nM; 5-HT_{2A}, K_i = 5.3 nM) displayed high affinities for the three receptors. The above observations indicated that introducing substituents at the 8-position is useful to increase affinity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors. Interesting, there results are consistent with our previously studies [28]. Introducing substituent at the 6-position derivative **29** is detrimental to the D_2, 5-HT_{1A} and 5-HT_{2A} receptors compare to compound **19**. The simple change n_1 from 1 (**20**) to 2 (**30**), produces a reduction of affinity for the three receptors. Finally, replacement of five or six-membered ring with phenyl,

Table 4
Binding affinities for the D_3 and H_1 receptors (K_i nM ± SEM) of compounds **12**, **19**, **27**, **28** and reference antipsychotics.[a]

Compound	K_i ± SEM (nM)[a]	
	D_3	H_1
Ripersidone	10.1 ± 0.9	22.9 ± 3.2
Clozapine	260.1 ± 30.5	6.8 ± 0.8
12	178.3 ± 19.2	699.1 ± 78.5
19	110.2 ± 12.1	21.3 ± 2.5
27	**13.6 ± 2.4**	**1825.3 ± 201.6**
28	13.9 ± 1.3	13.6 ± 2.1

[a] K_i values are taken from three experiments, expressed as means ± SEM.

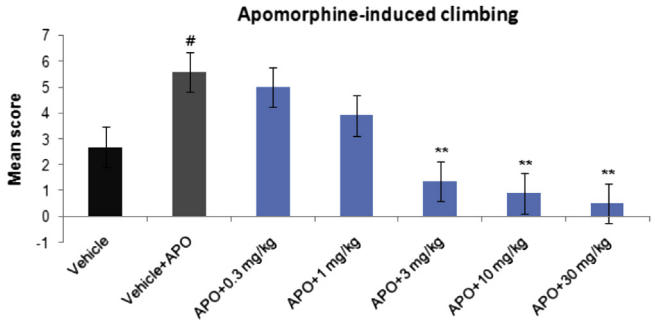


Fig. 3. Effect of compound **27** administered po on APO (apomorphine)-induced climbing in mice (10/group). Results are expressed as means SEM of score. Statistical significances of drug effects were analyzed by the nonparametric two-tailed Mann–Whitney *U*-test: $^{\#}p < 0.05$ versus vehicle treatment; $^{**}p < 0.01$, $^{*}p < 0.05$ versus apomorphine treatment.

compounds **34** and **35** resulted in the loss of activity for the three receptors.

Overall, compounds **12**, **19**, **27** and **28** exhibited high affinity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors. These four compounds showed higher affinities for 5-HT_{1A} receptors compared with clozapine (K_i = 185.6 nM) and risperidone (K_i = 190.2 nM). In particular, compound **12** (K_i = 0.2 nM) displayed strong affinities for 5-HT_{1A} receptors. Moreover, compounds **27** (K_i = 12.7 nM) and **28** (K_i = 18.9 nM) had higher affinities for D_2 receptors compared with clozapine (K_i = 130.7 nM). Furthermore, compounds **27** (K_i = 2.2 nM) and **28** (K_i = 5.3 nM) had higher affinities for 5-HT_{2A} receptors than clozapine (K_i = 12.9 nM). Therefore, compounds **12**, **19**, **27** and **28** were selected for additional studies of binding to the D_3 and H_1 receptors due to their high affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors.

The D_3 receptor was proposed as a target for atypical antipsychotic drugs and various pharmacological studies have suggested that D_3 antagonism might reduce catalepsy. Our results showed that compounds **27** (K_i = 13.6 nM) exhibit higher affinity for D_3 receptors compared with clozapine (K_i = 260.1 nM). Therefore, these results suggested that compound **27** may reduce catalepsy in patients with schizophrenia.

Treatment of schizophrenia with atypical antipsychotic drugs has been associated with weight gain. Histamine H_1 receptor is believed to be involved in this adverse event. As shown in Table 4, compounds **12** and **27** had lower affinities for H_1 receptors compared with risperidone (K_i = 22.9 nM) and clozapine (K_i = 6.8 nM). In particular, compound **27** showed significantly lower affinity for H_1 receptors (K_i > 1000 nM). According to the above results, compound **27** displayed a weak affinity for H1 receptors and exhibited low potential to elicit treatment-associated weight gain.

4.2. Acute toxicity

Taken together, these results indicated that compound **28** showed high affinity for D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors, as well as low affinity for the H_1 receptor. The acute toxicity of compound **27** was assayed in terms of LD_{50} values. Compound **27** displayed a good safety profile, even at the highest dose tested (LD_{50} > 2000 mg/kg).

4.3. Behavioral studies

Due to the marked effect of compound **27** on serotonergic and dopaminergic receptors, it was selected as a promising atypical antipsychotic agent and subjected to *in vivo* pharmacological characterization.

Inhibition or reversal of apomorphine-induced cage climbing behavior in mice by a test molecule is an indication of mesolimbic dopaminergic D_2 receptor antagonism. Reversal of apomorphine-induced climbing is predictive of efficacy against the positive symptoms of psychosis [32]. Compound **27** produced significant dose-dependent responses in this model (Fig. 3), with an ED_{50} value of 0.61 mg/kg (Table 5). In comparison, risperidone, clozapine and haloperidol reversed apomorphine-induced climbing with ED_{50} values of 0.02, 3.97 and 0.14 mg/kg, respectively. These results suggest that compound **27** was slightly more potent in terms of blocking D_2 receptors *in vivo* compared with clozapine.

The MK-801-induced hyperactivity model has been used to indirectly evaluate the ability of a compound to oppose cortical dopaminergic hypofunction induced by NMDA receptor blockade [33]. In this model, compound **27** showed significant dose-dependent responses (Fig. 4) with an ED_{50} value of 0.31 mg/kg (Table 5). In comparison, risperidone, clozapine and haloperidol yielded ED_{50} values of 0.017, 2.25 and 0.16 mg/kg, respectively. These results indicate that compound **27** was more potent than clozapine.

The catalepsy test is a common and widely used preclinical screening test for the propensity of an antipsychotic drug to induce EPS in humans [34]. In this model (Table 5), haloperidol had the highest propensity to induce catalepsy (ED_{50} 0.38 mg/kg), consistent with its marked ability to block D_2 receptors. In contrast, compound **27** exhibited a low potential for catalepsy (ED_{50}, 66.47 mg/kg), consistent with risperidone and clozapine (ED_{50}

Table 5
In *vivo* pharmacological profile of compound **27**. Inhibition of different behavioral responses after oral administration of the test and reference compounds.

Compound	APO[a]	MK-801[b]	CAT[c]	CAT/APO	CAT/MK-801
27	0.61 (0.46–0.76)	0.31 (0.23–0.44)	66.47	108.96	214.42
Risperidone	0.02 (0.01–0.03)	0.017 (0.011–0.029)	0.43	21.50	25.29
Clozapine	3.97 (2.06–6.96)	2.25 (1.22–6.79)	46.14	11.62	20.50
Haloperidol	0.14 (0.1–0.18)	0.16 (0.13–0.18)	0.38	2.71	2.38

[a] APO: apomorphine-induced climbing (mg/kg, po).
[b] MK-801: MK-801-induced hyperactivity (mg/kg, po).
[c] CAT: catalepsy (mg/kg, po).

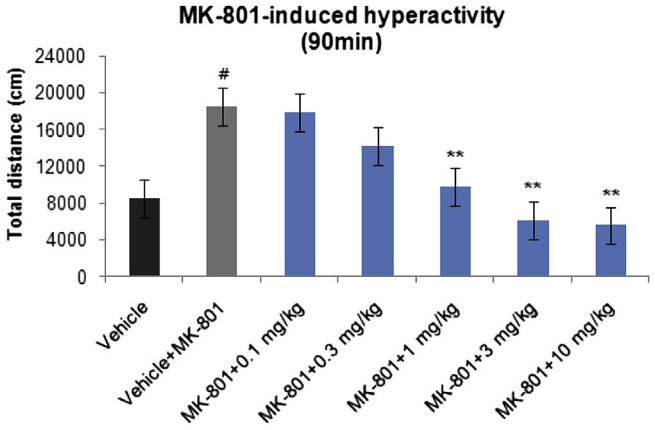


Fig. 4. Effect of compound **27** administered po on MK-801-induced hyperactivity in mice (10/group). Results are expressed as means SEM of distance traveled. Statistical evaluation was performed by two-way ANOVA followed by Tukey test for multiple comparisons. $^{\#}p < 0.05$ versus vehicle treatment; $^{**}p < 0.01$, $^{*}p < 0.05$ versus MK-801 treatment.

risperidone 0.43 mg/kg, clozapine 46.14 mg/kg). The therapeutic indices of compound **27** based on its efficacy (apomorphine or MK-801 models) and its side effects (catalepsy) were in the range 108–214, while the therapeutic indices of risperidone and clozapine are roughly 11–25. In contrast to risperidone and clozapine, compound **27** had a high threshold for catalepsy.

Therefore, compound **27** inhibited apomorphine-induced climbing behavior, MK-801-induced hyperactivity and the conditioned avoidance response. Compound **27** also demonstrated a low propensity to induce unwanted extrapyramidal motor disturbances at therapeutically useful doses.

5. Conclusion

In summary, we described the synthesis and pharmacological evaluation of a series of coumarin derivatives as potential multi-target antipsychotics. Among the derivatives synthesized, compound **27** showed high affinity for dopamine D_2 and D_3, serotonin 5-HT_{1A}, 5-HT_{2A} receptors, with a low affinity for the H_1 receptor. In *vivo* animal models showed that compound **27** had high potential for treating symptoms of schizophrenia without causing catalepsy. Compound **27** had a higher threshold for catalepsy induction compared with the two currently marketed atypical antipsychotics, risperidone and clozapine. Compound **27** might be useful for developing a novel class of drugs for the treatment of schizophrenia.

6. Experimental

6.1. Chemistry experimental

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian Inova Unity 200 spectrometer in $CDCl_3$ solution. Chemical shifts were given in δ values (ppm), using tetramethylsilane (TMS) as the internal standard; coupling constants (J) were given in Hz. Signal multiplicities were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). Reagents were all of analytical grade or of chemical purity. Analytical TLC was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. Purity analysis was carried out by LC-Mass analysis using an Agilent 1100 system. The HPLC conditions were as follows: column, Shim-pack ODS 5.0 μm × 150 mm × 2.0 mm I.D (SHIMADZU, Japanese); mobile phase, 0.0167% HCOOH (TEDIA Company, USA)/acetonitrile (Merck Company, Germany) 50/50; flow rate, 0.2 mL/min; column temperature, 40 °C. Compound purity is determined by high performance liquid chromatography (HPLC), and all final test compounds were >95% purity.

6.1.1. General procedure for the preparation of compounds **5**

To a solution of pimelic acid **1** (10 mmol) in 25 mL of methyl alcohol was slowly added 1 mL of concentrated sulfuric acid. The resulting mixture was stirred at reflux until that TLC analysis. Next, the mixture was poured into crushed ice and then, extracted with dichloromethane (5 × 50 mL). The organic layers were washed with 5% aq. $NaHCO_3$ solution. The organic layers were dried with anhydrous magnesium sulfate, the filtrate evaporated under reduced pressure to furnish the corresponding esters **3**.

To a suspension of anhydrous aluminum chloride (16 g, 120 mmol) in 50 mL of dichloromethane was added a solution of the corresponding pimelate ester derivative 2 (46 mmol) in 50 mL dichloromethane. After cooling the obtained mixture at 0 °C, 16 mL of triethylamine (120 mmol) was carefully added and reaction was stirred at room temperature until that TLC analysis indicated the total consumption of the starting material. Next, a 1:1 mixture of 10% aq. HCl and crushed ice (100 mL) was added and the reaction was extracted with dichloromethane (4 × 40 mL). The organic layers were washed with a saturated aq. oxalic acid solution. The organic layers were dried with anhydrous magnesium sulfate, the filtrate evaporated under reduced pressure to furnish the corresponding 2-carboalkoxycyclohexanone derivatives 4.

To the phenol (10 mmol) and β-ketoester **31** (10 mmol), the bismuth nitrate pentahydrate (5–10 mol %) was added and the contents were stirred in a pre-heated oil-bath at 80 °C. After completion of the reaction, the reaction mixture was cooled to room temperature and the contents were poured into ice-cold water. The products were collected by filtration, washed with water, and then recrystallized from ethanol to afford the coumarin derivatives **5**.

*6.1.1.1. 7-Hydroxy-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**5a**).* Yield: 71.3%; mp: 248–250 °C. MS (ESI) *m/z* 203 ($[M + H]^+$).

*6.1.1.2. 7-Hydroxy-6-methyl-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**5b**).* Yield: 61.9%; mp: 263–265 °C. MS (ESI) *m/z* 217 ($[M + H]^+$).

*6.1.1.3. 7-Hydroxy-6-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**5c**).* Yield: 49.2%; mp: 231–233 °C. MS (ESI) *m/z* 237 ($[M + H]^+$).

*6.1.1.4. 7-Hydroxy-8-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**5d**).* Yield: 56.5%; mp: 251–253 °C. MS (ESI) *m/z* 237 ($[M + H]^+$).

*6.1.1.5. 3-Hydroxy-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**5e**).* Yield: 62.3%; mp: 195–197 °C. MS (ESI) *m/z* 218 ($[M + H]^+$).

6.1.2. General procedure for the preparation of compounds **6**

1,4-Dibromobutane (1,3-dibromopropane or 1,5-dibromopentane) (2 mmol) was added to a solution of compounds 5 (1 mmol) and potassium carbonate in acetone (50 mL), and the mixture was refluxed for 4–6 h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the mixture was filtered, the solvent was evaporated and the residue was recrystallized from hexane/EtOH to yield compounds **6**.

*6.1.2.1. 7-(3-Bromopropoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6a**).* Yield: 62.1%; mp: 103–105 °C. ^{1}H NMR ($CDCl_3$) δ 2.16–2.34 (m, 4H), 2.88 (t, 2H, J = 6.4 Hz), 3.03 (t, 2H, J = 6.4 Hz), 3.60–3.63 (m, 1H), 3.75–3.78 (m, 1H), 4.16 (t, 2H, J = 6.4 Hz), 6.84–6.88 (m, 2H), 7.33–7.36 (m, 1H). MS (ESI) m/z 323.2 ($[M + H]^+$).

*6.1.2.2. 7-(4-Bromobutoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6b**).* Yield: 72.6%; mp: 81–83 °C. ^{1}H NMR ($CDCl_3$) δ 1.99–2.22 (m, 6H), 2.87 (t, 2H, J = 6.6 Hz), 3.04 (t, 2H, J = 8 Hz), 3.50 (t, 2H, J = 6.4 Hz), 4.04 (t, 2H, J = 6 Hz), 6.83–6.84 (m, 2H), 7.31–7.33 (m, 1H). MS (ESI) m/z 337.2 ($[M + H]^+$).

*6.1.2.3. 7-(4-Bromobutoxy)-9-methyl-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6c**).* Yield: 72.1%; mp: 91–93 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.88 (m, 4H), 1.99–2.16 (m, 4H), 2.34 (s, 3H), 2.75 (t, 2H, J = 6.4 Hz), 3.53 (t, 2H, J = 6.6 Hz), 4.09 (t, 2H, J = 6.4 Hz), 6.79–6.81 (m, 1H), 7.35–7.37 (m, 1H). MS (ESI) m/z 351.0 ($[M + H]^+$).

*6.1.2.4. 7-(4-Bromobutoxy)-8-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6d**).* Yield: 63.3%; mp: 104–105 °C. ^{1}H NMR ($CDCl_3$) δ 2.01–2.27 (m, 6H), 2.93 (t, 2H, J = 8 Hz), 3.07 (t, 2H, J = 8 Hz), 3.56 (t, 2H, J = 6.4 Hz), 4.20 (t, 2H, J = 6.4 Hz) 6.88–6.90 (m, 1H), 7.30–7.32 (m, 1H). MS (ESI) m/z 371.0 ($[M + H]^+$).

*6.1.2.5. 7-(4-Bromobutoxy)-6-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6e**).* Yield: 58.3%; mp: 94–96 °C. ^{1}H NMR ($CDCl_3$) δ 2.01–2.23 (m, 6H), 2.92 (t, 2H, J = 6.4 Hz), 3.05 (t, 2H, J = 6.4 Hz), 3.56 (t, 2H, J = 6.4 Hz), 4.20 (t, 2H, J = 6 Hz), 6.88 (s, 1H), 7.43 (s, 1H). MS (ESI) m/z 371.0 ($[M + H]^+$).

*6.1.2.6. 7-(4-Bromobutoxy)-6-methyl-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6f**).* Yield: 69.2%; mp: 104–106 °C. ^{1}H NMR ($CDCl_3$) δ 2.00–2.25 (m, 6H), 2.34 (s, 3H), 2.91 (t, 2H, J = 6.4 Hz), 3.05 (t, 2H, J = 6 Hz), 3.53 (t, 2H, J = 6.4 Hz), 4.10 (t, 2H, J = 6.4 Hz), 6.81–6.83 (m, 1H), 7.24–7.26 (m, 1H). MS (ESI) m/z 351.0 ($[M + H]^+$).

*6.1.2.7. 3-(4-Bromobutoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**6g**).* Yield: 52.0%; mp: 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 1.77–2.12 (m, 8H), 2.56 (t, 2H, J = 6.4 Hz), 2.74 (t, 2H, J = 6 Hz), 3.50 (t, 2H, J = 6.4 Hz), 4.04 (t, 2H, J = 6 Hz), 6.77–6.84 (m, 2H), 7.44–7.46 (m, 1H). MS (ESI) m/z 351.1 ($[M + H]^+$).

*6.1.2.8. 7-((5-Bromopentyl)oxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**6h**).* Yield: 62.8%; mp: 85–87 °C. ^{1}H NMR ($CDCl_3$) δ 1.62–1.67 (m, 2H), 1.83–1.97 (m, 4H), 2.16–2.20 (m, 2H), 2.86 (t, 2H, J = 6 Hz), 3.02 (t, 2H, J = 6 Hz) 3.45 (t, 2H, J = 6.4 Hz), 4.03 (t, 2H, J = 6 Hz) 6.80–6.84 (m, 2H), 7.30–7.32 (m, 1H). MS (ESI) m/z 351.2 ($[M + H]^+$).

*6.1.3. General procedure for the preparation of compounds **7**–**31***

To a suspension of compounds **6** (0.32 mmol) and K_2CO_3 (1.22 mmol) in acetonitrile (5.0 mL), arylpiperazine (piperidine) (0.32 mmol) and a catalytic amount of KI were added and the resulting mixture was refluxed for 7–9 h. After filtering, the resulting filtrate was evaporated to dryness under reduced pressure. The residue was suspended in water (10.0 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic layers were evaporated under reduced pressure, and the crude product was purified by means of chromatography (10% MeOH/$CHCl_3$) to yield compounds **7**–**30**.

*6.1.3.1. 7-(4-(4-Phenylpiperazin-1-yl)butoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**7**).* Yield: 64.2%; mp: 86–88 °C. ^{1}H NMR ($CDCl_3$) δ 1.74–1.90 (m, 4H), 2.17–2.22 (m, 2H), 2.50 (t, 2H, J = 6.0 Hz), 2.64–2.66 (m, 4H), 2.88 (t, 2H, J = 6.0 Hz), 3.03 (t, 2H, J = 6.0 Hz), 3.22 (t, 2H, J = 4.0 Hz), 4.05 (t, 2H, J = 6.0 Hz), 6.83–6.87 (m, 3H), 6.93 (d, 2H, J = 8.0 Hz), 7.25–7.26 (m, 2H), 7.32 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 419.2 ($[M + H]^+$).

*6.1.3.2. 7-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**8**).* Yield: 65.9%; mp: 115–116 °C. ^{1}H NMR ($CDCl_3$) δ 1.70–1.90 (m, 4H), 2.15–2.21 (m, 2H), 2.49 (t, 2H, J = 6.0 Hz), 2.65 (s, br, 3H), 2.87 (t, 2H, J = 4.0 Hz), 3.01–3.07 (m, 6H), 4.05 (t, 2H, J = 4.0 Hz), 6.82–6.84 (m, 2H), 6.94–6.97 (m, 1H), 7.13–7.14 (m, 2H), 7.30–7.32 (m, 1H). MS (ESI) m/z 487.1 ($[M + H]^+$).

*6.1.3.3. 7-(4-(4-(4-Fluorophenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclopent-a[c]chromen-4(1H)-one (**9**).* Yield: 54.3%; mp: 104–106 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.89 (m, 4H), 2.15–2.19 (m, 2H), 2.47 (t, 2H, J = 6.0 Hz), 2.62 (t, 4H, J = 4.0 Hz), 2.88 (t, 2H, J = 4.0 Hz), 3.03 (t, 2H, J = 4.0 Hz), 3.12 (t, 2H, J = 4.0 Hz), 4.05 (t, 2H, J = 6.0 Hz), 6.82–6.97 (m, 6H), 7.32 (d, 1H, J = 4.0 Hz) MS (ESI) m/z 437.2 ($[M + H]^+$).

*6.1.3.4. 7-(4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**10**).* Yield: 56.2%; mp: 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 1.75–1.93 (m, 4H), 2.17–2.19 (m, 2H), 2.24 (s, 3H), 2.28 (s, 3H), 2.53–2.56 (m, 3H), 2.69 (s, br, 3H), 2.89–2.97 (m, 6H), 3.04–3.08 (m, 2H), 4.07 (t, 2H, J = 6.0 Hz), 6.85–6.87 (m, 2H), 6.91–6.95 (m, 2H), 7.07–7.11 (m, 1H), 7.34–7.36 (m, 1H). MS (ESI) m/z 447.2 ($[M + H]^+$).

*6.1.3.5. 7-(4-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)butoxy)-2,3-dihydro-cyclopenta[c]chromen-4(1H)-one (**11**).* Yield: 66.1%; mp: 101–103 °C. ^{1}H NMR ($CDCl_3$) δ 1.76–1.92 (m, 4H), 2.17–2.25 (m, 2H), 2.51 (t, 2H, J = 6.0 Hz), 2.68–2.70 (m, 4H), 2.91 (t, 2H, J = 6.0 Hz), 3.05 (t, 2H, J = 6.0 Hz), 3.28–3.31 (m, 4H), 4.07 (t, 2H, J = 6.0 Hz), 6.84–6.87 (m, 2H), 7.07–7.12 (m, 3H),7.34–7.36 (m, 2H) MS (ESI) m/z 477.3 ($[M + H]^+$).

*6.1.3.6. 7-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**12**).* Yield: 69.3%; mp: 88–90 °C. ^{1}H NMR ($CDCl_3$) δ 1.69–1.89 (m, 4H), 2.09–2.17 (m, 2H), 2.48 (t, 2H, J = 6.0 Hz), 2.66 (br, 4H), 2.82–2.84 (m, 2H), 2.93–3.13 (m, 6H), 3.83 (s, 3H), 4.01 (t, 2H, J = 6.0 Hz), 6.76–6.91 (m, 6H), 7.23–7.254 (m, 1H). MS (ESI) m/z 449.3 ($[M + H]^+$).

*6.1.3.7. 7-(4-(4-(3-Methoxyphenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**13**).* Yield: 70.1%; mp: 86–88 °C. ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 1.72–1.89 (m, 4H), 2.16–2.21 (m, 2H), 2.48 (t, 2H, J = 6.0 Hz), 2.61–2.63 (m, 4H), 2.89–2.91 (m, 2H), 3.05 (t, 2H, J = 6.0 Hz), 3.09–3.12 (m, 4H), 3.78 (s, 3H), 4.06 (t, 2H, J = 6.0 Hz), 6.45 (d, 1H, J = 4.0 Hz), 6.49 (d, 1H, J = 4.0 Hz), 6.56–6.59 (m, 1H), 6.81–6.85 (m, 1H), 6.88 (d, 1H, J = 4.0 Hz), 7.19 (t, 1H, J = 4.0 Hz), 7.51 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 449.3.

*6.1.3.8. 7-(4-(4-(4-Methoxyphenyl)piperazin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**14**).* Yield: 64.2%; mp: 91–93 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.87 (m, 4H), 2.15–2.21 (m, 2H), 2.47 (t, 2H, J = 6.0 Hz), 2.62–2.64 (m, 4H), 2.88–2.90 (m, 2H), 3.04 (t, 2H, J = 6.0 Hz), 3.09–3.11 (m, 4H), 3.77 (s, 3H), 4.05 (t, 2H, J = 6.0 Hz), 6.82–6.92 (m, 6H), 7.32 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 449.3.

*6.1.3.9. 7-(4-(4-(Pyridin-2-yl)piperazin-1-yl)butoxy)-2,3-dihydrocyclopent-a[c]chromen-4(1H)-one (**15**).* Yield: 71.3%; mp: 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 1.65–1.79 (m, 4H), 2.05–2.08 (m, 2H), 2.37 (t, 2H, J = 6.4 Hz), 2.47–2.49 (m, 4H), 2.75 (t, 2H, J = 6.0 Hz),

2.89 (t, 2H, J = 6.0 Hz), 3.45–3.47 (m, 4H), 3.95 (t, 2H, J = 6.0 Hz), 6.50–6.55 (m, 2H), 6.70–6.74 (m, 2H), 7.18 (d, 1H, J = 4.0 Hz), 7.35–7.38 (m, 1H), 8.08 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 420.2.

*6.1.3.10. 7-(4-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)butoxy)-2,3-dihydro-cyclopenta[c]chromen-4(1H)-one (**16**).* Yield: 65.3%; mp: 96–98 °C. ^{1}H NMR ($CDCl_3$) δ 1.74–1.88 (m, 4H), 2.13–2.19 (m, 2H), 2.50 (t, 2H, J = 6.0 Hz), 2.68–2.70 (m, 4H), 2.85 (t, 2H, J = 6.0 Hz), 2.99 (t, 2H, J = 4.0 Hz), 3.55–3.57 (m, 4H), 4.03 (t, 2H, J = 6.0 Hz), 6.81–6.84 (m, 2H), 7.29–7.34 (m, 2H), 7.42–7.45 (m, 1H), 7.78 (d, 1H, J = 4.0 Hz), 7.88 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 476.2.

*6.1.3.11. 7-(4-(4-(Benzo[d]oxazol-2-yl)piperidin-1-yl)butoxy)-2,3-dihydro-cyclopenta[c]chromen-4(1H)-one (**17**).* Yield: 63.1%; mp: 98–99 °C. ^{1}H NMR ($CDCl_3$) δ 1.73–1.2.01 (m, 6H), 2.16–2.20 (m, 6H), 2.47 (t, 2H, J = 6.0 Hz), 2.89–3.06 (m, 7H), 4.06 (t, 2H, J = 6.0 Hz), 6.85–6.88 (m, 2H), 7.31–7.34 (m, 3H), 7.49–7.51 (m, 1H), 7.69–7.71 (m, 1H). MS (ESI) m/z 459.3.

*6.1.3.12. 7-(4-(4-(Benzo[d]thiazol-2-yl)piperidin-1-yl)butoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**18**).* Yield: 60.9%; mp: 110–112 °C. ^{1}H NMR ($CDCl_3$) δ 1.72–2.00 (m, 6H), 2.13–2.21 (m, 6H), 2.47 (t, 2H, J = 6.0 Hz), 2.88 (t, 2H, J = 6.0 Hz), 3.01–3.13 (m, 5H), 4.05 (t, 2H, J = 6.0 Hz), 6.83–6.85 (m, 2H), 7.31–7.33 (m, 2H), 7.43–7.45 (m, 1H), 7.85–7.86 (m, 1H), 7.96–7.97 (m, 1H). MS (ESI) m/z 475.2.

*6.1.3.13. 7-(4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**19**).* Yield: 72.2%; mp: 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 1.74–1.88 (m, 4H), 2.06–2.21 (m, 8H), 2.48 (t, 2H, J = 6.0 Hz), 2.87–2.89 (m, 2H), 3.02–3.10 (m, 5H), 4.06 (t, 2H, J = 6.0 Hz), 6.83–6.86 (m, 2H), 7.04–7.05 (m, 1H), 7.22–7.25 (m, 1H), 7.33 (d, 1H, J = 4.0 Hz), 7.68–7.71 (m, 1H). MS (ESI) m/z 477.3 ($[M + H]^+$).

*6.1.3.14. 7-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propoxy)-2,3-dihydrocyclo-penta[c]chromen-4(1H)-one (**20**).* Yield: 78.2%; mp: 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 2.05–2.21 (m, 4H), 2.62 (t, 2H, J = 6.0 Hz), 2.70 (br, 4H), 2.87–2.89 (m, 2H), 3.02–3.12 (m, 6H), 3.87 (s, 3H), 4.10 (t, 2H, J = 6.0 Hz), 6.84–7.00 (m, 6H), 7.32–7.34 (m, 1H). MS (ESI) m/z 435.2 ($[M + H]^+$).

*6.1.3.15. 7-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-6-methyl-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**21**).* Yield: 70.1%; mp: 117–119 °C. ^{1}H NMR ($CDCl_3$) δ 1.76–1.90 (m, 4H), 2.16–2.20 (m, 2H), 2.33 (s, 3H) 2.51 (t, 2H, J = 6.0 Hz), 2.67 (br, 4H), 2.87–2.90 (m, 2H), 3.00–3.10 (m, 6H), 3.86 (s, 3H), 4.08 (t, 2H, J = 6.0 Hz), 6.80–6.99 (m, 5H), 7.21–7.23 (m, 1H). MS (ESI) m/z 463.3 ($[M + H]^+$).

*6.1.3.16. 7-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-6-chloro-2,3-dihydr-ocyclopenta[c]chromen-4(1H)-one (**22**).* Yield: 75.1%; mp: 125–127 °C. ^{1}H NMR ($CDCl_3$) δ 1.78–1.96 (m, 6H), 2.18–2.22 (m, 2H), 2.51 (t, 2H, J = 6.0 Hz), 2.67 (br, 3H), 2.87–2.90 (m, 2H), 3.01–3.10 (m, 5H), 3.86 (s, 3H), 4.16 (t, 2H, J = 6.0 Hz), 6.87–6.93 (m, 5H), 7.26–7.27 (m, 1H). MS (ESI) m/z 483.3 ($[M + H]^+$).

*6.1.3.17. 7-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-8-chloro-2,3-dihydr-ocyclopenta[c]chromen-4(1H)-one (**23**).* Yield: 65.6%; mp: 131–133 °C. ^{1}H NMR ($CDCl_3$) δ 1.69–1.95 (m, 6H), 2.19–2.23 (m, 2H), 2.51 (t, 2H, J = 6.4 Hz), 2.68 (br, 3H), 2.88–2.91 (m, 2H), 3.00–3.10 (m, 5H), 3.86 (s, 3H), 4.12 (t, 2H, J = 6.0 Hz), 6.85–7.00 (m, 5H), 7.41 (s, 1H). MS (ESI) m/z 483.2 ($[M + H]^+$).

*6.1.3.18. 3-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**24**).* Yield: 66.3%; mp: 97–99 °C. ^{1}H NMR ($CDCl_3$) δ 1.73–1.87 (m, 8H), 2.47–2.74 (m, 10H), 3.11 (br, 4H), 3.86 (s, 3H), 4.04 (t, 2H, J = 6.0 Hz), 6.78–6.99 (m, 6H), 7.43–7.45 (m, 1H). MS (ESI) m/z 463.2 ($[M + H]^+$).

*6.1.3.19. 7-(3-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**25**).* Yield: 75.1%; mp: 118–120 °C. ^{1}H NMR ($CDCl_3$) δ 1.93–2.14 (m, 10H), 2.52 (t, 2H, J = 6.0 Hz), 2.77–2.81 (m, 2H), 2.93–3.02 (m, 5H), 4.02 (t, 2H, J = 6.0 Hz), 6.75–6.77 (m, 2H), 6.94–6.99 (m, 1H), 7.13–7.15 (m, 1H), 7.21–7.25 (m, 1H), 7.60–7.64 (m, 1H). MS (ESI) m/z 463.3 ($[M + H]^+$).

*6.1.3.20. 7-((5-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) pentyl)oxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**26**).* Yield: 75.6%; mp: 125–127 °C. ^{1}H NMR ($CDCl_3$) δ 1.52–1.64 (m, 2H), 1.85–2.01 (m, 2H), 2.07–2.22 (m, 8H), 2.44 (t, 2H, J = 6.6 Hz), 2.87–2.90 (m, 2H), 3.02–3.10 (m, 5H), 4.03 (t, 2H, J = 6.0 Hz), 6.83–6.85 (m, 2H), 7.03–7.07 (m, 1H), 7.22–7.24 (m, 1H), 7.32–7.34 (m, 1H), 7.70–7.72 (m, 1H). MS (ESI) m/z 491.3 ($[M + H]^+$).

*6.1.3.21. 7-(4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)-6-methyl-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**27**).* Yield: 80.3%; mp: 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 1.62–1.84 (m, 4H), 1.94–2.11 (m, 8H), 2.21 (s, 3H), 2.39 (t, 2H, J = 6.6 Hz), 2.76 (t, 2H, J = 8.0 Hz), 2.88–3.00 (m, 5H), 3.99 (t, 2H, J = 6.0 Hz), 6.701–6.72 (m, 1H), 6.91–6.96 (m, 1H), 7.10–7.12 (m, 2H), 7.58–7.61 (m, 1H). MS (ESI) m/z 491.3 ($[M + H]^+$).

*6.1.3.22. 7-(4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)-6-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**28**).* Yield: 78.1%; mp: 137–139 °C. ^{1}H NMR ($CDCl_3$) δ 1.76–2.23 (m, 12H), 2.51 (t, 2H, J = 6.0 Hz), 2.87–2.90 (m, 2H), 3.02–3.11 (m, 5H), 4.17 (t, 2H, J = 6.0 Hz), 6.88–6.91 (m, 1H), 7.04–7.05 (m, 1H), 7.22–7.25 (m, 2H), 7.68–7.72 (m, 1H). MS (ESI) m/z 511.2 ($[M + H]^+$).

*6.1.3.23. 7-(4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)-8-chloro-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**29**).* Yield: 78.8%; mp: 128–130 °C. ^{1}H NMR ($CDCl_3$) δ 1.79–2.23 (m, 12H), 2.51 (t, 2H, J = 6.4 Hz), 2.88–2.92 (m, 2H), 3.00–3.09 (m, 5H), 4.12 (t, 2H, J = 6.0 Hz), 6.88–6.89 (m, 1H), 7.03–7.05 (m, 1H), 7.23–7.27 (m, 1H), 7.42 (s, 1H), 7.68–7.72 (m, 1H). MS (ESI) m/z 511.3 ($[M + H]^+$).

*6.1.3.24. 3-(4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) butoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**30**).* Yield: 68.1%; mp: 103–105 °C. ^{1}H NMR ($CDCl_3$) δ 1.67–1.87 (m, 10H), 2.06–2.13 (m, 4H), 2.47 (t, 2H, J = 6.0 Hz), 2.56 (t, 2H, J = 4.0 Hz), 2.75 (t, 2H, J = 4.0 Hz), 3.07–3.10 (m, 3H), 4.05 (t, 2H, J = 6.0 Hz), 6.80–6.85 (m, 2H), 7.04–7.05 (m, 1H), 7.22–7.25 (m, 1H), 7.45 (d, 1H, J = 4.0 Hz), 7.68–7.71 (m, 1H). MS (ESI) m/z 491.2 ($[M + H]^+$).

6.1.4. 3-Hydroxy-6H-benzo[c]chromen-6-one (**32**) [31]

A solution of the 2-bromobenzoic acid (0.05 mol), resorcinol (0.1 mol), and sodium hydroxide (0.1 mol) in water (30 mL) was heated under reflux for 30 min. A 10% aqueous solution of copper sulfate (5 mL) was then added whereupon a slightly exothermic reaction ensued. The resulting mixture was heated under reflux for an additional 10 min and then cooled to 20 °C. The insoluble product was collected, washed well with water and dried. This material was of sufficient purity to be used as such for the preparations of the 3-hydroxy-6H-benzo[c]chromen-6-one, Yield: 66.3%; mp: 230–231 °C (lit [31] 234–236 °C).

*6.1.5. General procedure for the preparation of compounds **33a-b***

1,4-dibromobutane (1,3-dibromopropane) (2 mmol) was added to a solution of compound **33** (1 mmol) and potassium carbonate in acetone (50 mL), and the mixture was refluxed for 5 h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the mixture was filtered, the solvent was evaporated and the residue was recrystallized from EtOH to yield compounds 34.

*6.1.5.1. 3-(4-Bromobutoxy)-6H-benzo[c]chromen-6-one (**33a**).* Yield: 85.2%; mp: 101–103 °C. ^{1}H NMR ($CDCl_3$) δ 2.00–2.13 (m, 4H), 3.52 (t, 2H, J = 6.0 Hz), 4.06 (t, 2H, J = 6.0 Hz), 6.82–6.90 (m, 2H), 7.51 (t, 1H, J = 6.0 Hz), 7.78 (t, 1H, J = 6.0 Hz), 7.91 (d, 1H, J = 4.0 Hz), 7.98 (d, 1H, J = 4.0 Hz), 8.34 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 347.2 ($[M + H]^+$).

*6.1.5.2. 3-(3-Bromopropoxy)-6H-benzo[c]chromen-6-one (**33b**).* Yield: 76.9%; mp: 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 2.30–2.41 (m, 2H), 3.64 (t, 2H, J = 6.4 Hz), 4.17 (t, 2H, J = 6.8 Hz), 6.83–6.93 (m, 2H), 7.47–7.51 (m, 1H), 7.77 (t, 1H, J = 6.0 Hz), 7.90 (d, 1H, J = 4.0 Hz), 7.97 (d, 1H, J = 4.0 Hz), 8.33 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 333.1 ($[M + H]^+$).

*6.1.6. General procedures for the preparation of compounds **34**, **35***

To a suspension of compounds **33** (0.32 mmol) and K_2CO_3 (1.22 mmol) in acetonitrile (5.0 mL), arylpiperazine (piperidine) (0.32 mmol) and a catalytic amount of KI were added and the resulting mixture was refluxed for 6 h. After filtering, the resulting filtrate was evaporated to dryness under reduced pressure. The residue was suspended in water (10.0 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic layers were evaporated under reduced pressure, and the crude product was purified by means of chromatography (5% MeOH/$CHCl_3$) to yield compounds **34** and **35**.

*6.1.6.1. 3-(4-(4-(6-Fluoro-1H-inden-3-yl)piperazin-1-yl) butoxy)-6H-benzo[c]-chromen-6-one (**34**).* Yield: 68.9%; mp: 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 1.76–1.94 (m, 4H), 2.06–2.17 (m, 6H), 2.50 (t, 2H, J = 6.0 Hz), 3.05–3.12 (m, 3H), 4.08 (t, 2H, J = 6.6 Hz), 6.83–6.93 (m, 2H), 7.02–7.08 (m, 1H), 7.19–7.25 (m, 1H), 7.50 (t, 1H, J = 6.0 Hz), 7.70–7.80 (m, 2H), 7.92–8.00 (m, 2H), 8.34 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 487.2 ($[M + H]^+$).

*6.1.6.2. 3-(3-(4-(6-Fluoro-1H-inden-3-yl)piperazin-1-yl)propoxy)-6H-benzo[c]-chromen-6-one (**35**).* Yield: 71.2%; mp: 136–138 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.78 (m, 2H), 1.85–1.92 (m, 2H), 2.51 (t, 2H, J = 6.4 Hz), 2.67 (s, br, 3H), 3.07 (s, br, 4H), 4.05 (t, 2H, J = 6.0 Hz), 6.81–6.97 (m, 3H), 7.10–7.15 (m, 2H), 7.47 (t, 1H, J = 6.6 Hz), 7.75 (t, 1H, J = 6.0 Hz), 7.87–7.96 (m, 2H), 8.31 (d, 1H, J = 4.0 Hz). MS (ESI) m/z 473.3 ($[M + H]^+$).

6.2. Pharmacological methods

6.2.1. Animals

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. Animals were housed under standardized conditions for light and temperature and received standard rat chow and tap water and libitum. Animals were randomly assigned to different experimental groups, each kept in a separate cage. All research involving animals in this study follow the guidelines of the byelaw of experiments on animals, and have been approved by the Ethics and Experimental Animal Committee of Jiangsu Nhwa Pharmaceutical Co., Ltd.

6.2.2. In vitro binding assays

6.2.2.1. 5-HT_{1A} receptor [35]. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using an ULTRA TURAX homogeniser, and was then centrifuged at 32,000 g for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 32000 g for 10 min. The final pellet was resuspended in Tris–HCl buffer containing 10 μM Pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.5 nM [^{3}H]8-OH-DPAT (187.4 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer containing 10 μM Pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]8-OH-DPAT, 50 μL of 10 μM serotonin. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H] 8-OH-DPAT, 50 μL of new compounds or reference drug. The tubes were incubated at 37 °C for 30 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 mL) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.2.2.2. 5-HT_{2A} receptor [35]. Rat cerebral cortex was homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using an ULTRA TURAX homogeniser, and centrifuged at 32000 g for 20 min. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 volumes of the Tris–HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.6 nM [^{3}H] ketanserine (60.0 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]ketanserin, 50 μL of 10 μM methisergide. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]ketanserin, 150 μL of new compounds or reference drug. The tubes were incubated at 37 °C for 30 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 mL) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.2.2.3. Dopaminergic D_2 receptor [35]. Rat striatum was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7) using an ULTRA TURAX homogeniser, and centrifuged twice for 10 min at 48,000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 5 μM pargyline. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 0.5 nM [^{3}H]spiperone (16.2 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 5 μM pargyline. Non-specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]spiperone, 50 μL of 10 μM (+)-butaclamol. Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]spiperone, 50 μL of new compounds or reference drug. The tubes were incubated at 37 °C for 30 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 mL) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.2.2.4. Dopaminergic D_3 receptor [33]. Rat olfactory tubercle was homogenized in 20 volumes of ice-cold 50 mM Hepes Na (pH 7.5) using an ULTRA TURAX homogeniser, and centrifuged twice for 10 min at 48,000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM Hepes Na, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumin, and 200 nM eliprodil. Total binding each assay tube was added 900 μL of membranes, 50 μL of 0.6 nM [^{3}H] 7-OH-DPAT (50 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of 50 mM Hepes Na, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumin, 200 nM eliprodil. Non-specific binding each assay tube was added 900 μL of membranes, 50 μL of [^{3}H] 7-OH-DPAT (50 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of 1 μM dopamine. Specific binding each assay tube was added 900 μL of Membranes, 50 μL of [^{3}H] 7-OH-DPAT (50 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL of new compounds or reference drug. The tubes were incubated at 25 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 mL) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.2.2.5. Histamine H_1 receptor [36]. Guinea pig cerebellum was homogenized in 20 volumes of ice-cold 50 mM phosphate buffer (pH = 7.4) using an ULTRA TURAX homogeniser, and centrifuged twice for 10 min at 50,000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in phosphate buffer. Total binding each assay tube was added 900 μL of membranes 50 μL of 1 nM [^{3}H]mepyramine (20.0 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA), 50 μL phosphate buffer. Non-specific binding each assay tube was added 900 μL of membranes, 50 μL of [^{3}H] mepyramine, 50 μL of 1 μM promethazine. Specific binding each assay tube was added 900 μL of Membranes, 50 μL of [^{3}H]mepyramine, 50 μL of new compounds or reference drug. The tubes were incubated at 30 °C for 60 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL cold buffer and transferred to scintillation vials. Scintillation fluid (3.0 mL) was added and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

6.2.3. Behavioral tests

6.2.3.1. Acute toxicity. Mice (10 mice in each group) were orally dosed with increasing doses of the compound 27 (250, 500, 1000, 1500 and 2000 mg/kg). The number of surviving animals was recorded after 24 h of drug administration, and the percent mortality in each group was calculated. The LD_{50} values were calculated by using the program SPSS (Statistical Package for the Social Science).

6.2.3.2. MK-801-induced hyperactivity [33]. Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.1, 0.3, 1, 3.0 and 10 mg/kg), clozapine (0.3, 1, 3, 10 and 30 mg/kg), risperidone (0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg) and compound **27** (0.1, 0.3, 1, 3 and 10 mg/kg). Animals were placed in Plexiglas cages for evaluating locomotor activity. After 30 min, the animals were challenged with 0.3 mg/kg (sc) of MK-801 and the locomotor activity of each animal was recorded for 90 min. Statistical evaluation was performed by two-way ANOVA followed by Tukey test for multiple comparisons. $^{\#}p < 0.05$ versus vehicle treatment; $^{**}p < 0.01$, $^{*}p < 0.05$ versus MK-801 treatment.

6.2.3.3. Apomorphine-induced climbing [32]. Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.1, 0.3, 1 and 3 mg/kg), clozapine (3.0, 5.0, 10.0 and 30 mg/kg), risperidone (0.01, 0.03, 0.1 and 0.3 mg/kg), compound **27** (0.3, 1, 3, 10 and 30 mg/kg). Animals were then challenged at 30 min post-injection with 1.0 mg/kg of the apomorphine in 0.9% NaCl+ 0.1% ascorbic acid, placed in cylindrical wire cages (12 cm in diameter, 14 cm in height), and observed for climbing behavior at 10, 20 and 30 min post dose. The climbing behavior was scored as follows: 3, 4 paws on the cage floor = 0 score; 2 and 3 paws on the cage = 1 score; 4 paws on the cage = 2 score. The statistical significances of drug effects were analyzed by the nonparametric two-tailed Mann–Whitney *U*-test: $^{\#}p < 0.05$ versus vehicle treatment; $^{**}p < 0.01$, $^{*}p < 0.05$ versus apomorphine treatment.

6.2.3.4. Catalepsy test [34]. Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.1, 0.3, 1, 1.5 and 3.0 mg/kg), clozapine (12.5, 25, 50, 100 and 200 mg/kg), risperidone (0.1, 0.4, 0.75, 1.5, 3 and 6.0 mg/kg), compound **27** (5, 15, 45 and 100 mg/kg). Catalepsy was evaluated on a metal bar 0.3 cm in diameter positioned 4.5 cm above the tabletop. The test consisted in positioning the animal with its forepaws on the bar and recording how long it remained hanging onto the bar; the end-point was 60 s and an all-or-none criterion was used. A mean immobility score of 30 s was used as the criterion for the presence of catalepsy.

6.3. Statistics

To estimate the potency of test and reference compounds, the ED_{50} values and their 95% confidence limits were calculated by using the program SPSS (Statistical Package for the Social Science).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.012

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