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Isoquinolinone derivatives as potent CNS multi-receptor D_2/5-HT_{1A}/5-HT_{2A}/5-HT_6/5-HT_7 agents: Synthesis and pharmacological evaluation

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ABSTRACT

In this study, a series of novel Isoquinolinone derivatives were synthesized as potential multi-target antipsychotics. Among these, compound **13** showed high affinity for dopamine D_2 and serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors, showed low affinity for off-target receptors (5-HT_{2C}, H_1, and α_1), and negligible effects on ether-a-gogo-related gene (hERG; *i.e.*, reduced QT interval prolongation). An animal behavioral study revealed that compound **13** reversed APO-induced hyperlocomotion, MK-801-induced hyperactivity, and DOI-induced head twitch. Moreover, compound **13** exhibited a high threshold for acute toxicity, a lack of tendency to induce catalepsy, and did not cause prolactin secretion or weight gain when compared to risperidone. Furthermore, in the forced swim test, tail suspension test, and novel object recognition test, treatment with compound **13** resulted in improvements in depression and cognitive impairment. In addition, compound **13** had a favorable pharmacokinetic profile in rats. Thus, the antipsychotic drug-like effects of compound **13** indicate that it may be useful for developing a novel class of drugs for the treatment of schizophrenia.

1. Introduction

Schizophrenia is characterized by positive, negative, and cognitive symptoms. Approximately 20% of diagnosed individuals have permanent and severe symptoms that cause disability, and more than 50% have non-persistent symptoms that follow a long course.[1] Current antipsychotics (Cozapine, Olanzapine, Risperidone, etc, Fig. 1) are marginally effective in treating positive symptoms but are less effective against negative symptoms and cognitive dysfunction. Furthermore, the treatment of schizophrenia with antipsychotics is impaired by a number of side effects, such as motor side effects, weight gain, metabolic disturbances, cardiovascular risk factors, and abnormalities in prolactin secretion.[2–6] Therefore, novel drug types that are effective against not only positive symptoms but also negative symptoms and cognitive impairment would represent a significant advancement in schizophrenia treatment.

In our previous study, we found that compounds **A**–**D** exerted obvious multi-target characteristics (Fig. 2).[7-10] As shown in Table 1, Compared to compounds A-C, compound **D** exhibited high affinity for D_2, D_3, 5-HT_{1A}, and 5-HT_{2A}, but also had strong binding affinity to the $5HT_6$ receptor. Moreover, compound D was not only effective in the animal model of positive symptoms, but also significantly displayed procognition properties in a novel object recognition task in rats.[10] Compound **D** received China Food and Drug Administration (CFDA) approval for clinical trials in 2018. Aripiprazole was approved by the Food and Drug Administration for treatment of schizophrenia in 2002. Aripiprazole exhibits high affinity for dopamine D_2 and D_3 and serotonin 5-HT_{1A} and 5-HT_{2A} receptors, as well as moderate affinity for dopamine D_4, serotonin 5-HT_{2C} and 5-HT_7, alpha-$_1$ adrenergic, and histamine H_1 receptors. Aripiprazole functions as a partial agonist at dopamine D_2 and

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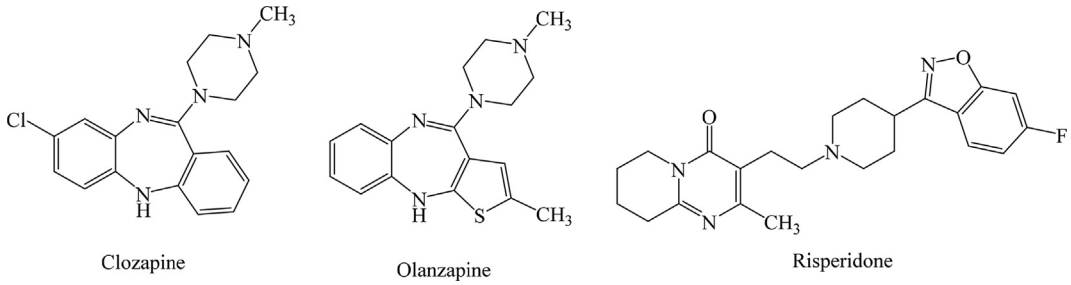


Fig. 1. Representative current antipsychotics.

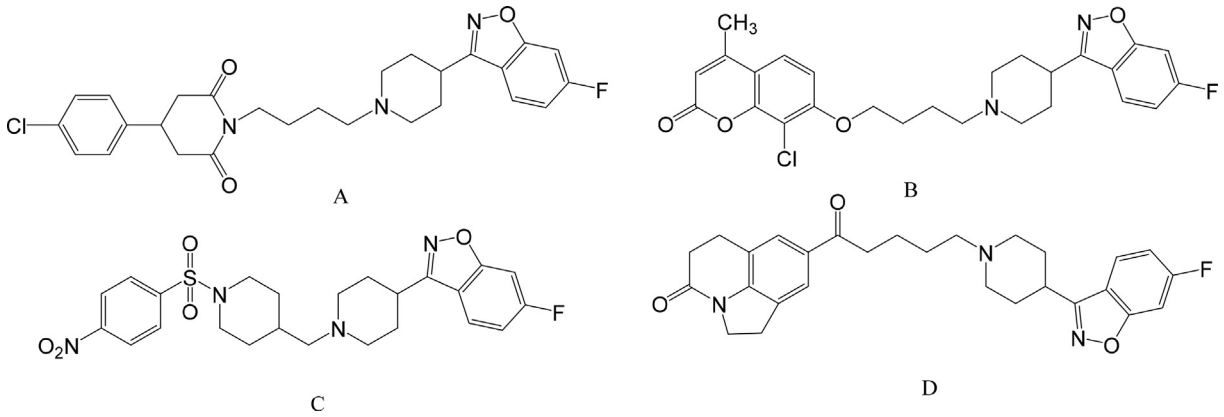


Fig. 2. Atypical antipsychotics of our previous study.

Table 1
Binding affinities of compounds A-D.

Compound	Ki(nM)						
	D_2	D_3	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	H_1	5-HT_6
A[8]	11.9	7.5	5.2	7.5	14.1	562.5	–
B[9]	2.6	4.3	3.3	0.3	1700.7	1125.3	–
C[10]	8.7	16.6	16.9	0.79	998.2	2845.2	–
D[11]	2.9	1.66	8.6	0.72	616	630.3	5.55

serotonin 5-HT_{1A} receptors, and as an antagonist at the serotonin 5-HT_{2A} receptor.[11] Therefore, it is considered to be a dopamine-serotonin system stabilizer, which has not been observed in the activity of any other antipsychotic drug.[12] Brexpiprazole is a partial agonist of 5-HT_{1A} and D_2 receptors with K_i values of 0.12 nM and 0.3 nM, respectively, as well as a 5-HT_{2A} receptor antagonist with a K_i of 0.47 nM.[13] RP5063, a novel multimodal dopamine and 5-HT modulator, shows high affinity for $D_{2,3,4}$ and 5-$HT_{2A,2B,7}$ receptors. As an adjunctive treatment, RP5063 has multifaceted effects in improving some of the cognitive deficits associated with schizophrenia.[14] These observations encouraged us to design multi-target ligands that can precisely modulate the activity of several monoaminergic receptors (dopamine D_2 and serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors), expecting that this multifunctional profile will contribute to the therapeutic potential for patients with schizophrenia.

Aripiprazole, brexpiprazole, and RP5063 contain lactam, with high affinity to 5-HT_{1A}, 5HT_7 and D_2 receptors. In this study, a new compound was designed to form lactam by reversing an amide bond (Fig. 3) and linking to an arylpiperazine (piperidine) group, which is one of the most important classes for CNS activity.[15] A series of new compounds were produced with an appropriate linker between the lactam molecule and privilege structures (Tables 1–3), and structure-activity relationship studies and competitive receptor-binding assays were performed to evaluate their pharmacological efficacy and relative affinity for the multi-receptor, respectively. The target compounds were subjected to preliminary pharmacological evaluation to determine their affinity for D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and H_1 receptors. Compound **13** exhibited higher affinity for D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors and low affinity for H_1 and 5-HT_{2C} receptors. Furthermore, compound **13** reduced apomorphine-induced climbing in animal behavioral studies as well as MK-801-induced hyperactivity, with a high threshold for inducing catalepsy. In addition, compound **13** lead to negligible weight gain and did not significantly influence serum prolactin levels compared with risperidone. Moreover, compound **13** displayed procognitive properties in a novel object recognition task in rats and showed favorable pharmacokinetic properties. Compared to compounds A-D, compound 13 showed also antipsychotic-like effects *in vivo* models in low doses, and it was also found effective in anti-depressant and procognitive tests. Thus, compound **13** was used to validate our novel approach to atypical antipsychotics based on its unique polypharmacological antipsychotic profile.

2. Results and discussion

2.1. Chemistry

The general strategy for the synthesis of the studied compounds is summarized in Schemes 1–6. As shown in Scheme 1, intermediate 2 was obtained under triethylamine conditions with ethoxyphenylamine and ethyl chloroformate. Under P_2O_5 and methanesulfonic acid conditions, intermediate 2 self-condensed to obtain intermediate 3, and reacted with iodomethane to obtain intermediate 4. Intermediate 4 was demethylated under HBr

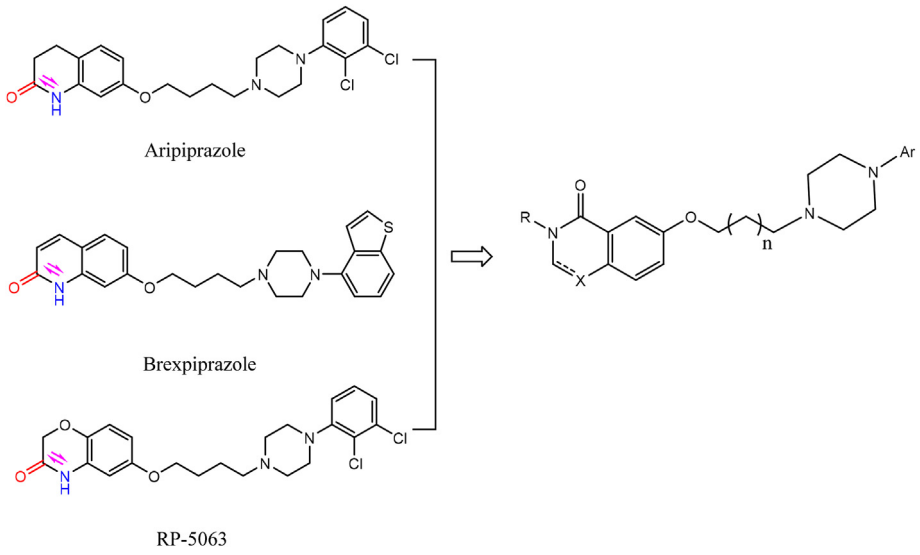


Fig. 3. Design of isoquinolinone derivatives.

conditions to obtain intermediate 5. Intermediate 5 reacted with 1,2-dibromoethane, 1,3-dibromopropane, and 1,4-dibromobutane to form intermediate 6. Compound 6 reacted with arylpiperazine (piperidine), yielding compounds **7**–**30**.

As shown in Scheme 2, intermediate 5 reacted with 2-(chloromethyl)oxirane to produce intermediate 31, and subsequently reacted with 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride to give compound **32**. Compound 32 reacted with diethylamino sulfur trivalent at −78 °C to obtain compound **33**. As shown in Scheme 3, intermediate 3 reacted with iodoethane, yielding intermediate 34. Intermediate 34 was demethylated under HBr conditions to obtain intermediate 35. Intermediate 35 reacted with 1,3-dibromopropane to form intermediate 36. Compound 36 then reacted with 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride to produce compound **37**. As shown in Scheme 4, carboxymethyl-5-methoxybenzoic acid [38] was condensed with methylamine to obtain intermediate 39. Intermediate 39 was placed under sodium borohydride conditions with dilute hydrochloric acid under the catalysis of the generated intermediate 40. Intermediate 40 was demethylated under HBr conditions to obtain intermediate 41. Intermediate 41 reacted with 1,3-dibromopropane to form intermediate 42. Compound 42 reacted with 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride to produce compound **43**.

As shown in Scheme 5, 2-bromo-5-methoxybenzoic acid [44] reacted with $SOCl_2$ to obtain acyl chloride 45, and then reacted with methylamine to obtain intermediate 46, which was condensed into intermediate 47 under cuprous bromide conditions. Intermediate 47 was demethylated under HBr conditions to obtain intermediate 48. Intermediate 48 reacted with 1,3-dibromopropane to form intermediate 49. Compound 49 reacted with 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride to produce compound **50**.

As shown in Schemes 6 and 2-(3-methoxyphenyl)ethan-1-amine [51] reacted with ethyl chloroformate, yielding intermediate 52. Under P_2O_5 conditions with methanesulfonic acid, intermediate 52 self-condensed to obtain intermediate 53, and reacted with iodomethane, yielding intermediate 54. Intermediate 54 was demethylated under HBr conditions to obtain intermediate 55. Intermediate 55 reacted with 1,3-dibromopropane to form intermediate 56. Compound 56 reacted with arylpiperazine (piperidine) to give compound **57**.

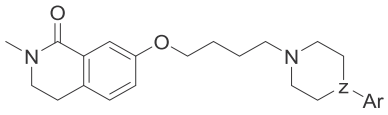
2.2. In vitro studies of new compounds

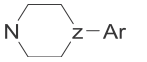
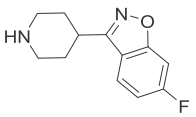
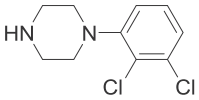
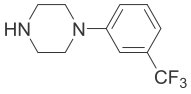
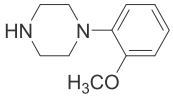
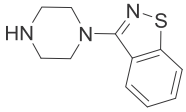
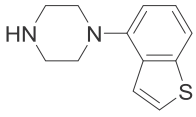
2.2.1. Structure-activity relationships

2.2.1.1. Effect of compounds with different amine moieties. In this work, our initial focus was to investigate the affinities of different amine moieties and four-carbon chain derivative compounds on the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors (Table 2, compounds **7**–**12**). As shown in Table 2, compound **7** bearing the (6-fluorobenzo[*d*]isoxazol-3-yl)piperidine moiety showed moderate affinity for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors (D_2, K_i = 41.6 nM; 5-HT_{1A}, K_i = 30.6 nM; 5-HT_{2A}, K_i = 28.3 nM). Moreover, compound **7** showed a higher affinity for 5-HT_{1A} receptors than risperidone (K_i = 182 nM). When the amine moieties were arylpiperazines, compounds **8**–**12** exhibited low affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors. These results indicate that compounds bearing (6-fluorobenzo[*d*]isoxazol-3-yl)piperidine (compound **7**) possess higher affinity for all three receptors compared with those with other amine moieties.

Next, we investigated the affinity of different amine moieties and three-carbon chain derivative compounds for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors (Table 3, compounds **13**–**29**). Notably, compound **13** bearing a three-carbon chain showed significantly higher affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors (D_2, K_i = 6.9 nM; 5-HT_{1A}, K_i = 9.1 nM; 5-HT_{2A}, K_i = 0.65 nM) compared with the four-carbon chain compound **7** (D_2, K_i = 41.6 nM; 5-HT_{1A}, K_i = 30.6 nM; 5-HT_{2A}, K_i = 28.3 nM). Moreover, compound **13** showed higher affinity for the 5-HT_{1A} receptor compared with risperidone (K_i = 182 nM). The 5-HT_{1A} receptor is implicated in the therapeutic efficacy of atypical antipsychotic drugs. Specifically, 5-HT_{1A} receptor activity reduces negative symptoms and the incidence of extrapyramidal symptoms (EPS) in patients with schizophrenia [32],[33]. Specifically, compound **13** also displayed higher affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors than aripiprazole (D_2, K_i = 9.7 nM; 5-HT_{1A}, K_i = 19.8 nM; 5-HT_{2A}, K_i = 12.1 nM). However, substitution of (6-fluorobenzo[*d*]isoxazol-3-yl)piperidine (compound **13**) with (2,4-difluorophenyl)(piperidin-4-yl)methanone (compound **14**) reduced the affinity for all three receptors. Compound **15** bearing the 3-(trifluoromethyl)phenyl)piperazine moiety displayed moderate affinity for the D_2 and 5-HT_{2A} receptors (D_2, K_i = 123.2 nM; 5-HT_{2A}, K_i = 101.5 nM). Compound **16** bearing 3-(piperazin-1-yl)benzo[*d*]isothiazole showed high affinity for the 5-

Table 2
Binding Affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds 7–12 and reference antipsychotics[a].



Compound		Receptor affinity Ki ± SEM(nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
7		41.6 ± 5.6	30.6 ± 3.1	28.3 ± 2.9
8		316.3 ± 42.1	119.2 ± 12.3	215.9 ± 33.9
9		261.4 ± 37.6	1039 ± 133	131.5 ± 15.3
10		>10000[b]	>10000[b]	>10000[b]
11		>10000[b]	1295.5 ± 139.6	890.2 ± 91.2
12		161.9 ± 28.6	431 ± 54	298 ± 32.1
risperidone	–	3.7 ± 0.3	182 ± 15	0.19 ± 0.02
aripiprazole		9.7 ± 1.1	19.8 ± 2.6	12.1 ± 1.6

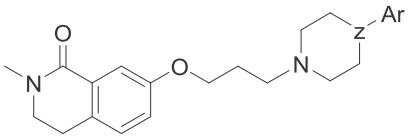
[a] Ki values are taken from three experiments, expressed as means ± SEM.
[b] The Ki values were not calculated because the inhibition percentages at 10 μM were too low.

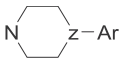
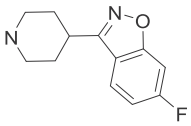
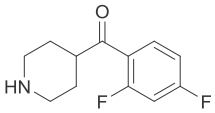
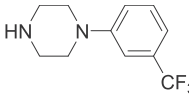
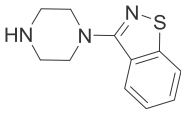
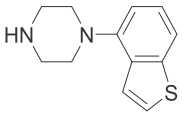
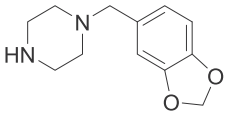
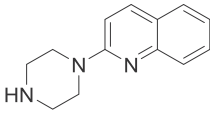
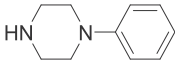
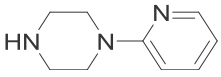
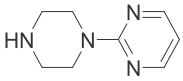
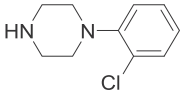
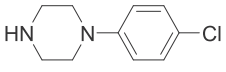
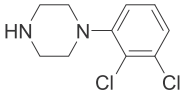
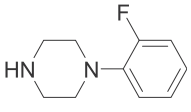
HT_{1A} and 5-HT_{2A} receptors (5-HT_{1A}, K_i = 12.3 nM; 5-HT_{2A}, K_i = 19.2 nM), but displayed weak affinity for the D_2 receptor (D_2, K_i = 923 nM). 1-(Benzo[*b*]thiophen-4-yl)piperazine derivative **17** showed moderate affinity for all three receptors (D_2, K_i = 29.3 nM; 5-HT_{1A}, K_i = 65.2 nM; 5-HT_{2A}, K_i = 75.9 nM). Compound **18** bearing 1-(benzo[d][1,3]dioxol-5-ylmethyl)piperazine showed low affinity for the D_2 receptor (K_i > 10,000 nM) and high affinity for 5-HT_{1A} (K_i = 18.3 nM) and 5-HT_{2A} (K_i = 25.9 nM). Compound **19** bearing the 2-(piperazin-1-yl)quinoline moiety showed high affinity for 5-HT_{1A} (K_i = 10.2 nM) and weak affinity for the D_2 and 5-HT_{2A} receptors. Phenylpiperazine derivative **20** and compound **21** bearing 1-(pyridin-2-yl)piperazine showed low affinity to the three receptors. When the 2-(piperazin-1-yl)pyrimidine moiety was the amine moiety, compound **22** exhibited moderate affinity for the 5-HT_{1A} and 5-HT_{2A} receptors and weak affinity for the D_2 receptor. Substitution of phenylpiperazine with the 1-(2-chlorophenyl)piperazine or 1-(4-chlorophenyl)piperazine groups (compounds **23** and **24**) improved the affinity for all three receptors. Moreover, compound **25** bearing 1-(2,3-dichlorophenyl)piperazine showed higher affinity for the three receptors (D_2, K_i = 68.2 nM; 5-HT_{1A}, K_i = 20.1 nM; 5-HT_{2A}, K_i = 16.8 nM) than compounds **23** and **24**. The phenyl ring substituted with F (compounds **26** and **27**) displayed moderate activity at all three receptors, while that substituted with methoxy (compounds **28** and **29**) showed lower activity at all three receptors compared with compounds **26** and **27**. These results indicate that compounds bearing electron-withdrawing groups possess higher affinity for all three receptors compared to those bearing electron-donating groups. According to our results, compounds bearing (6-fluorobenzo[*d*]isoxazol-3-yl)piperidine (compound **13**) possessed higher affinity for all three receptors compared to those with other amine moieties.

2.3. Effect of the lactam and piperidine ring linker

According to the above results, the compound bearing a three-carbon chain (compound **13**, D_2, K_i = 6.9 nM; 5-HT_{1A}, K_i = 9.1 nM; 5-HT_{2A}, K_i = 0.65 nM) possessed higher affinity for all three receptors compared with the four-carbon chain compound **7** (D_2, K_i = 41.6 nM; 5-HT_{1A}, K_i = 30.6 nM; 5-HT_{2A}, K_i = 28.3 nM). Therefore, we aimed to determine the effects of the length of the linker between the **lactam** and the piperidine ring. As shown in Table 4, a chain length of two carbon atoms (**30**) resulted in significantly reduced D_2, 5-HT_{1A}, and 5-HT_{2A} receptor binding. Introducing OH to the carbon chain of compound **32** resulted in

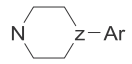
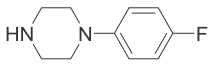
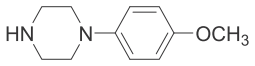
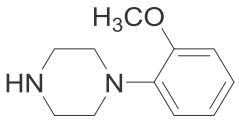
Table 3
Binding Affinities for D_2, 5-HT_{1A} and 5-HT_{2A} receptors of compounds 13–30 and reference antipsychotics[a].



Compound		Receptor affinity Ki ± SEM(nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
13		6.9 ± 0.7	9.1 ± 0.8	0.65 ± 0.1
14		902 ± 110	>10000[b]	>10000[b]
15		123.2 ± 21.5	1039 ± 133	101.5 ± 12.3
16		923 ± 108.2	12.3 ± 3.1	19.2 ± 2.8
17		29.3 ± 4.2	65.2 ± 8.9	79.1 ± 112.5
18		>10000[b]	18.3 ± 3.2	25.9 ± 4.5
19		>10000[b]	10.2 ± 2.1	>10000[b]
20		>10000[b]	>10000[b]	>10000[b]
21		>10000[b]	>10000[b]	1156.9 ± 26.8
22		1368 ± 236	59.3 ± 7.1	65.3 ± 8.2
23		391.2 ± 40.1	89.2 ± 10.4	179.3 ± 21.2
24		268.3 ± 39.2	356.2 ± 42.3	589.4 ± 69.1
25		68.2 ± 8.3	20.1 ± 2.9	16.8 ± 2.7
26		117.2 ± 19.1	262.2 ± 30.3	305.7 ± 41.8

(*continued on next page*)

Table 3 (*continued*)

Compound		Receptor affinity Ki ± SEM(nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
27		532.4 ± 63.5	158.2 ± 17.3	246.2 ± 36.9
28		>10000[b]	>10000[b]	>10000[b]
29		896 ± 101	1109 ± 153	992 ± 130
Risperidone	–	3.9 ± 0.3	182 ± 15	0.19 ± 0.02
Aripiprazole		9.7 ± 1.1	19.8 ± 2.6	12.1 ± 1.6

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

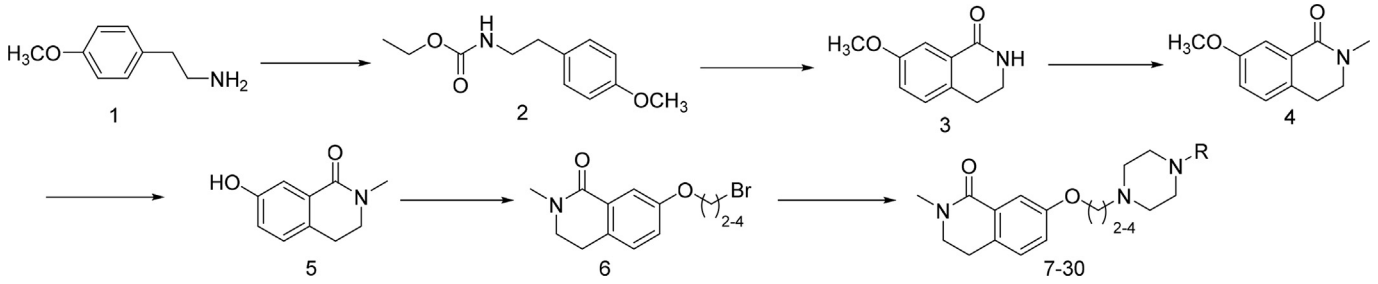
weak affinities of all three receptors. Moreover, compound **33** (replacement of the OH with F) showed moderate affinity for 5-HT_{1A} (K_i = 68.2 nM) and 5-HT_{2A} (K_i = 23.7 nM), and low affinity for the D_2 receptor. Taken together, these data indicate that a three-carbon chain length (compound **13**) resulted in the most active compound.

2.4. Effect of substitution on the 2-position (R)

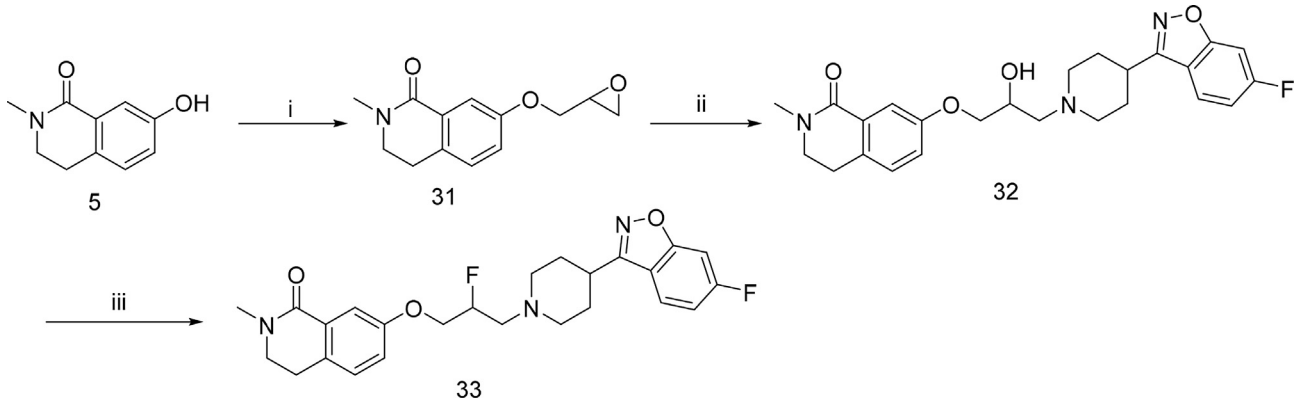
We investigated the effects of replacing the methyl (R) group with an ethyl group (Table 4, compound **37**). Compound **37** displayed moderate activity at all three receptors (D_2, K_i = 249.2 nM; 5-HT_{1A}, K_i = 198.3 nM; 5-HT_{2A}, K_i = 92.1 nM), and this replacement resulted in reduced affinity for the D_2 and 5-HT_{2A} receptors compared with compound **13**. Based on the above results, we observed that modifications of the alkyl group in the 4- position (R) of the **lactam** provided analogues with the following order of D_2, 5-HT_{1A} and 5-HT_{2A} receptors: methyl > ethyl.

2.5. Effect of the lactam double bond

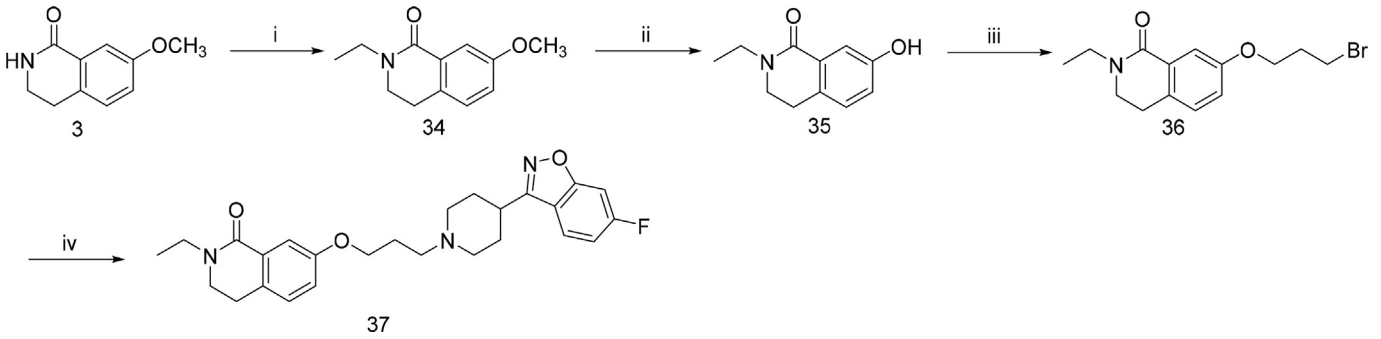
As shown in Table 4, replacing the single bond with double bond resulted in preserved high affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors (compound **43**, D_2, K_i = 9.2 nM; 5-HT_{1A}, K_i = 12.1 nM; 5-HT_{2A}, K_i = 3.5 nM).



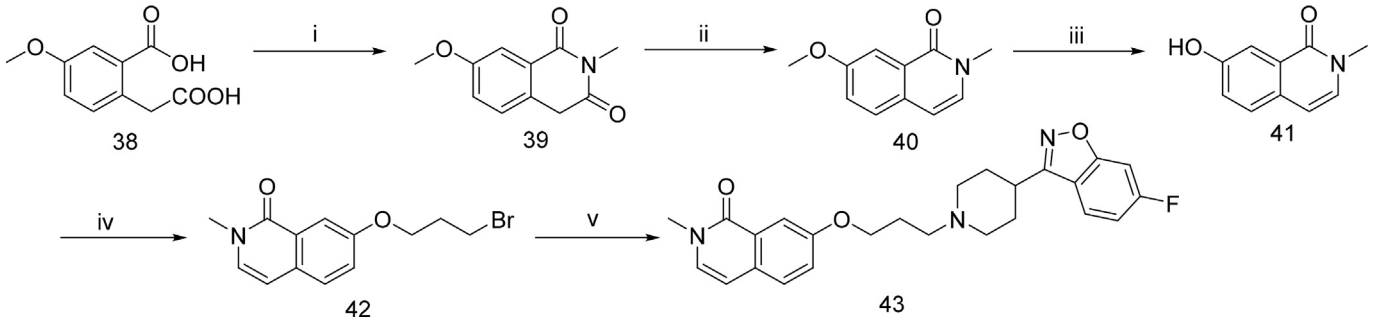
Scheme 1. Reagents and conditions: (i)ethyl chloroformate, Et_3N, CH_2Cl_2,0°C-rt; (ii), P_2O_5, MSA, 130 °C, 10h; (iii) CH_3I,NaH,DMF, 0°C-r.t.; (iv)HBr/H_2O, 100 °C; (v) K_2CO_3,acetone, TEBA, reflux; (vi)K_2CO_3,CH_3CN, reflux.



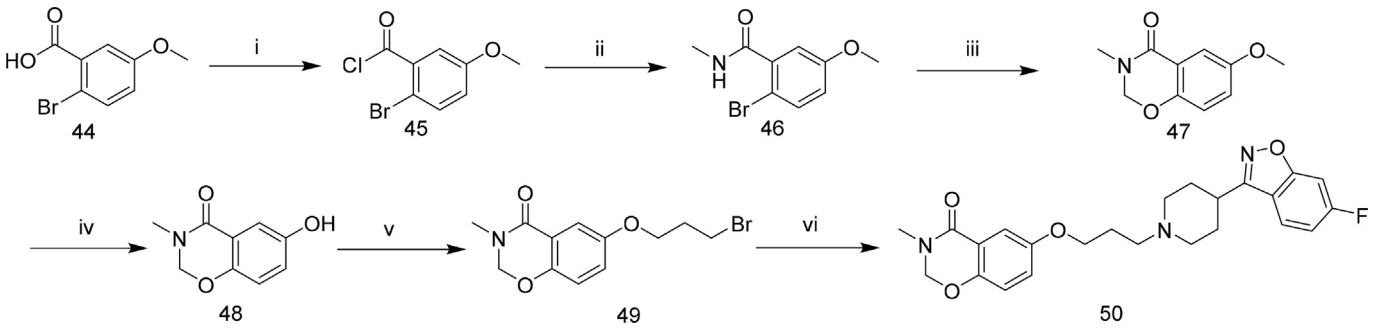
Scheme 2. Reagents and conditions: (i) 2-(cholomethyl)oxirane, K_2CO_3, KI, acetone, reflux; (ii)K_2CO_3, acetonitrile, reflux.; (iii) DAST, DCM, 0°C-rt.



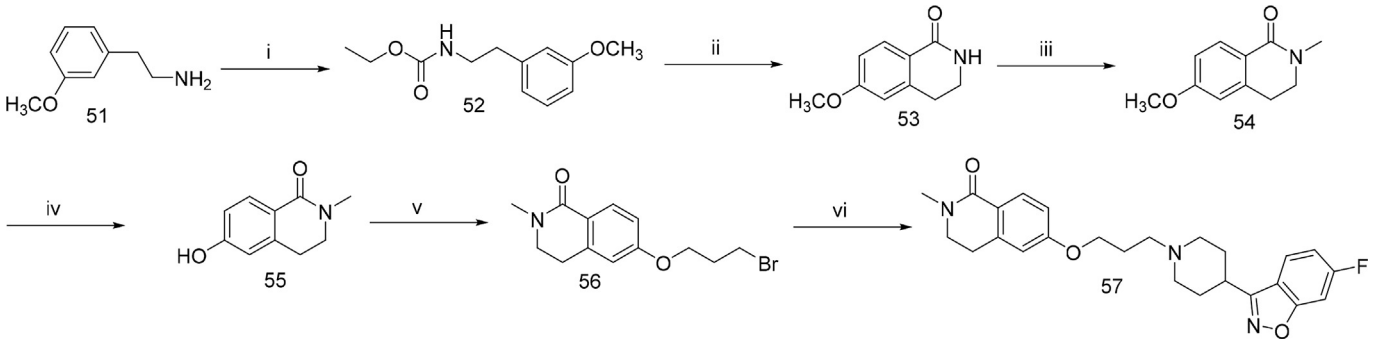
Scheme 3. Reagents and conditions: (i) CH_3CH_2I, NaH, DMF,0°C-r.t.; (ii) HBr/H_2O, 100 °C; (ii) K_2CO_3, acetone, reflux; (iv)K_2CO_3, CH_3CN,reflux.



Scheme 4. Reagents and conditions: (i) methylamine, MeOH, o-dichlorobenzene, reflux; (ii) $NaBH_4$, EtOH, 0 °C; (iii) HBr/H_2O, 100 °C; (iv) K_2CO_3, acetone, reflux; (v)K_2CO_3, CH_3CN, reflux.

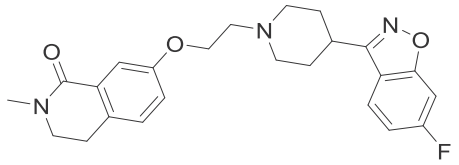
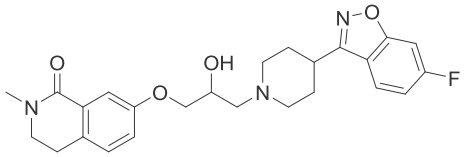
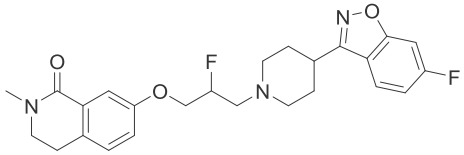
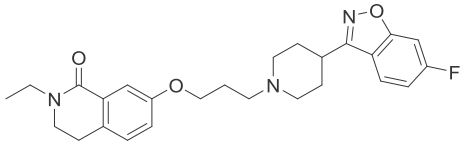
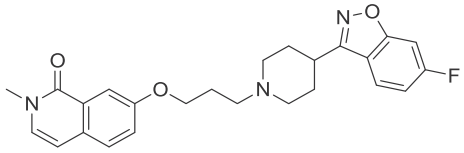
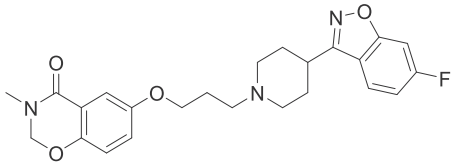
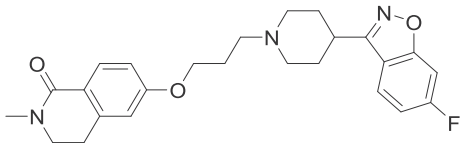


Scheme 5. Reagents and conditions: (i) $SOCl_2$, DCM; (ii) methylamine, Et_3N, DCM, 0°C-r.t.; (iii)CuBr, 1,10-Phenanthroline, CH_2Cl_2, DMSO, 100 °C; (iv) BBr_3, DCM; (v) $BrCH_2CH_2CH_2Br$, K_2CO_3, acetone, reflux; (vi)K_2CO_3, CH_3CN, reflux.



Scheme 6. Reagents and conditions: (i) ethyl chloroformate, Et_3N, CH_2Cl_2,0°C-r.t.; (ii) P_2O_5, MSA, 130 °C, 10h; (iii) CH_3I, NaH, DMF,0°C-r.t.; (iv) HBr/H_2O, 100 °C; (v) $BrCH_2CH_2CH_2Br$, K_2CO_3, acetone, reflux; (vi)K_2CO_3, CH_3CN,reflux.

Table 4
Binding Affinities for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors of compounds 30, 32, 33, 37, 43, 50, 57 and reference antipsychotics [a].

Compound	Structure	Receptor affinity Ki ± SEM(nM)		
		D_2	5-HT_{1A}	5-HT_{2A}
30		568.2 ± 69.5	>10000[b]	>10000[b]
32		1237 ± 145	856 ± 112	768 ± 97
33		359.2 ± 46.1	68.2 ± 8.7	23.7 ± 4.1
37		249.2 ± 35.6	198.3 ± 24.5	92.1 ± 11.2
43		9.2 ± 1.1	12.1 ± 1.3	3.5 ± 0.5
50		698 ± 79	187 ± 25.8	598 ± 72
57		>10000[b]	>10000[b]	692 ± 78
risperidone	–	3.9 ± 0.3	182 ± 15	0.19 ± 0.02
aripiprazole		9.7 ± 1.1	19.8 ± 2.6	12.1 ± 1.6

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

2.6. Effect of replacing the oxygen atom with methylene

We investigated the effects of replacing the oxygen atom in compound **13** with a CH_2 group (Table 4, compound **50**). Compound **50** displayed a decreased affinity for all three receptors compared with compound **13**.

2.7. Effect of changing the phenyl linker position

Finally, we changed the linker 7 position (compound **13)** to the 6 position (compound **57**), which caused a dramatic decrease in affinity for the D_2 and 5-HT_{1A} receptors.

Overall, compounds **13** and **43** exhibited high affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors. These two compounds showed higher affinity for 5-HT_{1A} receptors compared with aripiprazole (K_i = 19.8 nM) and risperidone (K_i = 182 nM). Furthermore, compounds **13** (K_i = 0.65 nM) and **43** (K_i = 3.5 nM) showed higher affinity for the 5-HT_{2A} receptor than aripiprazole (K_i = 12.1 nM). Therefore, compounds **13** and **43** were selected for additional studies to determine their affinity for the H_1, 5-HT_{2C}, α_1, 5-HT_6, and 5-HT_7 receptors due to their high affinity for the D_2, 5-HT_{1A}, and 5-HT_{2A} receptors.

Blockade of α_1 adrenergic receptors results in orthostatic hypotension[16], which is an unwanted side effect associated with

many antipsychotic agents. According to Table 4, compounds **13** (K_i = 991 nM) and **43** (K_i = 718 nM) showed lower affinity for α_1 receptors compared with risperdone (K_i = 3.2 nM) and aripiprazole (K_i = 8.9 nM), suggesting that compounds **13** and **43** may not cause orthostatic hypotension.

Treatment of schizophrenia with atypical antipsychotic drugs has been associated with weight gain. Several studies have demonstrated that there is significant correlation between affinity for H_1 and 5-HT_{2C} receptors and weight gain[17–19]. Thus, compound **13**, which had much lower affinity (K_i = 1196 nM) for H_1 receptors than risperidone (K_i = 32.6 nM) and aripiprazole (K_i = 48.9 nM) may have a lower propensity to induce weight gain (Table 4). Moreover, compound **13** had lower affinity for the 5-HT_{2C} receptor (K_i = 1687 nM) compared to risperidone (K_i = 20.9 nM) and aripiprazole (K_i = 18.2 nM). Therefore, compound **13** may exhibit a low potential to elicit treatment-associated weight gain.

Some preclinical studies have found that the 5-HT_7 receptor plays an important role in preventing depressive-like behaviors [20],[21] and in improving cognitive function[22],[23]. As shown in Table 4, compound **43**, risperidone, and aripiprazole display moderate affinity for the 5-HT_7 receptor. Interestingly, compound **13** (K_i = 4.9 nM) exhibited a higher affinity for the 5-HT_7 receptor than risperidone (K_i = 40.7 nM) and aripiprazole (K_i = 89.4 nM). The 5-HT_6 receptor has been proposed as a target for atypical antipsychotic drugs, and various pharmacological studies have suggested that 5-HT_6 antagonism might improve cognitive symptoms[24],[25]. As shown in Table 4, compounds **13** (K_i = 9.6 nM) and **43** (K_i = 167.2 nM) exhibit moderate affinity for the 5-HT_6 receptor compared with risperidone (K_i = 1329 nM) and aripiprazole (K_i = 318 nM). According to these results, compound **13** may improve cognitive symptoms and depressive-like behaviors.

2.8. Ether-a-gogo-related gene KC channels and acute toxicity

Recent studies have shown that a wide range of medications can prolong the QT interval, defined as the length of time between the start of the Q wave and the end of the T wave, on electrocardiogram. Specific examples include sertindole and grepafloxacin[26]. Efforts to predict increased risk for long QT syndrome have focused on assays that test hERG channel activity. As shown in Table 5, the affinity of compound **43** (IC_{50} = 598 nM) for hERG was comparable to that of risperidone (IC_{50} = 0.467 μM). Interestingly, the low affinity of compound **13** (IC_{50} = 2312 nM) for hERG compared with risperidone and aripiprazole may signify a decreased propensity for eliciting treatment-induced QT interval prolongation. More importantly, when tested *in vivo* in an anesthetized guinea pig model, compound **13** showed no QT prolongation or cardiac liability, even at the highest dose (0.04, 0.2, and 0.4 mg/kg, intraperitoneal, Supporting **Information**). Next, we determined the acute toxicity profiles of the compounds by determining their LD_{50}. Compound **13** exhibited low affinity for the H_1 and 5-HT_{2C} receptors and hERG, and displayed a good safety profile, even at the highest dose tested (LD_{50} > 2000 mg/kg).

2.9. Intrinsic activity of compound 13 at selected receptors

In an agonist assay including the D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors, compound **13** displaying <10% of the agonist efficacy at the 10 μM (Table 6). In an antagonist assay, compound **13** inhibited four receptors, D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7, by > 95%. Specifically, compound **13** showed antagonism against the following five receptors: D_2, IC_{50} = 6.3 nM; 5-HT_{1A}, IC_{50} = 56.9 nM; 5-HT_{2A}, IC_{50} = 46.3 nM; 5-HT_6, IC_{50} = 39.7 nM; and 5-HT_7, IC_{50} = 68.5 nM. Aripiprazole exhibits functions as a partial agonist at D_2 and 5-HT_{1A} receptors, and as an antagonist at the 5-HT_{2A} receptor,[11] however, compound 13 shows antagonism to D_2, 5-HT_{1A}, and 5-HT_{2A} receptors.

Overall, the binding profile of compound **13** showed high affinity for dopaminergic D_2 and serotonergic (5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7) receptors and low affinity for α_1, H_1, and 5-HT_{2C} receptors and hERG channels. Clozapine to reverse phencyclidine-elicited cortical desynchronization-a model of neural activity underlying negative symptoms-requires activation of 5-HT_{1A} receptors[27]. Clozapine can attenuate negative symptoms and cognitive decline through its actions as a 5-HT_{1A} partial agonist [28],[29]. Additionally, it has been argued that antagonism of serotonin 5-HT_{2A} receptors by second generation antipsychotics (SGAs) contributes to their efficacy, reducing the need for relatively extensive D_2 receptor occupancy, consequentially reducing side effects[30],[31]. Pimavanserin, a selective 5-HT_{2A} antagonist, effectively treats psychosis in Parkinson's disease without causing EPS[32],[33].5-HT_6 antagonists enhance dopamine release in the prefrontal cortex and regulate interactions between the cholinergic and glutamatergic systems, which is beneficial for the excitability and conductibility of neurotransmission and results in improved memory and cognitive functions[34].5-HT_7 receptor antagonists have been shown to produce a rapid-onset antidepressant effect in rodent models, while much more complex actions have been observed in cognitive assays.[35],[36] Therefore, combined with its affinity for 5-HT_6 and 5-HT_7, the actions of compound **13** at the desired receptors suggest a promising therapeutic potential in terms of enhancing cognitive function in subjects with schizophrenia as well as an adjunctive treatment for depression. Several studies have demonstrated that 5-HT_{2C} and H_1 antagonists likely lead to weight gain. The two antipsychotic drugs with the greatest effects on body weight, olanzapine and clozapine, also have high affinity for the 5-HT_{2C} and H_1 receptors, which implicates these receptors in antipsychotic-induced weight gain[37]. Compound **13** showed low affinity for 5-HT_{2C} and H_1, likely resulting in a lower propensity to induce weight gain.

2.10. In vivo studies

Initial behavioral screening was performed on compound **13** on the basis of its multiple receptor affinity profile. Atypical antipsychotics have been used to relieve positive symptoms at doses that do not result in EPS. In this study, the side effect profile of compound **13** was evaluated using the horizontal bar test, which is

Table 5
Binding affinities for D_3, 5-HT_{2C} and H_1 Receptors of compounds 13, 43 and reference antipsychotics[a].

Compound	Receptor affinity Ki ± SEM (nM)					(IC_{50}, nM)
	α_1	5-HT_{2C}	H_1	5-HT_7	5-HT_6	hERG
13	991 ± 122	1687 ± 159	1196 ± 122	4.9 ± 0.6	9.6 ± 1.1	2312 ± 298
43	718 ± 80	129.3 ± 14.9	117.9 ± 11.9	108.7 ± 16.8	167.2 ± 8.3	598 ± 65
risperidone	3.2 ± 0.5	20.9 ± 2.2	32.6 ± 4.7	40.7 ± 5.3	1329 ± 143	167 ± 23
aripiprazole	8.9 ± 1.2	18.2 ± 2.9	48.9 ± 5.8	89.4 ± 9.7	318 ± 49	896 ± 98

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

Table 6
Activities of compound 13 and reference compounds to D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptors.

Receptor	Compd	Activation (10 μM, %) (n = 3)	EC_{50} (nM)	Inhibition (10 μM, %) (n = 3)	IC_{50} (nM)
D_2	Dopamine	99.2 ± 5.3	18.2		
	SCH 2 3390			100.1 ± 2.4	25.8
	13	5.2 ± 0.5		99.9 ± 4.3	6.3
5-HT_{1A}	5-HT	98.3 ± 3.5	5.8		
	WAY-100635			98.8 ± 1.8	2.8
	13	5.8 ± 0.3		98.7 ± 2.5	56.9
5-HT_{2A}	5-HT	101.6 ± 1.2	1.1		
	ketanserin			99.4 ± 0.6	19.6
	13	22 ± 0.3		100.1 ± 1.4	46.3
5-HT_6	5-HT	96.1 ± 3.1	42.1		
	Clozapine			97.6 ± 5.3	20.3
	13	3.5 ± 0.3		99.5 ± 4.9	39.7
5-HT_7	5-CT	99.1 ± 4.9	5.1		
	Methiothepin			100.1 ± 4.2	11.3
	13	2.3 ± 0.3		99.1 ± 2.8	12.5

sensitive for catalepsy induced by dopamine D_2 receptor blockade. The antipsychotic potential of compound **13** was assessed using the apomorphine-induced climbing, dizocilpine (MK-801)-induced hyperactivity, and 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head twitch tests.

The apomorphine-induced climbing model has been classically linked to motor agitation as one of the schizophrenia positive symptoms[38]. Based on its binding profile (Table 6), compound **13** was selected for evaluation in a mouse model of the positive symptoms of schizophrenia (Fig. 4). Compound **13** exhibited dose-dependent antagonism of apomorphine-climbing behavior in mice, with higher doses producing greater inhibition (ED_{50}, 0.06 mg/kg). In comparison, risperidone, aripiprazole, and haloperidol produced reversal of apomorphine-induced climbing with ED_{50} values of 0.02, 0.25, and 0.09 mg/kg, respectively (Table 7).

MK-801, a highly selective non-competitive NMDA receptor antagonist, induces hyperlocomotion and other signs of disorganized behavior[39]. Compound **13** showed significant dose-dependent responses of MK-801-induced hyperactivity (Fig. 5), with an ED_{50} value of 0.08 mg/kg (Table 7). In comparison, risperidone, aripiprazole, and haloperidol yielded ED_{50} values of 0.04, 0.32, and 0.11 mg/kg, respectively. These results indicate that compound **13** was more potent than aripiprazole in inhibiting MK-801-induced hyperactivity.

2,5-DOI is a 5-$HT_{2A/2C}$ agonist that can elicit head twitch behavior mediated by activation of the 5-HT_{2A} receptor in mice[40]. To further evaluate the inhibitory effect of compound **13** on the 5-HT_{2A} receptor *in vivo*, mice were treated with compound **13** and the DOI-induced head twitch test was performed. As shown in Fig. 6, DOI caused head twitch responses in mice, which were suppressed by pretreatment with compound **13** in a dose-dependent manner (ED_{50}, 0.07 mg/kg) (Table 7). In comparison, risperidone and aripiprazole yielded ED_{50} values of 0.04 and 0.58 mg/kg, respectively. Based on the antagonistic effects of compound **13** on 5-HT_{2A} receptors observed *in vitro* (*i.e.*, antagonism of 5-HT_{2A} receptor in functional test) and *in vivo* (*i.e.*, suppressing DOI-induced head twitch behavior in mice) compared with aripiprazole, we speculate that the therapeutic potential of compound **13** in treatment-resistant depression largely may rely on its 5-HT_{2A} receptor antagonistic activity.

Catalepsy is often used as a measure to predict the incidence of extrapyramidal motor disorders. Haloperidol had the highest propensity to induce catalepsy (ED_{50}, 0.32 mg/kg), in agreement with the high affinity of this drug to block D_2 receptors (Table 7). By contrast, compound **13** exhibited a low potential to induce catalepsy, with an ED_{50} of 8.35 mg/kg (Table 7). Moreover, these results suggest that the therapeutic index of compound **13** as a function of its efficacy, measured with apomorphine, MK-801, and DOI tests, and its side effects, measured with the catalepsy test, was in the range of 119–139, while the therapeutic indices of both risperidone and aripiprazole were roughly 19–80. Thus, in contrast to risperidone and aripiprazole, compound **13** had a higher threshold for inducing catalepsy, which may translate into lower clinical EPS liability.

Overall, compound **13** significantly inhibited apomorphine-induced climbing behavior, MK-801-induced hyperactivity, and DOI-induced head twitch without causing catalepsy. These results suggest a preferential ability of compound **13** to modulate mesolimbic instead of nigrostriatal dopaminergic neurotransmission. These findings highlight its atypicality and low propensity to induce unwanted extrapyramidal motor disturbances at therapeutically useful doses.

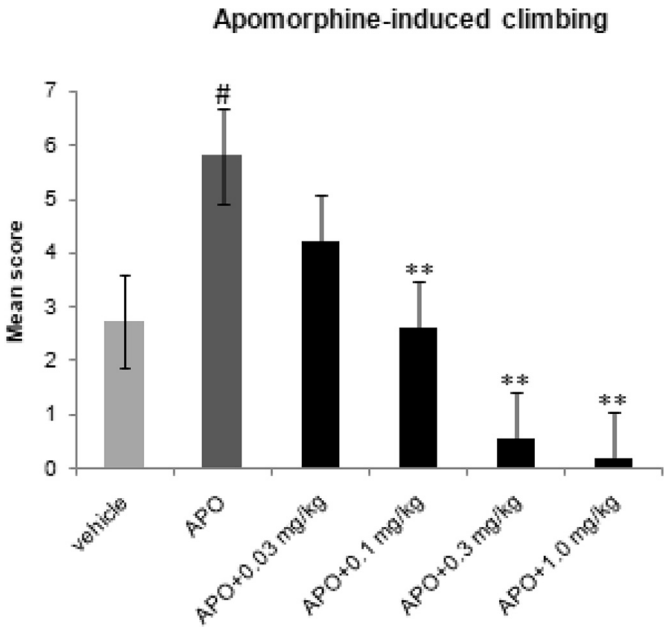


Fig. 4. Effect of compound 13 administered po on APO (apomorphine)-induced climbing in mice (1.0 mg/kg (sc) of the apomorphine,10/group). Results are expressed as means SEM of score. statistical significances of drug effects were analyzed by the nonparametric two-tailed Mann–Whitney *U* test: $^{\#}p < 0.05$ versus vehicle treatment; $^{**}p < 0.01$, $^{*}p < 0.05$ versus apomorphine treatment.

Table 7
In *vivo* pharmacological profile of compound 13. Inhibition of different behavioral responses after oral administration of the test and reference Compounds.

Compound	ED_{50}(mg/kg, po)				CAT/APO	CAT/MK-801	CAT/DOI
	APO[a]	MK-801[b]	DOI[c]	CAT[d]			
13	0.06	0.08	0.07	8.35	139.2	104.3	119.3
risperidone	0.02	0.04	0.04	1.62	81	40.5	40.5
aripiprazole	0.25	0.32	0.58	11.23	44.9	35.1	19.4
haloperidol	0.09	0.11	–	0.32	3.5	2.9	–

[a] **APO:**Apomorphine-induced climbing.
[b] MK-801-induced Hyperactivity.
[c] DOI: DOI-induced head twitch (mg/kg, po).
[d] Catalepsy.

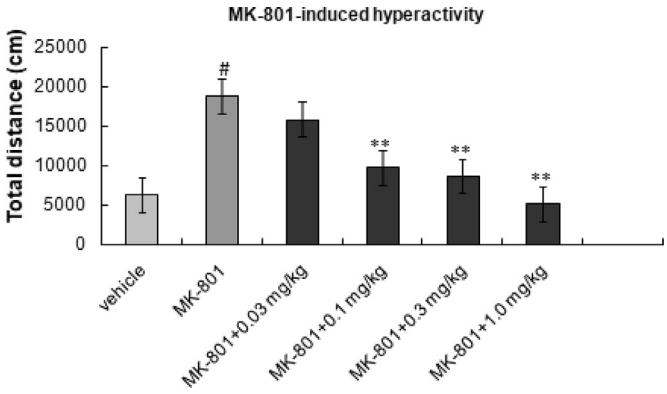


Fig. 5. Effect of compound 13 administered po on MK-801-induced hyperactivity in mice (0.3 mg/kg (sc) of MK-801, 10/group). Results are expressed as means SEM of distance traveled. Statistical evaluation was performed by two-way ANOVA followed by Tukey test for multiple comparisons. #p < 0.05 versus vehicle treatment; **p < 0.01, *p < 0.05 versus MK-801 treatment.

2.11. Forced swim test and tail suspension test in mice

The antidepressant-like activity of the test compound was screened using the forced swim test (FST)[41] and the tail suspension test (TST)[42]. In the FST and TST models, the 5-HT_7 receptor antagonist SB-269970 did not affect locomotor activity but reduced immobility time in mice[43]. Next, the FST and TST were performed in 5-HT_7 receptor gene knockout mice, resulting in significantly reduced immobilization time compared with wild-type mice, indicating that the 5-HT_7 receptor gene knockout resulted in an antidepressant effect[44]. Several studies have shown that 5-HT_7 antagonists result in excellent antidepressant-like effects during the FST[45],[46]. The antidepressant effects of compound **13** were evaluated using the TST and FST in a mouse model of acute depression with duloxetine as the positive control. As shown in Figs. 7 and 8, oral administration of duloxetine (20 mg/kg) decreased the duration of immobility. Compared to the control group, compound **13** caused a dose-dependent reduction in immobility time, with a lower MED (Minimum Effect Dose) of 0.06 mg/kg in both tests.

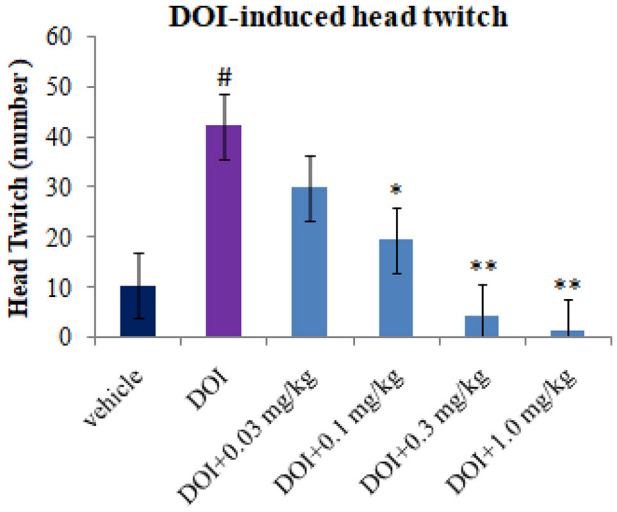


Fig. 6. Effect of compound **13** administered po on DOI (2,5-dimethoxy-4-iodoamphetamine)-induced head twitch in mice (1.0 mg/kg (ip) of DOI, 10/group). Results are expressed as means SEM of head twitch. Statistical evaluation was performed by two-way ANOVA followed by Tukey test for multiple comparisons. #p < 0.05 versus vehicle treatment; **p < 0.01, *p < 0.05 versus DOI treatment.

2.12. Evaluations of learning, memory, and cognitive function

The present *in vitro* results indicated that compound **13** exerted potential effects on 5-HT_6 (K_i = 9.6 nM) and 5-HT_7 (K_i = 4.9 nM), which are both associated with cognitive ability[36],[38]. The object recognition test, also known as the novel object recognition test (NOR), is a relatively fast and efficient means for testing different phases of learning and memory in mice[47], and can be used to predict the potential of a novel drug for cognitive enhancement[48].

In this model, compound **13** was orally administered 1 h prior to the acquisition trial, and the exploration times for the two identical objects were recorded (Fig. 9A). After a 24 h acquisition trial, one of the familiar objects was replaced with a novel object, the time spent investigating each of the objects was recorded (Fig. 9B), and the novelty discrimination index (NDI) was calculated as the percentage of novel object interaction time relative to total interaction time during the retention trial (Fig. 9C). Oral administration of compound **13** (0.02, 0.06, and 0.2 mg/kg) had no obvious influence on total exploration time (Fig. 9A), similar to oral administration of risperidone and rivastigmine (0.2 mg/kg). As a positive control, rivastigmine (0.2 mg/kg) exerted obvious influence on total exploration time (Fig. 9B). Furthermore, rats treated with 0.06 and 0.2 mg/kg of compound **13** during the retention trial (Fig. 9B) explored the novel object for a longer time period, indicating preserved memory for the familiar object presented during the acquisition trial. By contrast, rats treated with vehicle or dosed with 0.02 mg/kg of compound **13** did not exhibit differences in exploration time for the familiar and novel objects, indicating deterioration or loss of memory for the familiar object. Moreover, a 0.2-mg/kg oral dose of compound **13** and rivastigmine significantly increased the NDI (Fig. 9C). These results suggest that compound **47** enhanced recognition memory during the NOR task in rats. Taken together, these results demonstrate that compound **13** potentially plays a role in the promotion of recognition memory in this rat model, which suggests that this novel compound may improve cognitive function.

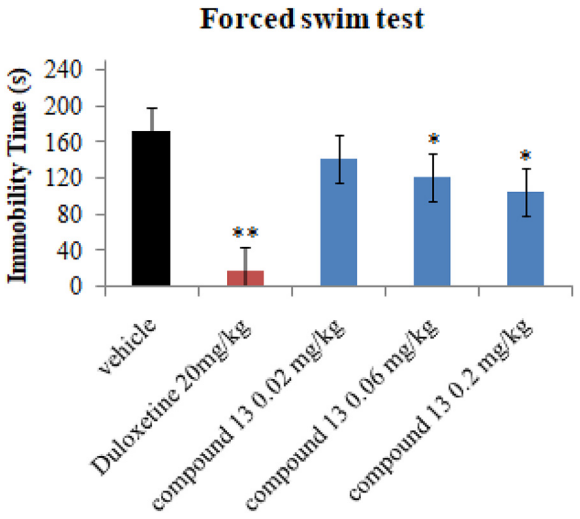


Fig. 7. Effect of treatment of mice with compound 13 given po at graded doses on the immobility time in the forced swim test. Results are represented as mean ± SEM. One-way ANOVA followed by Dunnett's test: **p < 0.01, *p < 0.05 compared with the vehicle control group (n = 10).

2.13. Locomotor activity test

The present study included a locomotor activity test to identify false positive effects and to check for the possible occurrence of drug-induced changes in locomotor activity that may have contributed to altered behavior in the models. Previous studies have shown that clonazepam can cause sedation[49], and thus, this drug was used as a reference compound. Locomotor activity analyses indicated that compound **13** does not significantly alter total travel distance at doses of 0.06, 0.2, and 0.6 mg/kg, which likely excludes false positive antipsychotic and antidepressant effects (Table 8).

2.14. Weight gain and serum prolactin

SGAs have many side effects, including weight gain and hyperprolactinemia[6]. As shown in Fig. 10, compound **13** resulted in

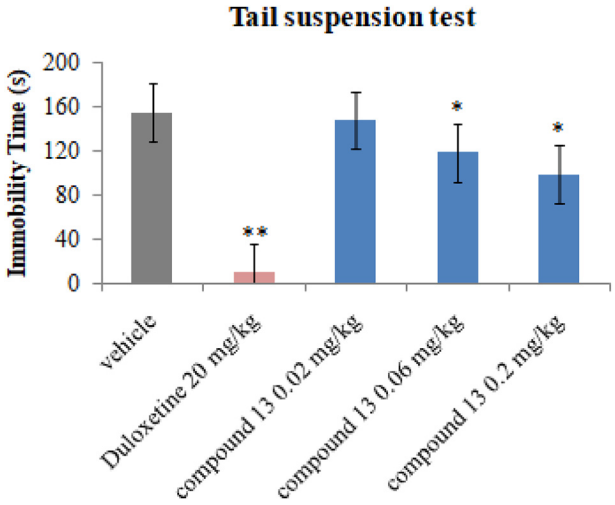


Fig. 8. Effect of treatment of mice with compound 13 given po at graded doses on the immobility time in the Tail suspension test. Results are represented as mean ± SEM. One-way ANOVA followed by Dunnett's test: **p < 0.01, *p < 0.05 compared with the vehicle control group (n = 10).

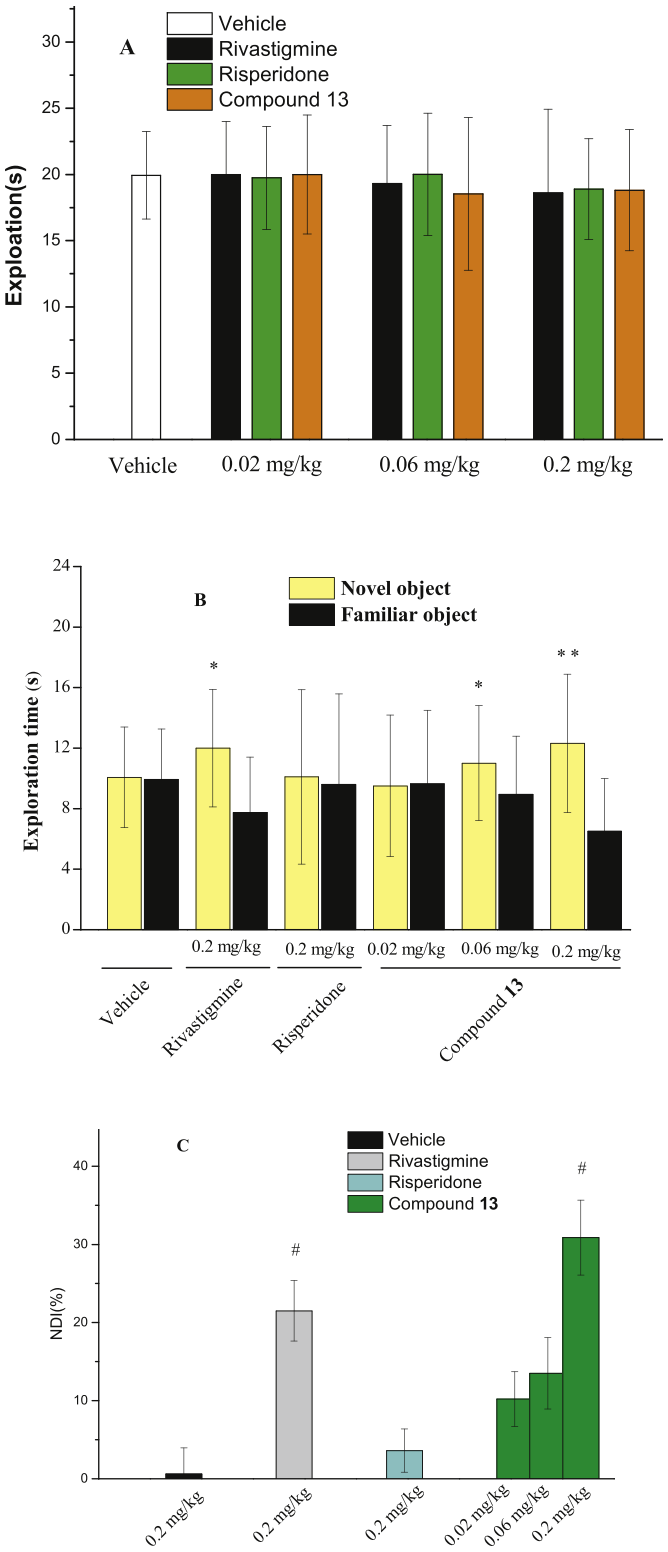


Fig. 9. Effects of compound **13** on NOR test in rats (10/group). Experimental results acquired 60 min after rats oral administration of vehicle and **13** (0.02, 0.0.6, and 0.2 mg/kg), rivastigmine (0.2 mg/kg) and risperidone (0.2 mg/kg). (A)The time spent in exploring two identical objects during acquisition trials; (B) The time spent in exploring a familiar and novel object during acquisition trials. (C) Novelty discrimination index (NDI) are shown as the mean ± SEM (n = 10). **, p < 0.01 , *, p < 0.05 vs familiar object by paired *t*-test; #, p < 0.05 vs vehicle by one-tailed Williams' test.

Table 8
Effects of the compounds 13, haloperidol and clonazepam on the spontaneous locomotor activity in mice (mean ± SD, n=8).

Compound	Dose (mg/kg)	Total distance (mm)
control	–	13579 ± 8864
clonazepam	1	12188 ± 5801
	3	5784 ± 2302*
	10	762 ± 253**
13	0.06	12697 ± 6818
	0.2	10829 ± 5512
	0.6	8876 ± 2095

**p < 0.01, *p < 0.05 *vs* control.

negligible weight gain in mice receiving chronic dosing for 28 days. Risperidone, used as a positive control, resulted in significantly increased weight gain in mice that received medium and high doses (Fig. 10). This was also consistent with the estimated K_i values for the H_1 (risperidone, 32.6 nM; compound **13**, 1196 nM) and 5-HT_{2C} (risperidone, 20.9 nM; compound **13**, 1687 nM) receptors. Moreover, compound **13** did not significantly influence serum prolactin levels compared with risperidone (Fig. 11).

2.15. Selectivity profile of compound 13

The interactions between compound **13** and other receptors related to CNS disorders were evaluated and a selectivity profile was created using additional receptors, including the D_1, D_3, α_2, H_3, SERT, NET, DAT, sigma-1, and sigma-2 receptors. Compound **13** showed moderate affinity for the D_3 receptor (K_i = 226 nM), with no significant affinity (K_i > 1000 nM) for any other putative target.

2.16. Pharmacokinetic studies of compound 13

The pharmacokinetic properties of compound **13** including its *in vitro* and *in vivo* efficacy and its safety profile were explored in rats (Table 9). Intravenous administration of compound **13** in rats (0.5 mg/kg, n = 6) resulted in detectable plasma levels [half-life ($t_{1/2}$) = 0.94 h]. Oral administration of compound **13** in rats (5 mg/kg, n = 6) resulted in a $t_{1/2}$ of 3.25 h. The area under the curve value of compound **13** was 2350 ng × h/mL after intravenous administration and 5194 ng × h/mL after oral administration. The C_{max} value after oral dosing was 1686 ng/mL, and the T_{max} value was 0.54 h. The bioavailability of compound **13** was 22.1%. These encouraging preclinical data suggest that compound **13** possesses desirable drug-like pharmacokinetic properties that may be applicable to humans.

3. Conclusion

In summary, we here described the synthesis and pharmacological evaluation of a series of Isoquinolinone derivatives as potential multi-target antipsychotics. Among the derivatives synthesized, compound **13** showed high affinity for dopamine D_2 and serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors, and a satisfactory selectivity profile for non-target receptors (5-HT_{2C}, H_1, and α_1) that have been closely linked to the negative side effects of other marketed antipsychotics. *In vivo* animal models showed that compound **13** reversed APO-induced hyperlocomotion, MK-801-induced hyperactivity, and DOI-induced head twitch. Moreover, compound **13** exhibited low levels of inhibition at the hERG channel, which is associated with no QT prolongation in a guinea pig model, a high threshold for acute toxicity, the lack of a tendency to induce catalepsy, and no effect on prolactin secretion or weight gain. Furthermore, the results of the FST, TST, and NOR tests demonstrated that compound **13** showed improvements in depression and cognitive impairment. Finally, pharmacokinetic studies demonstrated that compound **13** had a favorable drug-like pharmacokinetic profile. We anticipate that compound **13** might be useful for developing a novel class of drugs for the treatment of schizophrenia.

4. Experimental section

Chemistry. All commercially available chemicals and reagents were used without further purification. Reagents were all of analytical grade or of chemical purity (>95%). Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra was recorded on a Bruker Avance III 600 spectrometer at 400 MHz (^{1}H) using $CDCl_3$ as solvent. Chemical shifts were given in d 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). Analytical thin layer chromatography (TLC) was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. Compound purity is determined by high performance liquid chromatography (HPLC), and all final test compounds display purity higher than 95%. HPLC methods used the following: Shimadzu LC-20AD spectrometer; column, SHIMADZU VP-ODS (4.6 mm × 250 mm, 5 μm) C18-253; mobile phase A: 0.01 mol/L KH_2PO_4 (0.2%Et_3N, pH = 3.5) aq./CH_3OH = 90/10; mobile phase B: 0.01 mol/L KH_2PO_4 (0.2%Et_3N, pH = 3.5) aq./CH_3OH = 20/80; flow rate, 1.0 mL/min; column temperature, 35 °C. UV detection was performed at 210 nm.

4.1. Ethyl 4-methoxyphenethylcarbamate [2]

To a solution of ethyl chloroformate (300 mmol) in 200 mL of dry dichloromethane a solution of 1 (250mol) and triethylamine (375 mmol) in 600 mL of dichloromethane was added dropwise under ice-cooling. A white precipitate (the hydrochloride of the amine) formed immediately. After vigorous stirring for 1 h at r.t.,

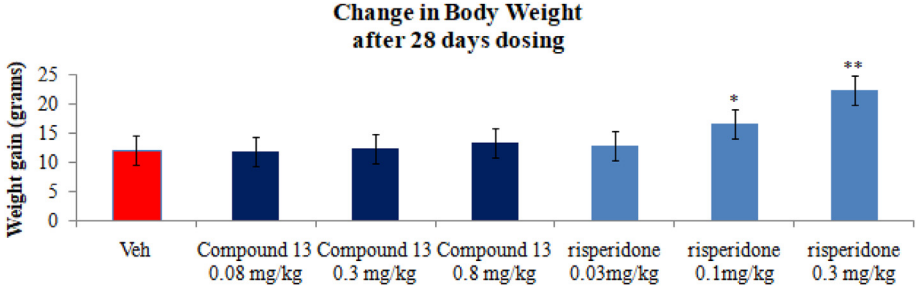


Fig. 10. Influence of compound 13 and risperidone on weight gain. Each value is the mean ± SEM of 10 mice per group. Student's *t*-test: **p < 0.01, *p < 0.05 versus vehicle group.

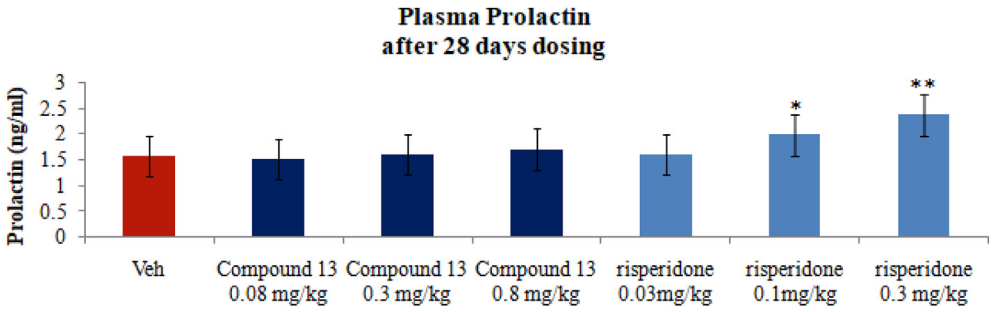


Fig. 11. Serum prolactin (PRL) multiple (28 days) administration of compound 13 and risperidone in mice. Each value is the mean ± SEM of 10 mice per group. Student's *t*-test: **p < 0.01, *p < 0.05 versus vehicle group.

the reaction mixture was quenched with water (100 mL), washed with 2 N HCl solution, brine and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The resulting oil solidified on standing. The crude mixture was purified with column chromatography (petroleum ether/EtOAc = 1: 20) to afford compound 2, as a pale-yellow oil (yield, 91.8%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 1H), 6.97 - 6.80 (m, 1H), 4.13 (q, *J* = 7.1 Hz, 1H), 3.81 (s, 1H), 3.41 (t, *J* = 6.7 Hz, 1H), 2.78 (t, *J* = 7.1 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 158.24, 156.67, 130.88, 129.76, 129.73, 114.02, 60.68, 55.25, 42.34, 35.27, 14.69. MS (ESI) *m/z*: 224.1 (calcd 224.2 for $C_{12}H_{17}NO_3^+$ $[M + H]^+$).

4.2. 7-Methoxy-3,4-dihydroisoquinolin-1(2H)-one [3]

To a solution of P_2O_5(300 mmol) in 70 mL of methanesulfonic acid, 2 (200 mmol) were added. The solution was heated for 2 h under 130 °C, excess methanesulfonic acid was removed in vacuo, and the resulting residue was quenched with ice-water. The solution was neutralised with $NaHCO_3$and extracted three times with dichloromethane. The combined organic phases were washed twice with water, dried with Na_2SO_4.The organic phase was evaporated under reduced pressure to give crude oil. The crude mixture was purified with column chromatography (petroleum ether/EtOAc = 1: 1) to compound 3, as a white yellow solid (yield, 52.5%), mp:117.5–118.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.58 (d, *J* = 2.8 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.83 (s, 4H), 3.54 (td, *J* = 6.6, 2.8 Hz, 2H), 2.90 (t, *J* = 6.6 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 166.78, 158.69, 131.14, 129.95, 128.40, 119.52, 111.07, 55.52, 40.29, 27.41. MS (ESI) *m/z*: 178.09 (calcd 178.1 for $C_{11}H_{13}NO_2$+$[M + H]^+$)

4.3. 7-Methoxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [4]

To a solution of sodium hydride (97.5 mmol) in 50 mL of dry N,N-dimethylformamide, a solution of compound 3 (65.0 mmol) in 30 mL of N,N-dimethylformamide and iodomethane (71.5 mmol) was added dropwise under ice-cooling. The reaction mixture was stirred at room temperature for 1.5 h. The reaction was quenched with 480 ml water, extracted with ethyl acetate (100 mL × 3), washed with brine, and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 10: 1) to afford compound 4, as a yellow oil (yield, 90.1%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 -7.57 (m, 1H), 7.10 - 6.78 (m, 2H), 3.80 (s, 3H), 3.52 - 3.47 (m, 2H), 3.11 (s, 3H), 2.91 - 2.87 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 164.66, 158.62, 130.15, 128.03, 118.79, 111.39, 55.44, 48.34, 35.17, 26.97, 21.00. MS (ESI) *m/z*: 192.0 (calcd 192.1 for $C_{10}H_{12}NO_2^+$ $[M + H]^+$).

4.4. Synthesis of 7-hydroxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [5]

To a stirred solution of compound 4 (41.9 mmol) was added hydrobromic acid in water solution (48%) (32.0 mL) and reaction mixture was refluxed at 100 °C overnight. The reaction mixture was quenched with water (64.0 mL)and then extracted with DCM (5 × 50 mL), The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to colorless solid. The solid was further purified with column chromatography (DCM/MeOH = 30:1) to afford compound 5, as a white solid. (yield, 83.8%), mp: 188–189 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.08 (d, *J* = 2.6 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.6 Hz, 1H), 3.58 (dd, *J* = 8.7, 4.8 Hz, 2H), 3.19 (s, 3H), 2.94 (t, *J* = 6.7 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ165.44, 156.22, 129.54, 129.02, 128.11, 119.52, 115.22,48.67, 35.39, 26.87. MS (ESI) *m/z*: 177.9 (calcd 178.1 for $C_{10}H_{11}NO_2^+$ $[M + H]^+$).

4.5. 7-(3-Bromopropoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [6]

The mixture of compound 5 (28.2 mmol),1,3-dibromopropane(56.5 mmol), K_2CO_3 (84.6 mmol), TEBA (1.4 mmol) and acetone (50.0 mL), was heated under reflux for 6 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 15: 1) to yield compound 6, as a yellow oil (yield, 87.3%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 - 7.60 (m, 1H), 7.16 - 7.04 (m, 1H), 6.99 - 6.95 (m, 1H), 4.18 - 4.12 (m, 2H), 3.63 - 3.51 (m, 4H), 3.26 - 3.09 (m, 2H), 3.02 - 2.84 (m, 2H), 2.35 - 2.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.63, 164.60, 157.75, 157.73, 130.44, 130.34, 130.33, 128.12, 119.21, 119.19, 119.15, 112.31, 65.54, 65.53, 48.39, 48.37,

Table 9
Pharmacokinetic profile of compound 13 in rats (n = 6/group).

Dose	C_{max} (ng/mL)	$T_{1/2}$ (h)	T_{max} (h)	CLz (mL/min/kg)	Vz (L/kg)	AUC(0-t) (ng × h/mL)	AUC(0-∞) (ng × h/mL)	F (%)
0.5 mg (i.v)	2566	0.94	0.08	14.7	1.16	2350	2370	–
5 mg (po)	1686	3.25	0.54	7.68	30.71	5194	5288	22.1

35.27, 35.25, 32.31, 32.29, 30.03, 27.06, 27.04. MS (ESI) *m/z*: 178.2 (calcd 178.1 for $C_{10}H_{12}NO_2$+[M + H]$^+$).

4.6. General procedures for the preparation of compounds 7-31

The mixture of compound 6 (16.8 mmol), arylpiperazine (piperidine) (18.5 mmol), K_2CO_3 (50.4 mmol) and acetonitrile (50.0 mL), was heated under reflux for 8 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give yellow solid, The residue was purified with column chromatography (DCM: MeOH = 20 : 1) to afford target compounds.

7-(4-(4-(6-Fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl) butoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [7] as a white solid (yield, 86.0%), mp: 130–132 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 - 7.68 (m, 1H), 7.66 - 7.56 (m, 1H), 7.27 - 7.16 (m, 1H), 7.11 - 7.00 (m, 2H), 7.00 - 6.92 (m, 1H), 4.10 - 3.99 (m, 2H), 3.58 - 3.48 (m, 2H), 3.20 - 3.01 (m, 6H), 2.98 - 2.86 (m, 2H), 2.56 - 2.39 (m, 2H), 2.23 - 2.00 (m, 6H), 1.92 - 1.79 (m, 2H), 1.73 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.31, 164.76, 163.85, 162.82, 161.10, 158.11, 130.17, 128.03 (s, 8H), 122.75, 119.34, 117.28, 112.45, 112.17, 97.51, 97.25, 67.93, 58.46, 53.49, 48.41, 35.25, 34.57, 30.44, 27.27, 27.05, 23.44. HRMS (ESI) *m/z*: 452.2339 (calcd 452.2349 for $C_{26}H_{31}FN_3O_3^+$ [M + H]$^+$).

7-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [8] as a white solid (yield, 82.5%), mp: 155–157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.8 Hz, 1H), 7.18 - 7.10 (m, 2H), 7.08 (s, 0.4H), 7.06 (s, 0.6H), 6.99 - 6.93 (m, 2H), 4.04 (t, *J* = 6.0 Hz, 2H), 3.53 (t, *J* = 6.8 Hz, 2H), 3.15 (s, 3H), 3.08 (s, 4H), 2.92 (t, *J* = 6.8 Hz, 2H), 2.66 (s, 4H), 2.49 (t, *J* = 7.2 Hz, 2H), 1.91 - 1.78 (m, 2H), 1.78 - 1.66 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.30, 163.95, 163.81, 162.81, 162.26, 161.11, 158.19, 131.12, 130.09, 127.46, 127.27, 122.91, 122.82, 122.71, 117.26, 112.41, 112.16, 108.22, 105.83, 97.50, 97.24, 66.70, 55.38, 53.60, 37.10, 34.67, 30.52, 26.79. HRMS (ESI) *m/z*: 462.1707 (calcd 462.1715 for $C_{24}H_{30}Cl_2N_3O_2^+$ [M + H]$^+$).

2-Methyl-7-(4-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl) butoxy)-3,4-dihydroisoquinolin-1(2H)-one [9] as a white solid (yield, 72.2%), mp: 100–101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 2.8 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.11 (s, 1H), 7.09 - 7.03 (m, 3H), 6.97 (d, *J* = 2.8 Hz, 0.6H), 6.95 (d, *J* = 2.8 Hz, 0.4H), 4.05 (t, *J* = 6.4 Hz, 2H), 3.58 - 3.48 (m, 2H), 3.27 - 3.20 (m, 4H), 3.14 (s,3H), 2.92 (t, *J* = 6.8 Hz, 2H), 2.66 - 2.56 (m, 4H), 2.51 - 2.40 (m, 2H), 1.89 - 1.78 (m, 2H), 1.76 - 1.65 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.78, 158.13, 151.42, 131.84, 131.52, 131.21, 130.89, 130.29, 130.09, 129.54, 128.44, 128.03, 125.73, 123.02, 120.31, 119.41, 118.62, 115.70, 115.66, 112.11, 112.05, 112.01, 67.92, 58.15, 53.03, 48.64, 48.43, 35.27, 27.22, 27.08, 23.40. HRMS (ESI) *m/z*: 462.2360 (calcd 462.2368 for $C_{25}H_{31}F_3N_3O_2^+$ [M + H]$^+$).

7-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [10] as a white solid (yield, 65.8%), mp: 98–100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.8 Hz, 1H), 7.06 (s, 0.4H), 7.04 (s, 0.6H), 7.01 - 6.88 (m, 4H), 6.86 (s, 0.6H), 6.84 (s, 0.4H), 4.04 (t, *J* = 6.4 Hz, 2H), 3.85 (s, 3H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.22–2.97 (m, 7H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.67 (s, 4H), 2.52 - 2.41 (m, 2H), 1.90 - 1.77 (m, 2H), 1.76 - 1.65 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.76, 158.13, 152.25, 141.36, 130.24, 130.04, 128.02, 122.86, 120.97, 119.37, 118.19, 112.11, 111.13, 67.97, 58.33, 55.36, 53.47, 50.65, 48.40, 35.26, 27.30, 27.05, 23.45. HRMS (ESI) *m/z*: 424.2593 (calcd 424.2600 for $C_{25}H_{34}N_3O_3^+$ [M + H]$^+$).

7-(4-(4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl)butoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [11] as a white solid (yield, 64.9%), mp: 114–116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 - 7.87 (m, 1H), 7.84 - 7.74 (m, 1H), 7.66 - 7.55 (m, 1H), 7.49 - 7.40 (m, 1H), 7.39 - 7.29 (m, 1H), 7.10 - 7.01 (m, 1H), 7.01 - 6.88 (m, 1H), 4.13 - 3.98 (m, 2H), 3.64 - 3.45 (m, 6H), 3.21 - 3.08 (m, 3H), 2.98 - 2.84 (m, 2H), 2.78 - 2.62 (m, 4H), 2.54 - 2.41 (m, 2H), 1.90 - 1.78 (m, 2H), 1.78 - 1.59 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.78, 163.97, 158.12, 152.71, 130.27, 130.07, 128.04, 127.54, 123.97, 123.90, 120.56, 119.37, 112.15, 67.94, 58.31, 53.06, 50.10, 48.43, 35.28, 27.23, 27.07, 23.40. HRMS (ESI) *m/z*: 451.2159 (calcd 451.2168 for $C_{25}H_{31}N_4O_2S^+$ [M + H]$^+$).

7-(4-(4-(Benzo[*b*]thiophen-4-yl)piperazin-1-yl)butoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [12] as a white solid (yield, 69.6%), mp: 118–120 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 2.8 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.39 (dd, *J* = 10.8, 5.2 Hz, 2H), 7.31 - 7.21 (m 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.96 (dd, *J* = 6.8, 1.6 Hz, 1H), 6.88 (d, *J* = 7.2 Hz, 1H), 4.05 (t, *J* = 6 Hz, 2H), 3.50 (t, *J* = 6.8 Hz, 2H), 3.25 - 3.09 (m, 7H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.70 (s, 4H), 2.54 - 2.46 (m, 2H), 1.90 - 1.78 (m, 2H), 1.78 - 1.67 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.79, 158.16, 148.54, 141.10, 134.08, 130.28, 130.09, 128.05, 125.06, 124.94, 121.99, 119.41, 116.96, 112.19, 112.15, 67.98, 58.31, 53.60, 53.53, 52.15, 48.43, 35.29, 27.30, 27.07, 23.48. HRMS (ESI) *m/z*: 450.2205 (calcd 450.2214 for $C_{26}H_{32}N_3O_2S$+[M + H]$^+$).

7-(3-(4-(6-Fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2*H*)-one [13] as a white solid (yield, 86.0%), mp: 150–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.61 (d, *J* = 2.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.10-7.00 (m, 2H), 6.96 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 2H), 3.52 (t, *J* = 6.6 Hz, 2H), 3.14 (s, 3H), 3.06 (d, *J* = 10.0 Hz, 3H), 2.91 (t, *J* = 6.6 Hz, 2H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.25 -1.88 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.27, 164.71, 163.90, 163.76, 162.78, 161.13, 158.12, 130.26, 130.08, 127.97, 122.80, 122.69, 119.31, 117.28, 117.27, 112.38, 112.24, 112.13, 97.46, 97.19, 66.58, 55.34, 53.60, 48.39, 35.21, 34.68, 30.57, 27.04, 26.86. HRMS (ESI) *m/z*: 438.2183 (calcd 438.2192 for $C_{25}H_{29}FN_3O_3$+[M + H]$^+$).

7-(3-(4-(2,4-difluorobenzoyl)piperidin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [14] as a white solid (yield, 76.2%), mp: 141–142 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 - 7.82 (m, 1H), 7.59 (d, *J* = 2.8 Hz, 1H), 7.08 (d, *J* = 8.3 Hz, 1H), 7.00 - 6.95 (m, 2H), 6.90 - 6.85 (m, 1H), 4.07 (t, *J* = 6.3 Hz, 2H), 3.54 (t, *J* = 6.7 Hz, 2H), 3.15 (s, 3H), 3.04 - 3.01 (m, 2H), 2.95 - 2.92 (m, 2H), 2.66 - 2.55 (m, 2H), 2.25 - 2.18 (m, 2H), 2.05 -1.05 (m, 4H), 1.85 - 1.76 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.65, 199.60, 164.72, 158.00, 133.00, 132.96, 132.90, 132.85, 130.24, 130.15, 128.03, 119.15, 112.48, 112.45, 112.35, 112.27, 112.24, 104.92, 104.67, 104.65, 104.39, 66.46, 55.28, 53.10, 48.40, 35.26, 27.79, 27.04, 26.58. HRMS (ESI) *m/z*: 438.2183 (calcd 438.2191 for $C_{25}H_{29}FN_3O_3$+[M + H]$^+$).

7-(3-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)propoxy)-2-Methyl-3,4-dihydroisoquinolin-1(2H)-one [15] as a white solid (yield, 76.2%), mp: 103–105 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.8 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.10 (s, 1H), 7.08 - 7.02 (m, 3H), 6.96 (d, *J* = 2.8 Hz, 0.6H), 6.93 (d, *J* = 2.8 Hz, 0.4H), 4.04 (t, *J* = 6.4 Hz, 2H), 3.55 - 3.47 (m, 2H), 3.28 - 3.21 (m, 4H), 3.16 (s,3H), 2.93 (t, *J* = 6.8 Hz, 2H), 2.67 - 2.58 (m, 4H), 2.52 - 2.42 (m, 2H), 1.91 - 1.86 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.72, 158.11, 151.41, 131.81, 131.51, 131.22, 130.87, 130.26, 130.06, 129.51, 128.41, 128.01, 125.70, 123.01, 120.30, 119.31, 118.51, 115.73, 115.61, 112.12, 112.03, 112.04, 67.91, 58.12, 53.01, 48.61, 48.39, 27.21, 27.19, 23.44. HRMS (ESI) *m/z*: 448.2204 (calcd 448.2212 for $C_{24}H_{29}F_3N_3O_2^+$ [M + H]$^+$).

7-(3-(4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [16] as a white solid (yield, 73.7%), mp: 122–124 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.09 (d, *J* = 8.3 Hz,2H), 6.99 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.11 (t, *J* = 6.2 Hz, 2H), 3.75-3.39 (m, 6H), 3.16 (s, 3H), 2.94 (t, *J* = 6.6 Hz, 2H), 2.81-2.43 (m, 6H), 2.15-1.90 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.74, 163.91, 158.08, 152.71,

130.19, 128.02, 127.51, 123.90, 120.55, 119.29, 112.26, 66.47, 55.23, 53.06, 50.04, 48.41, 35.26, 27.06, 26.69. HRMS (ESI) *m/z*: 437.2003 (calcd 437.2011 for $C_{24}H_{29}N_4O_2S^+$ $[M + H]^+$).

7-(3-(4-(Benzo[*b*]thiophen-4-yl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [17] as a white solid (yield, 68.5%), mp: 131–132 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d,*J* = 2.7 Hz, 1H), 7.60 - 7.58 (m, 1H), 7.47-7.41 (m, 2H), 7.34-7.29 (m, 1H), 7.13 - 7.10 (m, 1H), 7.04 - 7.01 (m, 1H), 6.96 - 6.9 (m, 1H), 4.17 - 4.13 (m, 2H), 3.99 - 3.53 (m, 2H), 3.27-3.18 (m, 7H), 2.99-2.94 (m, 2H), 2.79-2.67 (m, 6H), 2.13-2.06 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.76, 158.11, 148.43, 141.11, 134.09, 130.32, 130.18, 121.94, 119.35, 117.04, 112.27, 66.52, 55.23, 53.60, 52.01, 48.43, 35.29, 27.09, 26.70. HRMS (ESI) *m/z*: 436.2051 (calcd 436.2059 for $C_{25}H_{30}N_3O_2S$+$[M + H]^+$).

7-(3-(4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [18] as a white solid (yield, 77.2%), mp: 125.5–126.5 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.4 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 6.97 (dd, *J* = 5.6, 2.8 Hz, 1H), 6.91 (d, *J* = 1.6 Hz, 1H), 6.84 - 6.75 (m, 2H), 5.97 (s, 2H), 4.08 (t, *J* = 6.0 Hz, 2H), 3.61 - 3.46 (m, 4H), 3.17 (s, 3H), 2.95 (t, *J* = 6.8 Hz, 2H), 2.84 - 2.65 (m, 9H), 2.15 - 2.05 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 164.71, 157.86, 147.75, 147.05, 130.32, 130.26, 130.18, 128.10, 122.84, 119.23, 112.31, 109.77, 108.04, 101.04, 66.08, 62.15, 54.91, 53.53, 52.47, 51.69, 48.41, 35.30, 27.05, 25.97. HRMS (ESI) *m/z*: 438.2386 (calcd 438.2393 for $C_{25}H_{32}N_3O_4$+$[M + H]^+$).

7-(3-(4-(Quinolin-2-yl)piperazin-1-yl)propoxy)- 2-methyl-3,4-dihydroisoquinolin- 1(2H)-one [19] as a white solid (yield, 73.7%), mp: 121–123 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.65 -7.56 (m, 2H), 7.56 - 7.48 (m, 1H), 4.10 (t, *J* = 6.4 Hz, 2H), 3.83 - 3.69 (m, 4H), 3.53 (t, *J* = 6.8 Hz, 2H), 3.15 (s, 3H), 2.93 (t, *J* = 6.8 Hz, 2H), 2.71 - 2.41 (m, 6H), 2.06 - 2.00 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.79, 158.11, 157.43, 147.90, 137.45, 130.30, 130.15, 129.52, 128.05, 127.23, 126.64, 123.09, 122.36, 119.38, 112.24, 109.57, 66.51, 55.25, 53.23, 48.44, 45.10, 35.30, 27.08, 26.72.

HRMS (ESI) *m/z*: 431.2439 (calcd 431.2447 for $C_{26}H_{31}N_4O_2$+$[M + H]^+$).

7-(3-(4-Phenylpiperazin-1-yl)propoxy)- 2-methyl-3,4-dihydroisoquinolin- 1(2H)-one [20] as a white solid (yield, 85.3%), mp: 98.5–99.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 2.7 Hz, 1H), 7.37 - 7.26 (m, 2H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.05 - 6.95 (m, 3H), 6.90 (tt, *J* = 1.1, 7.3 Hz, 1H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.60 - 3.57 (m, 2H), 3.35 - 3.23 (m, 4H), 3.20 (s, 3H), 2.98 (t, *J* = 6.7 Hz, 2H), 2.77 - 2.44 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.79, 158.13, 151.36, 130.31, 130.13, 129.12, 128.02, 119.66, 119.39, 116.07, 112.24, 66.55, 55.20, 53.32, 49.15, 48.45, 35.30, 27.10, 26.76. HRMS (ESI) *m/z*: 430.2327 (calcd 380.2336 for $C_{23}H_{30}N_3O_2$+$[M + H]^+$)

7-(3-(4-(Pyridin-2-yl)piperazin-1-yl)propoxy)- 2-methyl-3,4-dihydroisoquinolin- 1(2H)-one [21] as a white solid (yield, 58.9%), mp: 85–87 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.64 (d, *J* = 2.5 Hz, 1H), 7.49 (dd, *J* = 9.2, 4.2 Hz, 1H), 7.10 (dd, *J* = 8.0, 2.7 Hz, 1H), 7.04 - 6.90 (m, 1H), 6.80 - 6.50 (m, 2H), 4.33 - 3.96 (m, 2H), 3.59 - 3.44 (m, 6H), 3.31 - 3.10 (m, 3H), 2.95 (d, *J* = 3.4 Hz, 2H), 2.62 (s, 6H), 2.05 (d, *J* = 6.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.73, 159.50, 158.07, 147.93, 137.47, 130.28, 130.14, 128.03, 119.32, 113.29, 112.23, 107.09, 66.46, 55.22, 53.06, 48.41, 45.14, 35.27, 27.06, 26.65. HRMS (ESI) *m/z*: 381.2282 (calcd 381.2291 for $C_{22}H_{29}N_4O_2$+$[M + H]^+$)

7-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [22] as a white solid (yield, 63.8%), mp: 105–107 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 - 8.13 (m, 2H), 7.64 - 7.63 (m, 1H), 7.11 - 7.08 (m, 1H), 7.01 - 6.97 (m, 1H), 6.53 - 6.50 (m, 1H), 4.14 - 4.10 (m, 2H), 3.98 - 3.81 (m, 4H), 3.64 - 3.45 (m, 2H), 3.25 - 3.07 (m, 3H), 2.98 - 2.93 (m, 2H), 2.74 - 2.53 (m, 6H), 2.12 - 2.05 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.73, 161.58, 158.01, 157.73, 130.30, 130.18, 128.04, 119.29, 112.25, 109.97, 66.33, 55.25, 53.04, 48.42, 43.40, 35.28, 27.07, 26.45. HRMS (ESI) *m/z*: 382.2235 (calcd 382.2243 for $C_{22}H_{28}N_5O_2$+$[M + H]^+$).

7-(3-(4-(2-Chlorophenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [23] as a white solid (yield, 71.9%), mp: 118–120 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 2.7 Hz, 1H), 7.40 (dd, *J* = 1.6, 7.9 Hz, 1H), 7.29-7.24 (m, 1H), 7.15 - 7.07 (m, 2H), 7.05 - 6.95 (m, 2H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.60 - 3.56 (m, 2H), 3.20 - 3.17 (s, 7H), 2.98 (t, *J* = 6.7 Hz, 2H), 2.82 - 2.44 (m, 6H), 2.26 - 1.88 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.76, 158.09, 149.21, 130.63, 130.31, 130.16, 128.78, 128.03, 127.63, 123.73, 120.47, 119.39, 112.22, 66.47, 55.19, 53.39, 51.03, 48.44, 35.29, 27.10, 26.60. HRMS (ESI) *m/z*: 414.1939 (calcd 414.1948 for $C_{23}H_{29}ClN_3O_2$+$[M + H]^+$).

7-(3-(4-(4-Chlorophenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [24] as a white solid (yield, 80.8%), mp: 146–147 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 2.7 Hz, 1H), 7.28 - 7.20 (m, 2H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.01 (dd, *J* = 2.7, 8.3 Hz, 1H), 6.93 - 6.83 (m, 2H), 4.13 (t, *J* = 6.3 Hz, 2H), 3.58 (dd, *J* = 6.3, 7.1 Hz, 2H), 3.31 - 3.10 (m, 7H), 2.98 (t, *J* = 6.7 Hz, 2H), 2.74 - 2.52 (m, 6H), 2.09 - 2.02 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.76, 158.11, 149.97, 130.32, 130.14, 128.94, 128.03, 124.42, 119.38, 117.21, 112.21, 66.49, 55.11, 53.14, 49.15, 48.44, 35.29, 27.09, 26.75. HRMS (ESI) *m/z*: 414.1937 (calcd 414.1948 for $C_{23}H_{29}ClN_3O_2$+$[M + H]^+$).

7-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [25] as a white solid (yield, 79.7%), mp: 111–113 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ7.66 (d, *J* = 2.9 Hz, 1H), 7.19 - 7.17 (m, 2H), 7.12 - 7.09 (m, 1H), 7.04 -6.91 (m, 2H), 4.33 - 3.96 (m, 2H), 3.71 - 3.45 (m, 2H), 3.24 - 3.04 (m, 7H), 2.97 (t, *J* = 6.6 Hz, 3H), 2.81 - 2.54 (m, 6H), 2.06 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.76, 158.09, 151.26, 133.99, 130.30, 130.15, 128.04, 127.49, 124.57, 119.40, 118.68, 112.18, 66.46, 55.10, 53.31, 51.23, 48.43, 35.28, 27.08, 26.66. HRMS (ESI) *m/z*: 448.1551 (calcd 448.1559 for $C_{23}H_{28}Cl_2N_3O_2$+$[M + H]^+$).

7-(3-(4-(2-Fluorophenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [26] as a white solid (yield, 80.2%), mp: 119–121 °C. ^{1}H NMR (400 MHz, CDCl3) δ 7.66 (t, *J* = 1.9 Hz, 1H), 7.19 - 6.78 (m, 6H), 4.24 - 4.02 (m, 2H), 3.60 - 3.55 (m, 2H), 3.20 -3.15 (m, 7H), 2.97 (m, 2H), 2.77 - 2.55 (m, 6H), 2.19 - 1.87 (m, 2H). ^{13}C NM (101 MHz, $CDCl_3$) δ 164.76, 158.12, 156.96, 154.51, 140.22, 140.14, 130.30, 130.12, 128.02, 124.49, 124.46, 122.43, 122.35, 119.40, 118.96, 118.93, 116.18, 115.98, 112.20, 66.52, 55.17, 53.34, 50.54, 50.51, 48.43, 35.28, 27.09, 26.72. HRMS (ESI) *m/z*: 398.2233 (calcd 398.2244 for $C_{23}H_{29}FN_3O_2$+$[M + H]^+$).

7-(3-(4-(4-Fluorophenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [27] as a white solid (yield, 79.2%), mp: 114–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 2.3 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 6.96 (t, *J* = 8.4 Hz, 3H), 6.91-6.82 (m, 2H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.53 (t, *J* = 6.7 Hz, 2H), 3.23-3.03 (m, 7H), 2.92 (t, *J* = 6.6 Hz, 2H), 2.78-2.47 (m, 6H), 2.21-1.80 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.72, 158.17, 155.89, 147.99, 130.19, 128.01, 119.31, 117.75, 115.56, 115.34, 112.19, 66.46, 55.07, 53.24, 50.10, 48.39, 35.24, 27.04, 26.70. HRMS (ESI) *m/z*: 398.2235 (calcd 398.2244 for $C_{23}H_{29}FN_3O_2$+$[M + H]^+$).

7-(3-(4-(4-Methoxyphenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [28] as a white solid (yield, 68.5%), mp: 96–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 2.7 Hz, 1H), 7.11 (dd, *J* = 3.4, 8.9 Hz, 1H), 7.04 - 6.75 (m, 6H), 4.14 - 4.10 (m, 2H), 3.89 - 3.71 (m, 3H), 3.59 - 3.54 (m, 2H), 3.26 - 3.11 (m, 7H), 2.98 - 2.93 (m, 2H), 2.78 - 2.55 (m, 6H), 2.16 - 1.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.74, 158.07, 153.82, 145.66, 130.30, 130.16, 128.04, 119.30, 118.25, 114.43, 112.27, 66.48, 55.58, 55.18, 53.36, 50.50, 48.42, 35.28, 27.08, 26.64. HRMS (ESI) *m/z*: 410.2438 (calcd 410.2444 for $C_{24}H_{32}N_3O_3$+$[M + H]^+$).

7-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [29] as a white solid (yield, 71.8%), mp: 99–101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 1H), 7.12 - 7.09 (m, 1H), 7.07 - 6.91 (m, 4H), 6.91- 6.87 (m, 1H), 4.15 - 4.10 (m, 2H), 3.96 - 3.74 (m, 3H), 3.58 - 3.54 (m, 2H), 3.31 - 3.10 (m, 7H), 3.03 - 2.67 (m, 8H), 2.18 - 2.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.71, 157.95, 152.23, 140.85, 130.31, 130.27, 128.08, 123.21, 121.04, 119.21, 118.38, 112.36, 111.19, 66.30, 55.41, 55.26, 53.32, 49.95, 48.41, 35.28, 27.07, 26.18. HRMS (ESI) *m/z*: 410.2436 (calcd 410.2444 for $C_{24}H_{32}N_3O_3$+[M + H]$^+$).

7-(2-(4-(6-Fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl) ethoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [30] as a white solid (yield, 65.3%), mp: 151–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.63 (d, *J* = 2.4 Hz, 1H), 7.26-7.16 (m, 1H), 7.15-6.81 (m, 3H), 4.19 (t, *J* = 5.7 Hz, 2H), 3.53 (t, *J* = 6.7 Hz, 2H), 3.30-3.00 (m, 6H), 2.94-2.86 (m, 4H), 2.36-2.30 (m, 2H), 2.19-1.88 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.27, 164.65, 163.88, 163.75, 162.78, 161.05, 157.84, 130.31, 130.28, 128.04, 122.73, 122.62, 119.38, 117.28, 117.27, 112.41, 112.34, 112.16, 97.49, 97.22, 66.20, 57.36, 53.87, 48.37, 35.23, 34.39, 30.48, 27.03. HRMS (ESI) *m/z*: 424.2029 (calcd 424.2036 for $C_{24}H_{27}FN_3O_3$+[M + H]$^+$).

4.7. 2-Methyl-7-(oxiran-2-ylmethoxy)-3,4-dihydroisoquinolin-1(2H)-one [31]

To a suspension of compound 5 (10 mmol) and 2-(chloromethyl) oxirane (11 mmol) in acetone (100 mL), potassium carbonate (20 mmol) and a catalytic amount of potassium iodide (1% mol) were added. The resulting mixture was heated and refluxed for 4–6 h, and the progress of the reaction was monitored by TLC. After cooling to room temperature, the mixture was filtered and the solvent was evaporated under reduced pressure. The crude product was purified by means of chromatography (petroleum ether/EtOAc = 40/1) to yield compound 31, as a pale-yellow oil (yield, 70.0%).

4.8. 7-(3-(4-(6-Fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)-2-hydroxypropoxy)-2-methyl-3,4- dihydroisoquinolin-1(2H)-one [32]

A mixture of 31 (5 mmol), 6-fluoro-3-(piperidin-4-yl)benzo[*d*] isoxazole hydrochloride (5.5 mmol) and potassium carbonate (10 mmol) in acetonitrile (50 ml) was stirred and heated to reflux for 6–8 h. The solvent was evaporated under reduced pressure, and the crude product was purified by means of chromatography (DCM/MeOH = 30/1) to yield compound 32, as a yellow oil (yield, 62.8%), mp: 131–133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.23 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.17-6.82 (m, 3H), 4.17-4.12 (m, 1H), 4.09-4.00 (m, 2H), 3.53 (t, *J* = 6.7 Hz, 3H), 3.31-2.97 (m, 6H), 2.92 (t, *J* = 6.7 Hz, 2H), 2.72-2.54 (m, 2H), 2.53-2.46 (m, 1H), 2.27-2.21 (m, 1H), 2.17-1.90 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.31, 164.62, 163.81, 162.82, 160.91, 157.84, 130.47, 130.26, 128.07, 122.53, 119.38, 117.24, 112.51, 112.23, 97.55, 97.28, 70.63, 65.73, 60.62, 54.94, 52.51, 48.35, 35.24, 34.25, 30.69, 30.46, 27.02. HRMS (ESI) *m/z*: 454.2136 (calcd 454.2142 for $C_{25}H_{29}FN_3O_4$+[M + H]$^+$).

4.9. 7-(2-Fluoro-3-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one [33]

At first, compound 32 (3.0 mmol) was added dropwise at −78 °C to a solution of diethylamino sulfur trifluoride (9.0 mmol) in CH_2Cl_2 (50 mL) and the reaction was stirred for 2.5 h, warming to room temperature. The resulting solution was stirred at room temperature for 18 h. The reaction was quenched by slowly pouring the solution onto an ice-cold saturated aqueous $NaHCO_3$ solution (100 mL). The layers were separated, and the aqueous was re-extracted into CH_2Cl_2. The organics were dried and concentrated. The light brown residue was purified by means of chromatography (DCM/MeOH = 40/1) to afford compound 33, as a yellow oil (yield, 33.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.09 (d, *J* = 8.3 Hz,2H), 6.99 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.11 (t, *J* = 6.2 Hz, 2H), 3.75-3.39 (m, 6H), 3.16 (s, 3H), 2.94 (t, *J* = 6.6 Hz, 2H), 2.81-2.43 (m, 6H), 2.15-1.90 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 164.74, 163.91, 158.08, 152.71, 130.19, 128.02, 127.51, 123.90, 120.55, 119.29, 112.26, 77.39, 77.08, 76.76, 66.47, 55.23, 53.06, 50.04, 48.41, 35.26, 27.06, 26.69. HRMS (ESI) *m/z*: 456.2091 (calcd 456.2099 for $C_{25}H_{28}F_2N_3O_3$+[M + H]$^+$).

4.10. 2-Ethyl-7-methoxy-3,4-dihydroisoquinolin-1(2H)-one [34]

To a solution of sodium hydride (75.0 mmol) in 50 mL of dry N, N-dimethylformamide, a solution of compound 3 (50.0 mmol) in 30 mL of N, N-dimethylformamide and iodoethane (60.0 mmol) was added dropwise under ice-cooling. The reaction mixture was stirred at room temperature for 1.5 h. The reaction was quenched with 480 ml water, extracted with ethyl acetate (100 mL × 3), washed with brine, and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 1: 5) to yield compound 34, as a yellow oil (yield, 89.0%).

4.11. 2-Ethyl-7-hydroxy-3,4-dihydroisoquinolin-1(2H)-one [35]

To a stirred solution of compound 34 (35.0 mmol) was added hydrobromic acid in water solution (48%) (70.0 mL) and reaction mixture was refluxed at 100 °C overnight.The reaction mixture was quenched with water (210.0 mL)and then extracted with DCM (5 × 50 mL), The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to colorless solid. The solid was further purified with column chromatography (DCM/MeOH = 30:1) to yield compound 35 as a white solid (yield, 80.8%), mp: 169-171 °C.

4.12. 7-(3-Bromopropoxy)-2-ethyl-3,4-dihydroisoquinolin-1(2H)-one [36]

The mixture of compound 35 (20 mmol),1,3-dibromopropane(40.0 mmol), K_2CO_3 (60.0 mmol), TEBA (0.2 mmol) and acetone (50.0 mL), was heated under reflux for 6 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 1: 10) to yield compound 36, as a yellow oil (5.36 g, 86.2%).

4.13. 2-Ethyl-7-(3-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)-3,4-dihydroisoquinolin-1(2H)-one [37]

The mixture of compound 36 (10.0 mmol),6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole hydrochloride (12.0 mmol), K_2CO_3 (30.0 mmol) and acetonitrile (30.0 mL), was heated under reflux for 8 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give yellow solid, The residue was purified with column chromatography (DCM/MeOH = 20 : 1) to yield compound 37, as a white solid (yield, 84.5%), mp: 135–137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.63 (d, *J* = 1.8 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.06 (dd, *J* = 12.1, 8.5 Hz, 2H), 6.97 (dd, *J* = 8.0, 2.1 Hz, 1H), 4.10 (t, *J* = 6.3 Hz, 2H), 3.65-

3.52 (m, 4H), 3.08 (d, J = 9.8 Hz, 3H), 2.92 (t, J = 6.6 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.23-1.90 (m, 8H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.28, 164.12,163.68, 162.79, 161.16, 158.13, 130.60, 130.09, 127.93, 122.76, 119.32, 117.27, 112.40, 112.17, 97.49, 97.23, 66.57, 55.37, 53.62, 45.79, 42.27, 34.71, 30.58, 27.34, 26.87, 12.77. HRMS (ESI) m/z: 452.2342 (calcd 452.2349 for $C_{26}H_{31}FN_3O_3$+[M + H]$^+$).

4.14. 7-Methoxy-2-methylisoquinoline-1,3(2H,4H)-dione(39)

To compound 38 (30.0 mmol) was added a 40% aq solution of methylamine (15 mL) at r.t. and the reaction mixture was stirred for 5 min. It was then concentrated in vacuo and dried at the vacuum pump. To the obtained salt was added o-dichlorobenzene (60 mL) and the mixture was vigorously refluxed for 10 h. The reaction mixture was allowed to cool to r.t. The silica gel column chromatographic purification of the resulting reaction mixture using petroleum ether/EtOAc (1:3) as an eluent compound 39, as a white solid (yield, 57.6%).

4.15. 7-Methoxy-2-methylisoquinolin-1(2H)-one [40]

To a stirred solution of compound 39 (15 mmol) in EtOH(200 mL) was added $NaBH_4$ (150 mmol) at 0 °C. The mixture was stirred under argon atmosphere for 7 h at 0 °C while 2 drops of a solution of aq 2 N HCl (20 mL) and EtOH (8 mL) were added at intervals of 15 min. The excess of $NaBH_4$ was quenched at 0 °C by the addition of aq 2 N HCl in EtOH (100 mL) until the mixture was acidic. The reaction mixture was concentrated in vacuo and the obtained residue was dissolved in EtOAc (25 mL). The organic layer was washed with H_2O (150 mL), brine (150 mL), and dried (Na_2SO_4). Concentration of the dried organic layer in vacuum followed by silica gel column chromatographic purification of the resulting residue using EtOAc/PE = 2:3 as a fluent gave the pure compound 40, as yellow oil (yield, 76.4%).1H NMR (400 MHz, CDCl3) δ 7.88 - 7.75 (m, 1H), 7.46 - 7.40 (m, 1H), 7.29 - 7.12 (m, 1H), 6.99 - 6.94 (m, 1H), 6.47 - 6.42 (m, 1H), 4.00 - 3.86 (m, 3H), 3.70 - 3.51 (m, 3H). 13C NMR (101 MHz, CDCl3) δ 162.24, 158.74, 131.20, 130.15, 127.51, 127.21, 122.67, 107.33, 105.85, 55.64, 37.15. MS (ESI) m/z: 190.2 (calcd 190.1 for $C_{11}H_{12}NO_2^+$ [M + H]$^+$).

4.16. 7-Hydroxy-2-methylisoquinolin-1(2H)-one [41]

To a stirred solution of compound 40 (10 mmol) was added hydrobromic acid in water solution (48%) (20.0 mL) and reaction mixture was refluxed at 100 °C overnight.The reaction mixture was quenched with water (60.0 mL)and then extracted with DCM (5 × 50 mL), The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to colorless solid. The solid was further purified with column chromatography (DCM/MeOH = 30:1) to yield compound 41, as a white solid. (yield, 76.0%), mp: 131–133 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.56 (d, J = 2.6 Hz, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.23 (d, J = 7.3 Hz, 1H), 7.17 (dd, J = 8.5, 2.6 Hz, 1H), 6.49 (d, J = 7.3 Hz, 1H), 3.47 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 161.42, 156.76, 130.84, 130.08, 128.26, 127.21, 122.35, 110.66, 105.29, 36.74. MS (ESI) m/z: 176.3 (calcd 176.1 for $C_{10}H_{10}NO_2^+$ [M + H]$^+$).

4.17. 7-(3-Bromopropoxy)-2-methylisoquinolin-1(2H)-one [42]

The mixture of compound 41 (7.0 mmol),1,3-dibromopropane(14.0 mmol), K2CO3 (21.0 mmol), TEBA (0.7 mmol) and acetone (30.0 mL), was heated under reflux for 6 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na2SO4 evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 1: 10) to yield compound 42, as a white oil (yield, 92.7%).1H NMR (400 MHz, CDCl3) δ 7.84 (d, J = 2.6 Hz, 1H), 7.44 (dd, J = 1.7, 8.7 Hz, 1H), 7.30 - 7.13 (m, 1H), 6.97 (dd, J = 1.8, 7.2 Hz, 1H), 6.45 (dd, J = 1.9, 7.3 Hz, 1H), 4.36 - 4.14 (m, 2H), 3.77 (td, J = 1.5, 6.4 Hz, 2H), 3.61 (d, J = 1.8 Hz, 3H), 2.28 (td, J = 1.7, 6.1 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.19, 157.79, 131.33, 130.29, 127.57, 127.21, 122.75, 108.25, 105.78, 64.63, 41.47, 37.14, 32.14. MS (ESI) m/z: 296.2 (calcd 296.0 for $C_{13}H_{15}BrNO_2^+$ [M + H]$^+$).

7-(3-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)-2-methylisoquinolin-1(2H)-one [43].

The mixture of compound 42 (5.0 mmol),6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride (6.0 mmol), K_2CO_3 (15.0 mmol) and acetonitrile (20.0 mL), was heated under reflux for 8 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure togive yellow solid, The residue was purified with column chromatography (DCM: MeOH = 20 : 1) to yield compound 43, as a white solid (yield, 68.2%). mp: 147–149 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 2.6 Hz, 1H), 7.74 (dd, J = 8.7, 5.1 Hz, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.33-7.19 (m, 2H), 7.06 (td, J = 8.9, 2.1 Hz, 1H), 6.98 (d, J = 7.3 Hz, 1H), 6.46 (d, J = 7.3 Hz, 1H), 4.19 (t, J = 6.4 Hz, 2H), 3.62 (s, 3H), 3.13-3.06 (m, 3H), 2.62 (t, J = 7.3 Hz, 2H), 2.32-2.04 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.30, 163.95, 163.81, 162.81, 162.26, 161.11, 158.19, 131.12, 130.09, 127.46, 127.27, 122.91, 122.82, 122.71, 117.26, 112.41, 112.16, 108.22, 105.83, 97.50, 97.24, 66.70, 55.38, 53.60, 37.10, 34.67, 30.52, 26.78. HRMS (ESI) m/z: 436.2029 (calcd 436.2036 for $C_{25}H_{27}FN_3O_3$+[M + H]$^+$).

4.18. 2-Bromo-5-methoxybenzoyl chloride [45]

To a solution of compound 44 (30.0 mmol) in 50 mL of dry CH_2Cl_2, a solution of thionyl chloride (90.0 mmol) was added dropwise under ice-cooling. The resulting mixture was stirred at room temperature for 4 h. The resulting mixture was concentrated under reduced pressure to afford compound 45, as a pale-yellow solid (6.34g, 85.6%).

4.19. 2-Bromo-5-methoxy-N-methylbenzamide [46]

To a solution of methylamine hydrochloride (25.0 mmol) and triethylamine (40.0 mmol) in 50 mL of dry CH_2Cl_2, a solution of compound 45 (20.0 mmol) was added dropwise under ice-cooling. The resulting mixture was stirred at room temperature for 8 h. The reaction mixture was quenched with water (100 mL), washed with 2 N HCl solution, brine and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The resulting oil solidified on standing. The crude mixture was purified with column chromatography (petroleum ether/EtOAc = 1: 20) to afford compound 46, as a yellow solid (yield, 77.8%). mp: 137–139 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 - 7.46 (m, 1H), 7.20 - 7.03 (m, 1H), 6.88 - 6.84 (m, 1H), 3.88 - 3.78 (m, 3H), 3.07 - 3.04 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ168.02, 158.93, 138.41, 134.14, 117.87, 117.84, 117.82, 114.72, 114.70, 114.69, 109.37, 77.38, 77.07, 76.75, 55.66, 26.78. MS (ESI) m/z: 244.2 (calcd 244.0 for $C_9H_{11}BrNO_2^+$ [M + H]$^+$).

4.20. 6-Methoxy-3-methyl-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one [47]

In a 250 mL tube were added compound 46 (15.0 mmol), 15 mL dichloromethane, LiOH (135.0 mmol), CuBr (0.8 mmol),1,10-Phenanthroline (1.5 mmol) and DMSO (50 mL). The vessel was

then sealed with a Teflon cap and heated up to100 °C and stirred at the same temperature for 12 h. After completion(TLC), the vessel was cooled down to room temperature, and water (150 mL) was added. The resulting suspension was extracted with ethyl acetate (10 mL × 3). The organic layer was combined and dried with anhydrous Na_2SO_4. After filtration, the solvent in the acquired solution was removed under reduced pressure. The residue obtained therein was subjected to silica gel column chromatography(petroleum ether/EtOAc = 1: 10)to afford compound 47, as a pale-yellow oil (yield, 77.8%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 3.1 Hz, 1H), 7.01 (dd, J = 8.9, 3.1 Hz, 1H), 6.89 (d, J = 8.9 Hz, 1H), 3.82 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.47, 154.99, 151.82, 121.78, 119.04, 117.39, 110.14, 55.85, 31.42. MS (ESI) m/z: 194.1 (calcd 194.1 for $C_{10}H_{12}NO_3^+$ $[M + H]^+$).

4.21. 6-Hydroxy-3-methyl-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one [48]

A solution of borontribromide in dichloromethane (1 M solution, 40 mmol) was added dropwise with stirring to an ice-cooled solution of compound 47 (10 mmol) in dichloromethane (120 ml). After stirring at room temperature for 16 h the reaction mixture was poured onto a mixture of crushed-ice and aqueous ammonia (400 ml). The resulting mixture was extracted with dichloromethane (3 × 200 ml).he combined organics were washed with brine (200 ml) then dried (Na_2SO_4) and the solvent evaporated in vacuum to afford compound 48, as a pale yellow solid (yield, 78.8%), mp: 148−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 3.0 Hz, 1H), 7.05 (dd, J = 8.8, 3.0 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 3.18 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 151.91, 151.43, 122.06, 118.87, 117.43, 113.72, 79.50, 31.60. MS (ESI) m/z: 180.3 (calcd 180.1 for $C_9H_{10}NO_3^+$ $[M + H]^+$).

4.22. 6-(3-Bromopropoxy)-3-methyl-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one [49]

The mixture of compound 48 (6.0 mmol),1,3-dibromopropane(12.0 mmol), K_2CO_3 (18.0 mmol), TEBA (0.6 mmol) and acetone (30.0 mL), was heated under reflux for 6 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 1: 15) to yield compound 49, as white oil (1.61 g, 89.9%).

4.23. 6-(3-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)propoxy)-3-methyl-2,3-dihydro-4H-benzo[e][1,3]oxazin-4-one [50]

The mixture of compound 49 (5.0 mmol),6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride (6.0 mmol), K_2CO_3 (15.0 mmol) and acetonitrile (20.0 mL), was heated under reflux for 8 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure togive yellow solid, The residue was purified with column chromatography (DCM/MeOH = 20 : 1) to yield compound 50, as a white solid (yield, 55.0%), mp: 152−154 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, J = 8.7, 5.1 Hz, 1H), 7.44 (d, J = 2.9 Hz, 1H), 7.22 (dd, J = 8.5, 1.6 Hz, 1H), 7.07-6.99 (m, 2H), 6.88 (d, J = 8.9 Hz, 1H), 5.12 (s, 2H), 4.05 (t, J = 6.3 Hz, 2H), 3.28-2.85 (m, 6H), 2.57 (t, J = 7.2 Hz, 2H), 2.27-1.76 (m, 8H).^{13}C NMR (100 MHz, $CDCl_3$) δ 165.27, 163.83, 162.78, 162.47, 161.11, 154.41, 151.75, 122.72, 122.19, 119.03, 117.29, 112.40, 112.15, 111.03, 97.49, 97.23, 79.49, 66.98, 55.33, 53.59, 34.64, 31.41, 30.54, 26.83. HRMS (ESI) m/z: 440.1981 (calcd 440.1986 for $C_{24}H_{27}FN_3O_4$+$[M + H]^+$).

4.24. Ethyl (3-methoxyphenethyl)carbamate [52]

To a solution of ethyl chloroformate (300 mmol) in 200 mL of dry dichloromethane a solution of compound 51(250 mol) and triethylamine (375 mmol) in 600 mL of dichloromethane was added dropwise under ice-cooling. A white precipitate (the hydrochloride of the amine) formed immediately. After vigorous stirring for 1 h at r.t., the reaction mixture was quenched with water (100 mL), washed with 2 N HCl solution, brine and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The resulting oil solidified on standing. The crude mixture was purified with column chromatography (petroleum ether/EtOAc = 1: 20) to afford compound 52, as a pale-yellow oil (yield, 95.9%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (t, J = 7.8 Hz, 1H), 6.90 - 6.70 (m, 3H), 4.14 (q, J = 7.1 Hz, 2H), 3.83 (s, 3H), 3.47 (q, J = 6.7 Hz, 2H), 2.83 (t, J = 7.0 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.80, 156.64, 140.49, 129.62, 121.12, 114.49, 111.83, 60.73, 55.17, 42.04, 36.23, 14.68. MS (ESI) m/z: 224.4 (calcd 224.1 for $C_{12}H_{18}NO_3^+$ $[M + H]^+$).

4.25. 6-Methoxy-3,4-dihydroisoquinolin-1(2H)-one (53)

To a solution of P_2O_5 (300.0 mmol) in 70 mL of methanesulfonic acid, compound 52 (200.0 mmol) were added. The solution was heated for 2 h under 130 °C, excess methane sulfonic acid was removed in vacuo, and the resulting residue was quenched with ice-water. The solution was neutralised with $NaHCO_3$ and extracted three times with dichloromethane. The combined organic phases were washed twice with water, dried with Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The crude mixture was purified with column chromatography (petroleum ether/EtOAc = 1: 1) to afford compound 53, as pale-yellow solid (yield, 54.4%), mp: 133-135 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14−7.89 (m, 1H), 6.89 (dd, J = 8.7, 2.5 Hz, 1H), 6.74 (t, J = 2.2 Hz, 1H), 3.98 - 3.77 (m, 3H), 3.61 - 3.57 (m, 2H), 3.01 - 2.97 (m, 2H). ^{13}C NMR (100 MHz,$CDCl_3$) δ 162.56, 141.07, 130.02, 121.85, 112.48, 112.33, 55.42, 40.23, 28.75. MS (ESI) m/z: 178.3 (calcd 178.1 for $C_{10}H_{12}NO_2^+$ $[M + H]^+$).

4.26. 6-Methoxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (54)

To a solution of sodium hydride (75.0 mmol) in 50 mL of dry N, N-dimethylformamide, a solution of compound 53 (50.0 mmol) in 30.0 mL of N, N-dimethylformamide and iodomethane (60.5 mmol) was added dropwise under ice-cooling. The reaction mixture was stirred at room temperature for 1.5 h. The reaction was quenched with 480 ml water, extracted with ethyl acetate (100 mL × 3), washed with brine, and dried over Na_2SO_4. The organic phase was evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 10: 1) to afford compound 54, as yellow oil (yield, 93.5%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 - 7.77 (m, 1H), 6.82 (dd, J = 8.6, 2.5 Hz, 1H), 6.64 (d, J = 2.6 Hz, 1H), 3.85- 3.78 (m, 3H), 3.53 (t, J = 6.7 Hz, 2H), 3.11 (s, 3H), 2.95 (d, J = 4.4 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 164.85, 162.53, 162.08, 140.07, 130.09, 122.24, 112.37, 111.86, 77.50, 77.18, 77.18, 76.86, 55.34, 48.11, 36.49, 34.99, 31.40, 28.20. MS (ESI) m/z: 192.2 (calcd 192.1 for $C_{11}H_{14}NO_2^+$ $[M + H]^+$).

4.27. 6-Hydroxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (55)

To a stirred solution of compound 54 (40.0 mmol) was added hydrobromic acid in water solution (48%) (30.0 mL) and reaction mixture was refluxed at 100 °C overnight. The reaction mixture was quenched with water (60.0 mL)and then extracted with DCM

(5 × 50 mL), The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to colorless solid. The solid was further purified with column chromatography (DCM/MeOH = 30:1) to afford compound 55, as yellow solid (yield, 83.1%), mp:124–126 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, J = 8.5 Hz, 1H), 6.69 (dd, J = 8.4, 2.3 Hz, 1H), 6.61 (d, J = 2.3 Hz, 1H), 3.46 (td, J = 6.7, 3.0 Hz, 2H), 2.96 (s, 3H), 2.86 (td, J = 6.7, 2.4 Hz, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 164.28, 160.69, 141.10, 129.88, 120.90, 114.19, 113.73, 47.82, 34.81, 27.88. MS (ESI) m/z: 178.3 (calcd 178.1 for $C_{10}H_{12}NO_2^+$ $[M + H]^+$).

4.28. 6-(3-Bromopropoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (56)

The mixture of compound 55 (20.0 mmol),1,3-dibromopropane(40.0 mmol), K_2CO_3 (60.0 mmol), TEBA (2.0 mmol) and acetone (50.0 mL), was heated under reflux for 6 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give crude oil. The residue was purified with column chromatography (petroleum ether/EtOAc = 15: 1) to yield compound 56, as pale-yellow oil (3.89 g, 65.5%).

4.29. 6-(3-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl) propoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (57)

The mixture of compound 56 (10.0 mmol),6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride (12.0 mmol), K_2CO_3 (30.0 mmol) and acetonitrile (40.0 mL), was heated under reflux for 8 h. Water (300 ml) was added to the reaction solution, which was then extracted with DCM, The organic phase was dried over Na_2SO_4 evaporated under reduced pressure to give yellow solid, The residue was purified with column chromatography (DCM: MeOH = 20 : 1) to afford compound 57, as white solid (yield, 52.2%), mp: 116–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.62 (m, 2H), 7.24 (dd, J = 8.5, 1.8 Hz, 1H), 7.08-7.03 (m, 1H), 6.95 (dd, J = 8.3, 1.7 Hz, 1H), 6.84-6.79 (m, 1H), 4.11 (t, J = 6.2 Hz, 2H), 3.11 (d, J = 10.2 Hz, 3H), 2.94 (s, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.24-2.00 (m, 7H), 1.95-1.82 (m, 2H), 1.43 (s, 2H), 0.38 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 167.84, 165.31, 163.83, 162.82, 162.18, 161.05, 151.59, 124.74, 122.55, 117.28, 114.31, 112.46, 112.20, 106.53, 97.55, 97.28, 66.67, 64.99, 55.33, 53.59, 34.51, 30.49, 29.95, 26.84, 24.66, 23.78, 7.52. HRMS (ESI) m/z: 438.2185 (calcd 438.2193 for $C_{25}H_{29}FN_3O_3$+$[M + H]^+$).

5. In vitro binding assays

5.1. General procedures for the binding assays

All of the new compounds were solved in 50% (v/v) DMSO, and the compound concentration was 2×10^{-3} M; dilution to the initial concentration of the new compound, 2×10^{-4} M, contained 5% DMSO. The following specific radioligands and tissue sources were used:

(a) the serotonin 5-HT_{1A} receptor, [^{3}H]8-OH-DPAT, from rat brain cortex; (b) the serotonin 5-HT_{2A} receptor, [^{3}H]ketanserin in the present of 4-dione hydrochloride hydrate (35 nM), from rat brain cortex; (c) the serotonin 5-HT_{2C} receptor, [^{3}H]mesulergine in the present of spiperone (40 nM), from rat brain cortex; (d) the serotonin 5-HT_6 receptor, [^{3}H]lysergic acid diethylamide, from 5-HT_6-C3 cells (CHO-K1); (e) the serotonin 5-HT_6 receptor, [^{3}H]-5-CT, from rat cerebral cortex. (f) the dopamine D_2 receptor, [^{3}H] spiperone, from rat striatum; (g) the histamine H_1 receptor, [^{3}H] mepyramine, from guinea pig cerebellum; (h) the adrenergic α_1 receptor, [^{3}H]prazosin, from rat cerebral cortex; For 5-HT_{1A} receptor binding assays, total binding (TB) was determined in the presence of the radioligand [^{3}H]8-OH-DPAT. Nonspecific binding (NB) was determined in the presence of the radioligand [^{3}H]8-OHDPAT and serotonin, whereas compound binding (CB) was determined in the presence of the radioligand [^{3}H]8-OH-DPAT and the compound of interest. Each specific binding (SB) was calculated as the total binding (TB) minus the nonspecific binding (NB) at a particular concentration of radioligand. Each percentage of inhibition (%) was calculated as follows: percentage of inhibition (%) = [(TB - CB)/(TB - NB)] × 100.

Blank binding experiments contained 0.25% (v/v) DMSO were performed; DMSO had no effect. All compounds were tested at least three times over a 6-fold concentration range (10^{-5} M to 10^{-10} M). IC^{50} values were determined by nonlinear regression analysis with fitting to the Hill equation curve. Ki values were calculated using the Cheng and Prussoff equation, $Ki = IC_{50}/(1 + C/K_d)$, where C represents the concentration of the hot ligand used and Kd the receptor dissociation constant of each labeled ligand. The mean Ki values and SEM were derived in at least three independent experiments.

5.1.1. 5-HT_{1A} receptor binding assay[8,10]

Rat cerebral cortex was homogenized in 20 vol of ice-cold Tris–HCl buffer (50 mM, pH 7.7) using an ULTRA TURAX homogeniser, and was then centrifuged at 32000 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, PerkinElmer 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 0.5 nM [^{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 0.5 nM [^{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 PE Microbeta 2450 liquid scintillation counter.

5.1.2. 5-HT_{2A} receptor binding assay [8,10]

Rat cerebral cortex was homogenized in 20 vol 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 20min. The final pellet was resuspended in 50 vol 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]ketanserin (60.0 Ci/mmol, PerkinElmer 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 0.6 nM [^{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 0.6 nM [^{3}H]ketanserin, 150 μL of new compounds or reference drug.The tubes were incubated at 37 °C for 15 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 PE Microbeta 2450 liquid scintillation counter.

5.1.3. 5-HT_{2C} receptor binding assay[8,10]

Rat cerebral cortex was homogenized in 20 vol of ice-cold Tris−HCl buffer (50 mM, pH7.7) using 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 20min. The final pellet was resuspended in 50 vol of the Tris−HCl buffer. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]mesulergine (85.4 Ci/mmol; PerkinElmer 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 1 nM [^{3}H]mesulergine, 50 μL of 10 μM mianserin.Specific binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]mesulergine, 50 μL of new compounds or reference drug.The tubes were incubated at 37 °C for 15 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 PE Microbeta 2450 liquid scintillation counter.

5.1.4. 5-HT_6 receptor binding assay [8,10]

Membranes were prepared from CHO-5-HT_6 cells stably transected with the human serotonin 5-HT_6 receptor cell. The harvested cells are suspended in 1 volume of fresh physiological phosphate buffered saline (PBS) solution and centrifugedat 1000 g. This homogenate was centrifuged at 100000 g for 60 min, the resulting pellet was suspended in Tris-HCl (pH 7.4) to obtain a concentration corresponding to 4×10^7 cells/ml and aliquots were stored at −80 °C. Total binding each assay tube was added 900 μL of the tissue suspension, 50 μL of 2 nM [^{3}H]lysergic acid diethylamide (84.0 Ci/mmol, PerkinElmer 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]lysergic acid diethylamide, 50 μL of 10 mM serotonin. Compound binding each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H] lysergic acid diethylamide, 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 (1.0 mL) was added and the radioactivity bound was measured using a PE Microbeta 2450 liquid scintillation counter.

5.1.5. 5-HT_7 receptor binding assay[8,10]

Rat cerebral cortex was homogenized in 20 vol of ice-cold membrane buffer (50 mM Tris-HCl, pH 7.7 containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline) using an ULTRA TURAX homogeniser, and centrifuged at 50,000g for 12 min at 4 °C. The resulting membrane pellets were washed by resuspension in membrane buffer (50 mM Tris-HCl, pH 7.7 containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline) and centrifuged for 20 min at 37 °C. The final pellet was resuspended in 50 vol of the Tris-HCl buffer containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline. For total binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of 5 nM [^{3}H]-5-CT, and 50 μL of membrane buffer. For nonspecific binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of 5 nM [^{3}H]-5-CT, and 50 μL of 1 μM5-HT. For specific binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of 5 nM [^{3}H]-5-CT, 50 μL of compound **47** solution in various concentrations (10^{-5} mol to 10^{-10} mol). The tubes were incubated at 37 °C for 30 min. The incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Scintillation fluid(1.0 mL) was added, and the radioactivity bound was measured using a PE Microbeta 2450 liquid scintillation counter.

5.1.6. Dopaminergic D_2 receptor binding assay[8,10]

Rat striatum was homogenized in 20 vol 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; PerkinElmer 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 0.5 nM [^{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 0.5 nM [^{3}H]spiperone, 50 μL of new compounds or reference drug.The tubes were incubated at 37 °C for 15 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 PE Microbeta 2450 liquid scintillation counter.

5.1.7. Histamine H_1 receptor binding assay[8,10]

Guinea pig cerebellum was homogenized in 20 vol 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] pyrilamine (20.0 Ci/mmol; PerkinElmer 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] pyrilamine, 50 μL of 1 μM promethazine. Specific binding each assay tube was added 900 μL of Membranes, 50 μL of [^{3}H] pyrilamine, 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 PE Microbeta 2450 liquid scintillation counter.

5.1.8. Adrenergic α_1 receptor binding assay [8,10]

Rat cerebral cortex was homogenized in 20 vol of ice-cold Tris-HCl buffer containing 5 mM EDTA (50 mM, pH 7.4) using an ULTRA TURAX homogeniser and centrifuged at 44000 g for 20 min at 4 °C. The resulting pellet was resuspended in the same quantity of the buffer centrifuged for 20 min. The final pellet was resuspended in 50 vol of the Tris-HCl buffer. For total binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H] prazosin (85.4 Ci/mmol; PerkinElmer Life Sciences, Boston, MA, USA), and 50 μL of Tris-HCl buffer. For nonspecific binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of 1 nM [^{3}H]prazosin, and 50 μL of 10 mMprazosin. Compound binding, to each assay tube was added 900 μL of the tissue suspension, 50 μL of [^{3}H]prazosin, 50 μL of new compounds, or reference drug. The tubes were incubated at 25 °C for 60 min. The incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Scintillation fluid (1.0 mL) was

added, and the radioactivity bound was measured using a PE Microbeta 2450 liquid scintillation counter.

5.1.9. Experimental in vitro pharmacology for intrinsic activity Assessment[10]

CHO-K1 cells expressing five receptors (HEK293/D_2, HEK293/5-HT_{1A}, HEK293/5-HT_{2A}, HEK293/h5-HT_6 and HEK293/h5-HT_7) were seeded in a 384-well black-walled, clear bottom plate at a density of 20,000 cell per well in 20 μL of growth medium, 18 h prior to the day of experiment, and maintained at 37 °C/5% CO_2.

For the agonist assay, 20 μL of dye-loading solution (20 mM HEPES/HBSS with 2.5 mM Probenecid, pH 7.4) was added to the well. Then, the plate was placed into a 37 °C incubator for 60 min, followed by 15 min at room temperature. Finally, 10 μL of compounds or control agonist were added into respective wells of the assay plate.

For the antagonist assay, 20 μL of dye-loading solution (20 mM HEPES/HBSS with 2.5 mM Probenecid, pH 7.4) and 10 μL of compound or control antagonist solution were added into the well. Then, the plate was placed into a 37 °C incubator for 60 min, followed by 15 min at room temperature. Finally, 12.5 μL of control agonist was added into respective wells of the assay plate during reading in FLIPR.

For the agonist test, the compounds or agonist plate contained compounds or control agonist at 5 × final test concentration and were placed at Source 2. Compounds or control agonist was added to the reading plate at 20 s and the change in cell fluorescence signal was measured in a FLIPR (FLIPR Calcium 4 assay kit, Molecular Devices) for an additional 100 s (21–120 s). For the antagonist test, only compounds or control agonist solution at the Source 2 plate was replaced with control agonist solution at 5 × EC_{80} concentration.

Data were recorded by ScreenWorks (version 3.1) as FMD files with FLIPR. Data acquisition and analyses were performed using the ScreenWorks (version 3.1) program and exported to Excel. The average value of 20 (1 s–20 s) seconds of reading was calculated as the baseline reading and the relative fluorescent unit (ΔRFU) intensity values were calculated with the maximal fluorescent units (21 s–120 s) subtracting the average value of baseline reading.

The % activation of the compound was calculated from the following equation:

$$\%\ \text{activation} = \{(\Delta RFU_{Compound} - \Delta RFU_{Background})/(\Delta RFU_{Agonist\ control} - \Delta RFU_{Background})\} \times 100$$

The % activation was then plotted as a function of the log of the cumulative doses of compounds.

The % inhibition of the test article was calculated from the following equation:

$$\%\ \text{inhibition} = \{1 - (\Delta RFU_{Compound} - \Delta RFU_{Background})/(\Delta RFU_{Agonist\ control} - \Delta RFU_{Background})\} \times 100$$

The % inhibition was then plotted as a function of the log of the cumulative doses of compounds.

5.1.10. hERG Affinity[10]

Ability to block hERG potassium channels was determined using the whole-cell patch clamp method and cloned hERG potassium channels (expressed in HEK 293 cells) as biological material. For this purpose, the patch clamp amplifier (Axopatch 200B, Molecular Devices) and digital converter (Digidata 1440A, Molecular Devices) were used. Recording electrodes were made from borosilicate glass with filament (BF120-94-15, Sutter Instrument Company). Creation of voltage-clamp command pulse protocols and data acquisition were controlled by pCLAMP software (version 10.1, Molecular Devices). The bath solution consisted of 137 mM NaCl, 5.4 mM KCl, 10 mM glucose, 10 mM HEPES, and 2 mM $CaCl_2$. The pH was adjusted to 7.5 by addition of NaOH. The pipet filling solution consisted of 140 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, and 5 mM Na_2ATP. The pH was adjusted to 7.2 by addition of KOH.

To study voltage dependence of steady-state block of hERG channels on different drug concentrations (0.3, 1, 3, and 10 μM) in HEK cells, the holding membrane potential was switched from −80 to +50 mV for 2 s following return to −50 mV for 3 s (sampling rate of 4 kHz, low-pass filtered at 1 kHz) in intervals of 30 s. Tail currents were measured at −50 mV in control and in the presence of the drug at concentrations determined empirically. All raw measurements were performed using Clampfit (version 10.2), a part of pCLAMP software (version 10.1). Results were transferred to the program Statistical Package for the Social Sciences (SPSS) spreadsheets for further analysis.

6. Behavioral tests

6.1. 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 studies involving animals in this research follow the guidelines of the bylaw of experiments on animals and have been approved by the Ethics and Experimental Animal Committee of Jiangsu Nhwa Pharmaceutical Co., Ltd.

6.1.1. Acute toxicity study[8,10]

Mice (5 mice in each group) were orally dosed with increasing doses of the compound 13 (100, 200, 500, 1000, 1500 and 2000 mg/kg). The number of surviving animals was recorded after 24h 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).

6.1.2. MK-801-induced hyperactivity[8,10]

Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.06, 0.2, 0.6, 2.0 and 6 mg/kg), aripiprazole (0.3, 1.0, 3.0 and 10 mg/kg), risperidone (0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg) and compound 13 (0.03, 0.1, 0.3 and 1.0 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.

6.1.3. Apomorphine-induced climbing[8,10]

Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.1, 0.13, 0.17, 0.23 and 0.3 mg/kg), aripiprazole (0.3, 1.0, 3.0 and 10 mg/kg), risperidone (0.01, 0.03, 0.1 and 0.3 mg/kg), compound 13 (0.03, 0.1, 0.3 and 1.0 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.

6.1.4. DOI-induced head twitch [50]

Mice (10 mice in each group) were orally dosed with increasing doses of the aripiprazole (0.3, 1.0, 3.0 and 10 mg/kg), risperidone (0.01, 0.03, 0.1 and 0.3 mg/kg), compound 13 (0.03, 0.1, 0.3 and 1.0 mg/kg). or vehicle 60 min before i.p. administration of DOI (1 mg/kg). Immediately after DOI treatment, animals were individually placed into observation chambers; after a 5-min wait, the number of head twitch responses that occurred during the next 15 min was counted. The mean (SEM) number of head twitches was calculated for each group.

6.1.5. ***Forced swim test (FST)*** [51]

A total of 110 naive mice were randomly assigned to eleven groups consisting of vehicle control, duloxetine (20 mg/kg), and compound 13 (0.02, 0.06 and 0.2 mg/kg) with each group containing 10 mice. One hour after oral administration of test compounds, each mouse was forced to swim in an open cylindrical container (diameter of 10 cm, height of 25 cm, containing 15 cm of water with temperature maintained at 24 ± 1 °C). The duration of immobility during the last 4 min of total 6 min was recorded, and animals were judged to be immobile when they floated motionless, making only necessary movements to keep their heads above the water.

6.1.6. Tail suspension test (TST) [52]

A total of 110 naive mice were randomly assigned to eleven groups consisting of vehicle control, duloxetine (20 mg/kg), and compound **13** (0.02, 0.06 and 0.2 mg/kg) with each group containing 10 mice. After oral administration of compounds for 60 min, each mouse was suspended on the top of apparatus using adhesive tape placed approximately 1 cm from the tail tip. The immobility duration of the last 4 min of total 6 min period was recorded. Mice were considered to be immobile when hung passively without moving.

6.1.7. Catalepsy test [8,10]

Mice (10 mice in each group) were orally dosed with vehicle or increasing doses of the haloperidol (0.18, 0.35, 0.75, 1.5 and 3.0 mg/kg), aripiprazole (5, 15, 30, 100 and 150 mg/kg), risperidone (0.1, 0.6, 1.2, 2.5 and 5.0 mg/kg), compound 13 (0.5, 1.5, 3, 15 and 30 mg/kg). Catalepsy was evaluated on a metal bar 0.6 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.

6.2. Locomotor activity

Mice (8 mice in each group) were orally dosed with vehicle or increasing doses of the clonazepam (1, 3 and 10 mg/kg) and compound 13 (0.06, 0.2 and 0.6 mg/kg). Locomotor activity was assessed in automated activity frames equipped with an automated video tracking system. Test compounds were orally administered by intragastric administration 1 h before test start. Mice were acclimated to the test chamber for 30 min before starting the experiment. Locomotor activity was then measured for 10 min. The horizontal distance traveled in centimeters by the animals was analyzed.

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).

6.3.1. Weight gain and serum prolactin [8,10]

Mice (10 mice in each group) were orally dosed with vehicle or increasing doses as follows: risperidone (0.03, 0.1, and 0.3 mg/kg) and **13** (0.08, 0.3 and 0.8 mg/kg). The animals received multiple doses (28 day) of each compound. The weight of mice was tested every day. The mice were killed by decapitation 180 min after the last treatment. Blood samples (2 mL) were collected and centrifuged (300 g for 30 min), and the resulting serum samples were stored at −20 °C until analyzed for prolactin (PRL). Serum PRL was determined by an EIA-kit from Amersham. Data were analyzed by Student's *t*-test ($^{\#\#}p < 0.01$).

6.3.2. Pharmacokinetics study in rats [8,10]

The HPLC conditions were as follows: column, XSELECT CSH XP C18 (2.1 mm × 50 mm, 2.5 μm); mobile phase, 0.025% FA and 1 mM NH4OAc (ROE SCIENTIFIC INC, USA) in water/acetonitrile (Merck Company, Germany) (v:v, 45:55); flow rate, 0.6 mL/min; column temperature, 50 °C. UV detection was performed at 210 nm. For routine compound **13** screening, rats (n = 6/group) were dosed via the lateral tail vein at the indicated dose for iv administration (0.5 mg/kg, 100% saline) or via oral gavage (5 mg/kg, suspension in 0.5% methylcellulose). At 30 min and 1, 2, 3, 4, 5, 6, 8 and 24 h after administration, serial blood samples were collected from the lateral tail vein into heparinized collection tubes (approximately 0.25 mL). The plasma was separated by centrifugation, and the sample was prepared for LC/MS analysis by protein precipitation with acetonitrile. The plasma samples were analyzed for drug and internal standard via the LC−MS/MS protocol.

6.3.3. Novel object recognition training and testing [10]

Rats were acclimated for 1 week prior to the experiment. The rats were housed in groups of two/cage in a light-controlled room (12 h light/dark cycles with lights on at 07:00). Food and water were provided ad libitum. Rats were habituated to handling and an empty test arena (a gray-colored polyvinyl chloride box ($40 \times 40 \times 50\ cm^3$)) for 7 min on each of 3 consecutive days. On the training day, the drug was administered p.o. in a vehicle of physiological saline at a volume of 1 mL/kg 1h after dosing, each rat was placed in the test arena, which now contained two identical objects located centrally in the arena. Rats were given either 3 min explore the arena and objects. Memory retention was tested 24 h after training. Rats were placed back in the arena with one "familiar" (previously trained) and one "novel" object and given 5 min to explore. The spatial position of objects left−right position) and which object was novel (ball or cube) was counterbalanced across subjects. Objects and arenas were cleaned with diluted 75% alcohol solution between trials to remove rat feces and urine. To determine memory performance, the novelty discrimination index (NDI) was calculated using the following equation: novel object interaction time/total interaction time × 100 (%). Rats were excluded from the analysis if total exploration time in the acquisition trial was less than 10 s. Numbers of rats treated with vehicle and those treated with 0.02, 0.06, and 0.2 mg/kg of compound **13** were 14, 15, 15, and 14, respectively. 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).

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112709.

Abbreviations used

D_2	dopamine-2
D_3	dopamine-3
5-HT_{1A}	serotonin-1A
5-HT_{2A}	serotonin-2A
5-HT_6	serotonin-6
5-HT_{2C}	serotonin-2C
5-HT7	serotonin-7
H_1	histamine-1
α_1	adrenergic-1
EPS	extrapyramidal symptoms
TD	tardive dyskinesia
hERG	ethera-go-go-related gene
CNS	central nervous system
NMDA	N-methyl-D-aspartate
ED_{50}	50% effective dose
LD_{50}	median lethal dose
PD	Parkinson's disease
AD	Alzheimer disease
DMF	N N-dimethylformamide
DMSO	Dimethyl sulfoxide
DOI	2,5-Dimethoxy-4-iodoamphetamine
PCP	phencyclidine
APO	apomorphine
FST	Forced swim test
TST	Tail suspension test
PCP	dizocilpine
NDI	novelty discrimination index
SPSS	Statistical Package for the Social Sciences

Supporting information

Selectivity of Compound 13 for Additional Receptors; Anesthetized Rat Model for the Assessment of QT Prolongation; ^{1}H NMR, ^{13}C- NMR, HR-MS, and HPLC of compound 13 and 43.

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