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Discovery of aryl-piperidine derivatives as potential antipsychotic agents using molecular hybridization strategy

Chen Zhu, Xinwei Li, Bangyi Zhao, Weiqing Peng, Wei Li, Wei Fu

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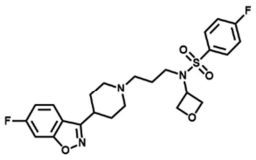


D_2R IC_{50} = 3.0 nM

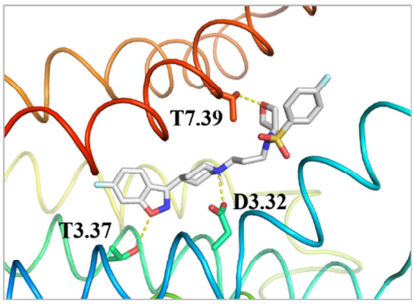
5-$HT_{2A}R$ IC_{50} = 15.1 nM

in vivo $t_{1/2}$ = 0.54 h (i.v.)

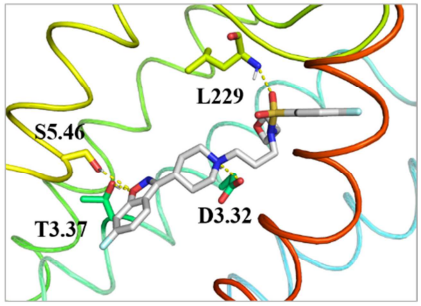
in vivo $t_{1/2}$ = 2 h (p.o.)

K_p = 1.72~4.03

Showed sedative effect in mice



The binding mode of 9f with D_2 receptor



The binding mode of 9f with 5-HT_{2A} receptor

Discovery of Aryl-piperidine Derivatives as Potential Antipsychotic Agents Using Molecular Hybridization Strategy

Chen Zhu[#], Xinwei Li[#], Bangyi Zhao, Weiqing Peng, Wei Li, Wei Fu*

School of Pharmacy & Minhang Hospital, Fudan University, Shanghai 201301, P. R. China

[#] These authors contributed equally in this work.

*Corresponding author: *Tel & Fax: +86-21-51980096, E-mail address: wfu@fudan.edu.cn (Wei Fu)*

Abstract

Schizophrenia is a chronic, disabling mental disorder that affects about one percent of world's population. Drugs acting on multiple targets have been demonstrated to provide superior efficacy in schizophrenia than agents acting on single target. In this study, based on **FW01**, a selective potent 5-HT_{1A} receptor agonist discovered via dynamic pharmacophore-based virtual screening, molecular hybridization strategy was employed to optimize its *in vitro* activity over D_2 and 5-HT_{2A} receptors. The optimized compound **9f** was found to show dual potent D_2 and 5-HT_{2A} receptors antagonistic activity. In addition, compound **9f** showed good *in vivo* metabolic stability with $t_{1/2}$ of 2 h in ICR mice and good capability to penetrate the blood-brain barrier with K_p value of 4.03. These results demonstrated that the dual D_2 and 5-HT_{1A} receptor antagonist **9f** could serve as a promising lead compound to discover potent antipsychotic agents.

Keywords

D_2 receptor antagonist, 5-HT_{2A} receptor antagonist, molecular hybridization, multi-target strategy, antipsychotic

1. Introduction

Schizophrenia is a chronic, disabling mental disorder with high risk of suicide,

affecting more than one percent of world's population.[1] The symptoms of schizophrenia can be divided into three categories: positive symptoms, including hallucination and delusion; negative symptoms, such as motivation and diminished expression; and cognitive deficits like deficit in learning, memory and attention.[2, 3] The dopaminergic hypothesis of schizophrenia postulates a hyperactivity of dopamine in the mesolimbic pathway, and a hypofunctionality of dopamine in other brain areas like the prefrontal cortex.[4] Typical antipsychotics, such as haloperidol **(Figure 1)**, producing therapeutic effects by the blockage of D_2 receptor, are effective for controlling positive symptoms, but exert severe extrapyramidal side effects (EPS) and hyperprolactinemia.[5] The D_2 binding property is the key therapeutic mechanism of typical antipsychotics in ameliorating at least the positive symptoms of schizophrenia.[6] Accumulating reports showed that multiple receptors, including dopamine receptors and serotonin receptors in the central nervous system (CNS), contribute to the complicated pathophysiological behaviors and various symptoms of schizophrenia.[7, 8]

Atypical antipsychotics, such as clozapine and risperidone **(Figure 1)**, have equivalent or superior efficacy than typical antipsychotics but with lower EPS and hyperprolactinemia. The general idea is that the blockage of 5-HT_{2A} receptor enhances dopamine efflux, compensates for the effect of blocking dopamine receptors, thus dampening the deleterious effect associated with the blockade of D_2 receptor.[9, 10] Previous studies showed that 5-HT_{2A} receptor antagonist could enhance the antipsychotic effect of typical and atypical antipsychotics, and was effective in attenuating negative and cognitive symptoms of schizophrenia.[11, 12] As for the role of 5-HT_{1A}, accumulated data supported the assertion that there is key involvement of 5-HT_{1A} receptors in the pathophysiology and treatment-related facets of schizophrenia, particularly negative symptomatology. The combined D_2 receptor antagonists and 5-HT_{1A} receptor agonists **SLV313 (Figure 1)** augmented extracellular microdialysate dopamine and acetylcholine levels in medial prefrontal cortex.[13] The enhanced dopaminergic and cholinergic tone induced by the stimulation of 5-HT_{1A} receptor would translate into efficacy in the cognitive and negative

symptom domains.[14] The allosteric methods were expected to expedite the discovery of dopamine and serotonin regulators.[15,16,17]

Though atypical antipsychotics are widely used in clinical practice, they still remain some severe side effects such as weight gain and cardiovascular risk.[18, 19]

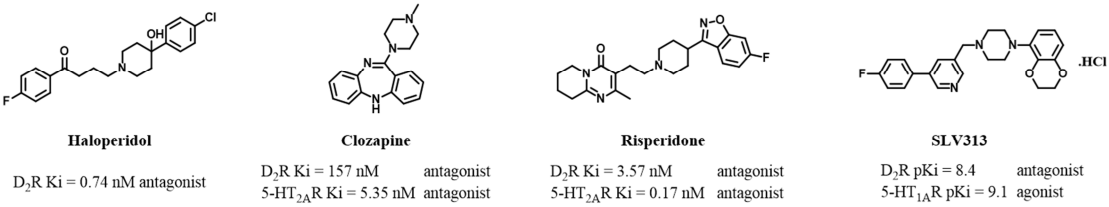


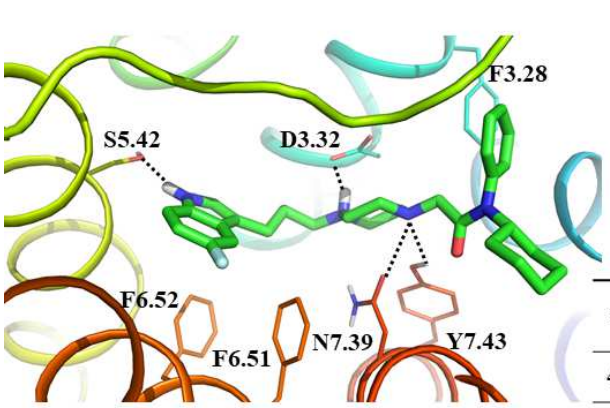
Figure 1. Structures and biological activity of antipsychotics

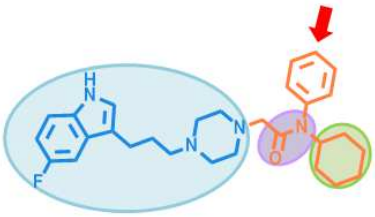
In this study, based on **FW01**, a selective potent 5-HT_{1A} receptor agonist discovered by dynamic pharmacophore-based virtual screening, molecular hybridization strategy was employed with the eventual aim to discover promising multi-target antipsychotic agents with dual D_2 and 5-HT_{2A} antagonistic action.

2. Result and discussion

2.1 Compound design

Our previous study discovered a selective 5-HT_{1A} receptor agonist **FW01** (Figure 1) through the dynamic pharmacophore-based virtual screening.[20] **FW01** displayed high binding affinity for 5-HT_{1A} receptor with K_i of 51.9±16.4 nM, moderate affinity for 5-HT_{2A} receptor with K_i of 206.71±7.46 nM and weak affinity for dopamine D_3 receptor with K_i of 2161.35±25.55 nM. **FW01** bound to 5-HT_{1A} receptor via multiple interaction forces. Nitrogen of indole and piperazine formed hydrogen bonding with S5.42, Y7.43 and N7.39, respectively. Protonated nitrogen of piperazine participated in the salt bridge with D3.32 (**Figure 2**). We employed hybridization strategy with an aim to discover multi-target antipsychotic agents towards both serotonin receptors and dopamine receptors, especially D_2 receptor.





Predicted metabolic sites of FW01

MW	HBA	HBD	AlogP	[^{3}H]-8-OH-DPAT K_i(nM)	[^{35}S]GTPγS EC_{50}(nM)
476.6	5	1	5.84	51.9 ± 16.4	7

Figure 2. Binding mode of **FW01** (green, sticks) with 5-HT_{1A} receptor (cartoon).

The binding pocket of the dopamine receptor was divided into two parts, the orthosteric binding site (OBS), in which dopamine was also thought to bind, enclosed by transmembrane segments (TMs) 3, 5, 6, and 7, and a second binding site (SBS) enclosed by TMs 1, 2, 3 and 7.[21] In Newman's study, the 2,3-dichloro-4-phenylpiperazine terminus (Ⅱ) of compound Ⅰ, a highly selective D_3 receptor agonist, maintained high binding affinities for both D_2 and D_3 receptors, while the isolated 2-butyl-indole amide terminus (Ⅲ) showed weak binding affinities for D_2 and D_3 receptors.[22] The predicted binding pose of compound Ⅰ showed that the 2,3-dichloro-4-phenylpiperazine can penetrate into the OBS, with the 2-indole terminus extended into SBP, suggesting that the binding in the OBS is critical to potency and efficacy for dopamine receptors (**Figure 3B**). The docking results of **FW01** with D_2 receptor represented that **FW01** was inactive for the indole moiety of **FW01** collides with amino acid residues in the OBS (**Figure 3D**). Based on this finding, we considered replacing the indolepiperazine moiety of the **FW01** with phenylpiperazine moiety of D_2 antagonist Ⅰ to introduce the efficacy for D_2 receptor (Figure 3A). Considering the substitution on the benzene moiety may affect activity for D_2 receptor by altering the binding pose within the OBS, the *o*-methoxy substitution on the benzene can constrain the bioactive conformation of benzene, compounds **5a**-**5h** were designed. Next, we studied the binding mode of atypical antipsychotic iloperidone with D_2 receptor. It showed that the 6-fluoro-1,2-benzisoxazolyl piperidine moiety bound in the OBS (**Figure 3C**).

Therefore, we replaced the indolepiperazine moiety of the **FW01** with the 6-fluoro-1,2-benzisoxazolyl piperidine moiety of iloperidone in order to introduce the dual D_2 and 5-HT_{2A} receptors antagonist activity. Meanwhile, we reversed the atom order of the amide bond of **FW01** to avoid the possible toxic aniline of the newly compounds. Besides, we changed the cyclohexyl ring to alkyl substituents, in order to reduce the ClogP value of new compounds (**Figure 3E**). Finally, compounds **9a-9f** were designed, which is more structurally related to iloperidone and the dual D_2/5-HT_{2A} receptors antagonistic activity were successfully introduced.

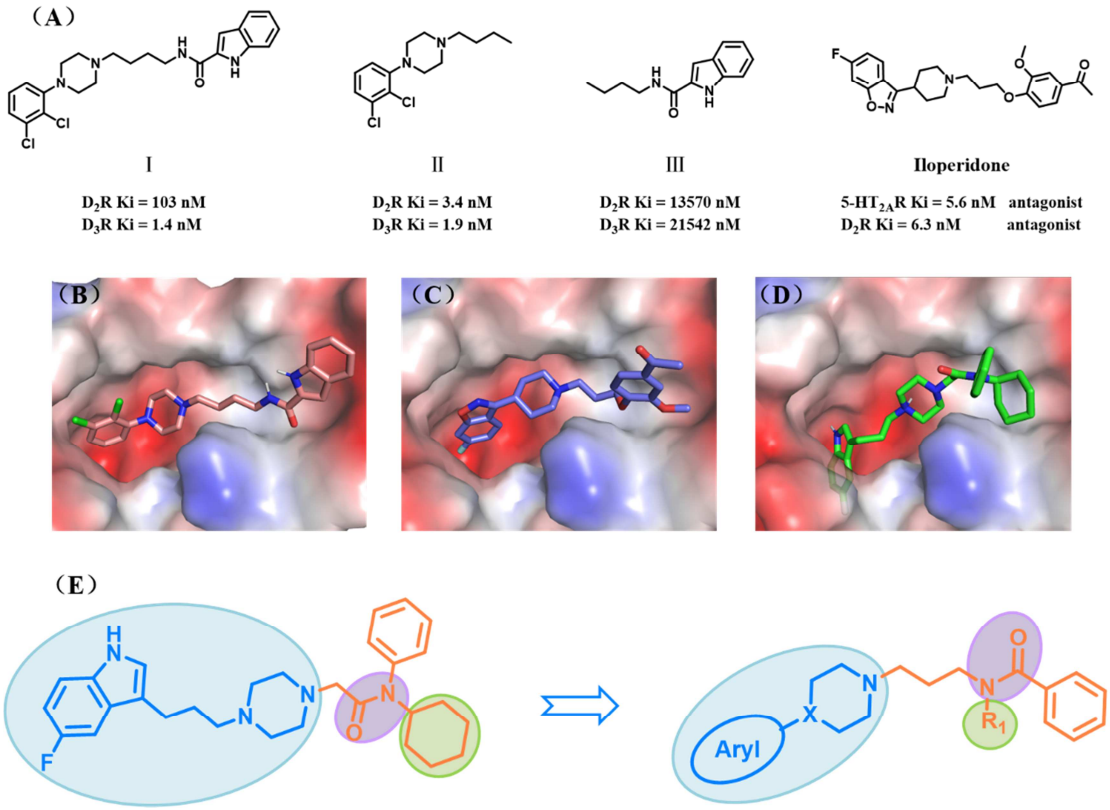
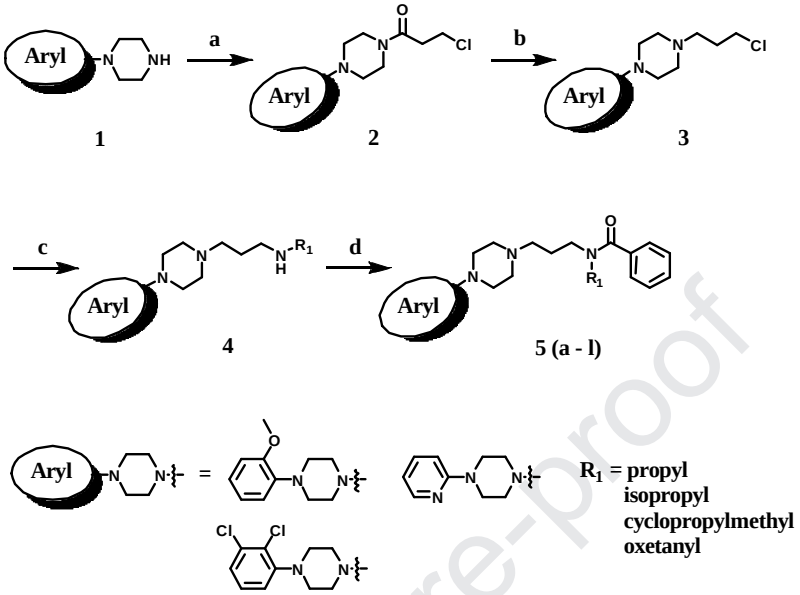


Figure 3. (A) Structures of lead compounds. (B) Electrostatic potential surface of compound □ (pink) with D_2 receptor. (C) Electrostatic potential surface of **iloperidone** (blue) with D_2 receptor. (D) Electrostatic potential surface of **FW01** (green) with D_2 receptor. (E) Design of aryl-piperazine/piperidine derivatives.

2.2 Chemical synthesis

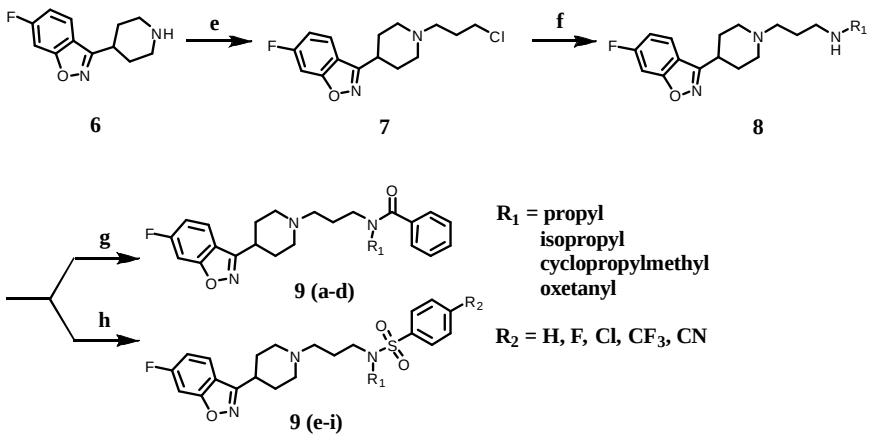
Synthesis of **5a-l** was shown in **scheme 1**. Commercially available arylpiperazines were first acylated with 3-chloropropionyl chloride to obtain compound **2**, which was

subjected to reduction by borane-methyl sulfide complex to give compound **3**. Compound **4**, which was obtained via nucleophilic substitution of compound **3** with corresponding amines, underwent condensation with benzoic acid to give **5a-l**.



Scheme 1. Reagents and conditions: (a) 3-chloropropionyl chloride, TEA, DCM, r.t., 10 min; (b) borane-methyl sulfide complex, THF, 80□, 2 h; (c) R_1-NH_2, K_2CO_3, CH_3CN, 75□, 8 h; (d) benzoic acid, HBTU, HOBT, DIEA, DCM, r.t., 6 h.

Synthesis of **9a-i** were shown in **scheme 2**. Commercially available 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole underwent alkylation with 1-bromo-3-chloropropane to give **7**, followed by amination with corresponding amines to afford compound **8**. **9a-d** were obtained by condensation of **8** with benzoic acid and **9e-i** were obtained by reaction with corresponding arylsulfonyl chloride with **8**. Structure of all compounds were confirmed by ESI-MS, HRMS, ^{1}H NMR and ^{13}C NMR.



Scheme 2. Reagents and conditions: (e) 1-bromo-3-chloropropane, K_2CO_3, DMF, r.t., 17 h; (f) R_1-NH_2, K_2CO_3, CH_3CN, 75□, 8 h; (g) benzoic acid, HBTU, HOBT, DIEA, DCM, r.t., 6 h; (h) benzenesulfonyl chloride derivatives, DIEA, DCM, r.t., 10 h.

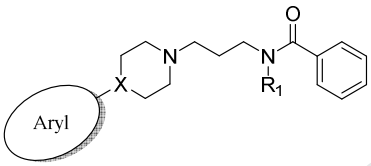
2.3 Biological evaluation

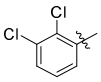
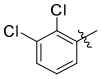
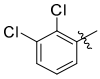
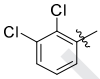
After these new derivatives were synthesized, they were subjected to evaluation for the functional activity toward 5-HT_{1A}, 5-HT_{2A} and D_2 receptors. To our delight, as shown in **Table 1**, in addition to 5-HT_{1A} receptor agonistic activity, **5a**-**5d** displayed moderate D_2 receptor and weak 5-HT_{2A} receptor antagonistic activity, which confirmed the reliability of the design idea. However, in sharp contrast to **FW01**, which served as a selective 5-HT_{1A} receptor agonist, **5e**~**5h** only showed moderate antagonistic activity for D_2 receptor and no activity for 5-HT_{1A} and 5-HT_{2A} receptors. Compound **5g** showed moderate D_2 receptor antagonistic activity, indicating that cyclopropylmethyl is superior to n-propyl, iso-propyl and oxetanyl. Compounds **5i**~**5l** bearing pyridine-2-yl displayed as selective 5-HT_{1A} receptor agonists, with similar activity profile to **FW01**. **5j** and **5k** bearing isopropyl and cyclopropylmethyl, respectively, had higher potency than **5i** and **5l**. Different activity profile of *o*-methoxylphenyl and pyridine-2-yl against 5-HT_{1A}, 5-HT_{2A} and D_2 receptors suggested that this part plays a pivotal role in potency and efficacy.

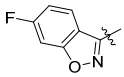
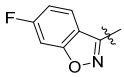
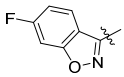
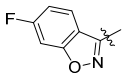
As for **9a**~**9d** that bear 6-fluorobenzo[d]isoxazo-2-lyl, they showed greatly reduced activity for 5-HT_{1A} receptor, and greatly improved antagonistic activity for D_2 receptor and 5-HT_{2A} receptor comparing with **5i**~**5l**. Among **9a**~**9d**, while **9c** displayed highest binding affinity for D_2 receptor, **9b** displayed highest binding

affinity for 5-HT_{2A} receptor. Due to high activity for 5-HT_{2A} receptor and D_2 receptor, we next evaluated *in vitro* metabolic stability of **9a**~**9d** in mice hepatic microsome assay. As shown in **Table 1**, compound **9d** was most stable among tested compounds with $t_{1/2}$ of 173.2 min, indicating that oxetanyl was metabolically stable superior to isopropyl, cyclopropylmethy and propyl.

Table 1 Biological activity of compounds **5a~5l**, and **9a~9d** for D_2, 5-HT_{1A} and 5-HT_{2A} receptors.



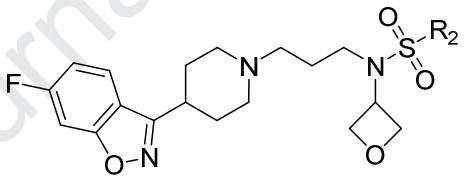
Compounds	Aryl	X	R_1	D_2 IC_{50} (nM)	5-HT_{1A} EC_{50} (nM)	5-HT_{2A} IC_{50} (nM)	in *vitro* $t_{1/2}$
FW01	--	--	--	2161.35±25.55 [a]	7	206.71±7.46 [a]	--
5a		N		484.7	451.6	3249	--
5b		N		528	**46.1**	2008	--
5c		N		**459.2**	**175.1**	768.1	--
5d		N		1878	583.2	2177	--
5e		N		466.9	NA	NA	--
5f		N		823.5	NA	NA	--
5g		N		**288.7**	NA	NA	--
5h		N		3765	NA	NA	--
5i		N		6935	54.5	7549	--
5j		N		NA	22.8	NA	--
5k		N		NA	24.9	7719	--
5l		N		NA	373.8	NA	--

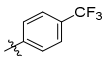
9a		C		18.5	NA	73.1	6.2
9b		C		59.3	NA	11.6	31.5
9c		C		10.8	NA	40.8	6.9
9d		C		156.0	NA	18.8	173.2

a: Ki value; NA: no activity.

However, compounds **9a**~**9d** displayed moderate antagonistic activity for D_2 receptor. We next made an attempt to improve D_2 and 5-HT_{2A} receptor activity by substitution of benzoyl group with arylsulfonyl group while keeping oxetanyl constant. As shown in **Table 2**, all compounds showed improved antagonistic activity for D_2 receptor but varying activity for 5-HT_{2A} receptor. Compound **9e** bearing phenylsulfonyl was found to have highest activity for D_2 receptor as well as 5-HT_{2A} receptor. Substitutions of H with F, Cl, CF_3 or CN were also tolerated with minor reduced activity for D_2 receptor and moderate reduced activity for 5-HT_{2A} receptor.

Table 2 Biological activity of compounds **9e~9i** for D_2, 5-HT_{1A} and 5-HT_{2A} receptors.



Compounds	**R_2**	**D_2 IC_{50} (nM)**	**5-HT_{1A} EC_{50} (nM)**	**5-HT_{2A} IC_{50} (nM)**
9e		1.9	NA	9.3
9f		3.0	NA	15.1
9g		2.0	NA	34.6
9h		2.3	NA	92.7
9i		3.6	NA	11.3

NA: no activity

Given potential metabolically labile of *para*-phenyl of **9e**, reduced activity of **9g** and **9h** for 5-HT_{2A} receptor, potential hydrolysis of **9i** *in vivo*, **9f** was finally chosen to

evaluate its *in vivo* metabolic stability in ICR mice. As showed in **Table 3**, **9f** was rapidly eliminated with $t_{1/2}$ of 0.54 h after i.v. injection. However, **9f** displayed improved $t_{1/2}$ with 2.0 h after p.o. administration. Despite its relatively higher $t_{1/2}$, its oral availability was low with F of 6.3%.

Table 3. Pharmacokinetics data of compound **9f** measured in ICR mice (n = 6/group).

Dose (mg/kg)	AUC_{0-24h} (ng×h/ml)	$t_{1/2}$ (h)	C_{max} (ng/ml)	T_{max} (h)	F (%)
5 (i.v.)	829.1	0.54	-	-	-
25 (p.o.)	259.4	2.00	141.7	0.25	6.3

We also tested its blood brain barrier (BBB) penetration ability in ICR mice. As showed in **Table 4**, **9f** displayed K_p value of 1.72 after 1 hour and 4.03 after 4 hours later, demonstrating that **9f** was easy to pass BBB. As showed in **Figure S1,** compound **9f** displayed sedative effect in the forced swimming test, the mice keep immobile within 2 hours after administration of **9f** (i.g.).

New second-generation antipsychotic medications, iloperidone, asenapine and lurasidone, approved by The Food and Drug Administration (FDA), are potent dual 5-HT_{2A} and D_2 receptors antagonists (**Table S1**).[23,24,25,26] Among them, lurasidone shows weak 5-HT_{1A} agonistic activity.[26] Ileridone and lurasidone is indicated for the treatment of schizophrenia in adults,[27,28] asenapine has regulatory approval for the indications of schizophrenia and bipolar mania/mixed episodes.[29] In this work, **9f** shows potent dual D_2 and 5-HT_{2A} receptors antagonistic activity *in vitro*, good capability to penetrate the blood-brain barrier with K_p value of 4.03 and potent sedative activity *in vivo*, we speculate **9f** has potential to treat schizophrenia, especially to treat the positive symptom of schizophrenia. In the course of the next research, further pharmacological studies would be carried on **9f** to test its antipsychotic effect.

2.4 SAR discussion

Among all the above compounds，compounds **5a~5d** bearing 2,3-dichlorophenyl

showed moderate D_2 and weak 5-HT_{2A} receptor antagonistic activity, moderate 5-HT_{1A} agonistic activity. Compounds **5e~5h** bearing 2-methoxyphenyl served as moderate selective D_2 receptor antagonists. Compounds **5i~5l** bearing pyridine-2-yl displayed as selective 5-HT_{1A} receptor agonists. Compounds **9a~9i** bearing 6-fluorobenzo[d]isoxazo-2-lyl displayed potent antagonistic activity towards D_2 and 5-HT_{2A} receptors. To understand the structure and activity relationship (SAR) between compounds **5a~5d, 5e~5h, 5i~5l** and **9a~9i**, the binding modes of representative compound from every group **5c, 5g, 5h and 9f** with dopamine and serotonin subtypes were studied by molecular docking. Docking of compounds **5c, 5g, 5h** and **9f** was performed using the GOLD 5.0.1 suit to the binding sites of the X-ray structure of D_2 receptor (PDB code: 6CM4), 5-HT_{1A} receptor that was obtained by homology modeling from the X-ray structure of 5-HT_{1B} receptor (PDB code: 4IAR) and 5-HT_{2A} receptor (PDB code: 6A93).[30,31,32] The results were shown in **Figure 4**.

For compound **5c,** the protonated nitrogen atom interacts with residue D3.32 and the benzene in the tail part formed π-π interaction with W7.40 of D_2 receptor **(Figure 4A)**. In the active pocket of the 5-HT_{1A} receptor, the protonated nitrogen atom of **5c** had interaction with residue D3.32 of D_2 receptor, the benzene in the head part formed π-π interaction with F6.52 and the benzene in the tail part of **5c** formed π-π interaction with W7.40 (**Figure 4B**). In the case of binding mode of compound **5c** with 5-HT_{2A} receptor (**Figure 4C**), the protonated nitrogen atom formed a hydrogen bond with residue D3.32 and the benzene in the head part formed π-π interaction with F6.52. Thus compound **5c** was found to display more potent activity toward 5-HT_{1A} receptor than that of D_2 and 5-HT_{2A} receptor. In terms of a selective and moderate D_2 receptor antagonist **5g**, the protonated nitrogen atom formed strong salt bridge with D3.32, the benzene in the head formed π-π interaction with F5.47 and F6.52, the benzene in the tail formed π-π interaction with W100 (**Figure 4D**). While in terms of the binding mode of **5g** with 5-HT_{1A} and 5-HT_{2A} receptors, the phenylpiperazine moiety of **5g** is not able to reach into the deep pocket of receptors due to the existence of 2-methoxyphenyl, leading to the cyclopropylmethyl in **5g** collapsed with C187 and

C227 in the active pocket of 5-HT_{1A} and 5-HT_{2A} receptors, respectively (**Figure 4E** and **4F**). Thus, **5g** lost activity toward 5-HT_{1A} and 5-HT_{2A} receptors. For selective 5-HT_{1A} receptor agonist 5**k,** the cyclopropylmethyl collapsed with V3.29 in the pocket of D_2 receptor (**Figure 4G**), which is caused by the lack of conformational constraint since there is no substituent in the head part. While in terms of **5k** with 5-HT_{1A} receptor (**Figure 4H**), the protonated nitrogen atom of **5k** formed strong salt bridge with D3.32 of 5-HT_{1A} receptor, the benzene in the head formed π-π interaction with W6.48 and F6.52. However, in terms of binding of **5k** with 5-HT_{2A}, the only interaction comes from the interaction of its protonated nitrogen atom with D3.32 in 5-HT_{2A} receptor. In contract, **9f** showed potent activity toward D_2 and 5-HT_{2A} receptors, while it missed the activity to 5-HT_{1A}. In terms of the binding of **9f** with D_2 receptor (**Figure 4J**), the protonated nitrogen atom of **9f** formed strong salt bridge with residue D3.32. The benzisoxazole moiety of **9f** inserted deeply into the OBS. The oxygen atom of the benzisoxazole moiety of **9f** formed a hydrogen bond with T3.37 and the oxygen atom of the oxetanyl moiety also formed a hydrogen bond with T7.39. As for the docking of **9f** with 5-HT_{2A} receptor (**Figure 4L**), the protonated nitrogen atom formed a hydrogen bond with the carboxylic acid side chain of D3.32 of 5-HT_{2A} receptor. The oxygen atom of the benzisoxazole moiety formed strong hydrogen bonds with T3.37 and S5.46, and the oxygen atom of the sulfonamide moiety also formed a hydrogen bond with the NH group of L229. While in the case of the interaction of **9f** with 5-HT_{1A} receptor (**Figure 4K**), the benzoisoxazole moiety of **9f** collapsed with F6.44 and C3.36 in the active pocket of 5-HT_{1A}, that is why **9f** missed the binding affinity for 5-HT_{1A} receptor.

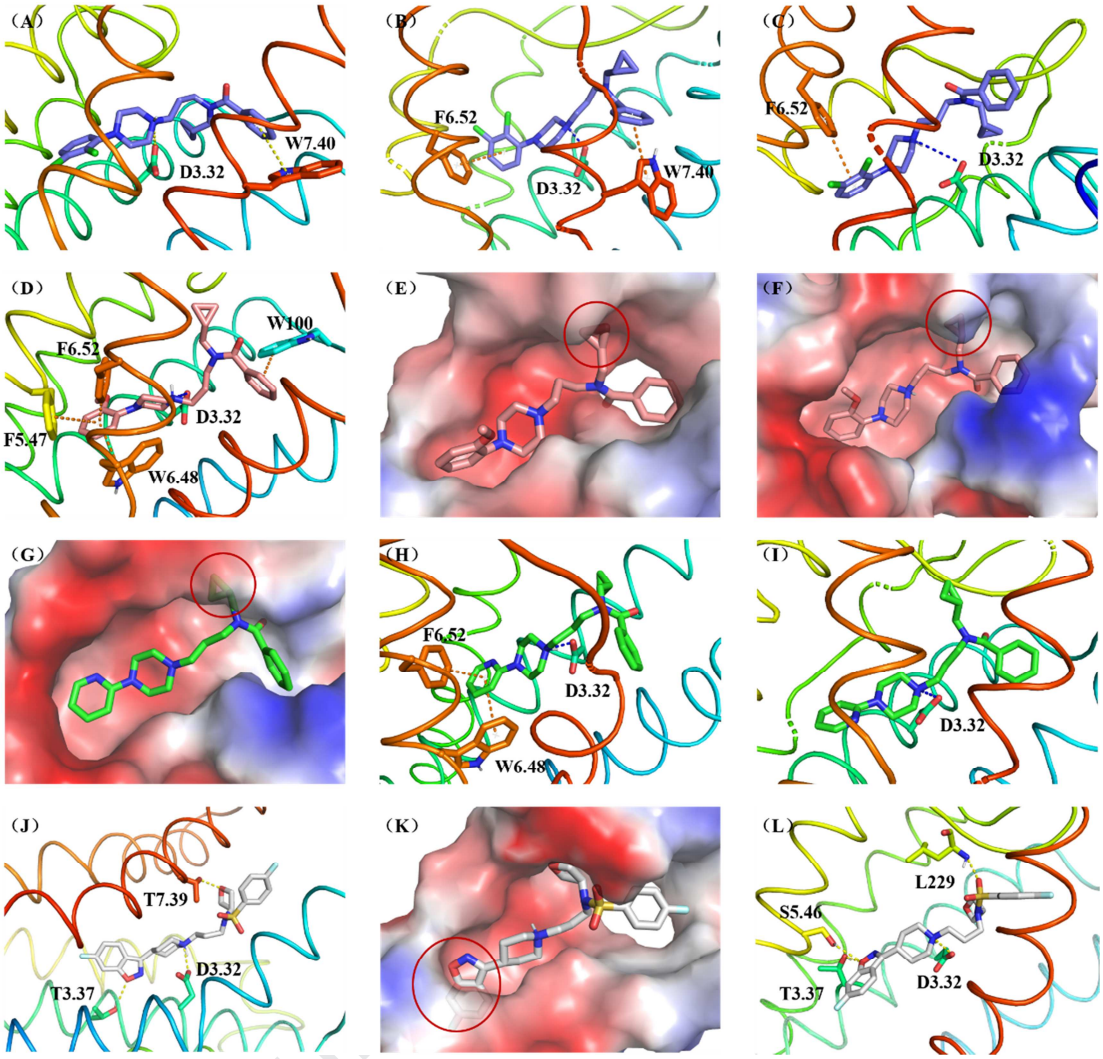


Figure 4. (A) The binding mode of compound **5c** with D_2 receptor. (B) The binding mode of compound **5c** with 5-HT_{1A} receptor. (C) The binding mode of compound **5c** with 5-HT_{2A} receptor. (D) The binding mode of compound **5g** with D_2 receptor. (E) Electrostatic potential surface of compound **5g** with 5-HT_{1A} receptor. (F) Electrostatic potential surface of compound **5g** with 5-HT_{2A} receptor. (G) Electrostatic potential surface of compound **5k** with D_2 receptor. (H) The binding mode of compound **5k** with 5-HT_{1A} receptor. (I) The binding mode of compound **5k** with 5-HT_{2A} receptor. (J) The binding mode of compound **9f** with D_2 receptor. (K) Electrostatic potential surface of compound **9f** with 5-HT_{1A} receptor. (L) The binding mode of compound **9f** with 5-HT_{2A} receptor. Compound **5c** was shown as blue sticks. Compound **5g** was shown as pink sticks. Compound **5k** was shown as green sticks. Compound **9f** was shown as white sticks.

3. Conclusion

In summary, a series of aryl-piperazine/piperidine compounds were designed,

synthesized and evaluated activity over D_2, 5-HT_{1A} and 5-HT_{2A} receptors. Among all the compounds, compound **9f**, displayed potent antagonist activity towards D_2 and 5-HT_{2A} receptors, good *in vivo* metabolic stability with $t_{1/2}$ of 2 h in ICR mice after oral administration and nice capability to penetrate the blood-brain barrier with K_p value of 4.03. Molecular docking studies elucidated the driving force for the strong association of **9f** with D_2 and 5-HT_{2A} receptors. Thus, **9f** could be used as a lead compound for further pharmacological studies and to discover potent antipsychotic agents acting on both D_2 and 5-HT_{2A} receptors.

4. Experimental protocols

4.1. Chemistry

Reagents and solvents were purchased from Adamas-beta and used without further purification. Analytical thin-layer chromatography was performed on HSGF 254 (0.15-0.2 mm thickness, Yantai Jiangyou Company, Yantai, Shandong, China). Column chromatography was carried out on silica gel (200-300 mesh). ^{1}H NMR spectra was recorded on a Brucker AMX-400/600 instrument, using TMS as an internal standard and the chemical shifts were reported in parts per million (ppm). Proton-coupling patterns were described as singlet, doublet, triplet, quartet, multiplet, and coupling constants (*J*) values are given in hertz (Hz). Mass spectra were given with an electric ionization (ESI) produced by HP5973N analytical mass spectrometer. All tested compounds had a purity of >95% determined by HPLC.

4.1.1. General procedures for the preparation of compound **2**.

To a solution of corresponding aryl-piperazine (2.6 mmol) and triethylamine (3.1 mmol) in anhydrous dichloromethane (5 ml), 3-chloropropionyl chloride (3.1 mmol) was added at 0°C and then stirred at room temperature for 10 min. After completion of the reaction, 20 ml water was added and the aqueous phase was washed with dichloromethane (3×15 ml). The organic phases were combined, washed with saturated brine, and dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 5:1, V/V) to yield compound **2**.

4.1.2. General procedures for the preparation of compound **3**.

To a solution of **2** (2.0 mmol) in 20 ml anhydrous tetrahydrofuran, borane-methyl sulfide complex (8.0 mmol) was added at 0°C under N_2 protection over 30 min and then stirred at 70 □ for 2 h. 40 ml methanol was then added and the reaction mixture was stirred at 70 □ for another 1 h. After completion of the reaction, the solvent was evaporated and 40 ml dichloromethane was added and the organic phase was washed with water (3×20 ml), saturated brine. The organic phases were combined and dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 5:1, V/V) to yield compound **3**.

4.1.3. General procedures for the preparation of compound **4**.

To a solution of **3** (2.0 mmol) in acetonitrile (5 ml), potassium carbonate (3.0 mmol) and corresponding amine (3.0 mmol) were added at room temperature. The reaction mixture was refluxed at 85 ^{0}C for 8 h. After reaction was completed, the solvent was evaporated and 40 ml dichloromethane was added and the organic phase was washed with water (3×20 ml), saturated brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane: methanol = 40:1, V/V) to yield compound **4**.

4.1.4. General procedures for the preparation of compounds **5a~5l**.

To a solution of **4** (2.0 mmol) in dichloromethane (6 ml), benzoic acid (2.4 mmol), HBTU (3.0 mmol), 1-hydroxybenzotriazole (3.0 mmol) and *N*, *N*-diisopropylethylamine (4.0 mmol) were added. The reaction mixture was stirred at room temperature for 6 h. After completion of reaction, 10 ml dichloromethane was added and the organic phase was washed with water (3×10 ml), saturated brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane: methanol = 40:1, V/V) to yield compounds **5a~5l**.

4.1.4.1. *N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)-*N*-propylbenzamide (**5a**). Compound **5a** was prepared from 3-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*-propylpropan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 80.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43

(d, *J* = 4.0 Hz, 3H), 7.30-7.34 (m, 4H), 7.12 (d, *J* = 32.0 Hz, 1H), 3.41 (d, *J* = 20.0 Hz, 3H), 3.17 (d, *J* = 32.0 Hz, 2H), 3.00 (s, 1H), 2.78 (s, 2H), 2.58 (s, 1H), 2.42 (s, 1H), 2.22 (d, *J* = 48.0 Hz, 4H), 1.80 (s, 1H), 1.61 (s, 3H), 1.49 (s, 1H), 0.92 (s, 2H), 0.67 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6, 151.3, 137.6, 132.8, 129.0, 128.6, 128.5, 126.4, 126.1, 124.5, 119.7, 54.7, 52.6, 51.0, 45.9, 40.2, 25.5, 20.6, 11.4. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 434.1760, found: 434.1761.

4.1.4.2. *N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)-*N*-isopropylbenzamide (**5b**). Compound **5b** was prepared from 3-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*-isopropylpropan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 81.7%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.43 (d, *J* = 4.0 Hz, 3H), 7.30-7.34 (m, 4H), 7.13 (d, *J* = 28.0 Hz, 1H), 3.80 (s, 1H), 3.30 (s, 1H), 3.16 (s, 1H), 3.01 (s, 3H), 2.76 (s, 1H), 2.57 (s, 2H), 2.42 (s, 2H), 2.13 (d, *J* = 20.0 Hz, 2H), 1.81 (s, 1H), 1.53 (s,1H), 1.24 (s, 2H), 1.10 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.4, 151.3, 137.9, 132.8, 129.0, 128.6, 126.1, 126.0, 124.5, 119.7, 55.6, 52.9, 51.1, 50.0, 38.4, 26.2, 20.8. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 434.1760, found: 434.1764.

4.1.4.3. *N*-(cyclopropylmethyl)-*N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)benzamide (**5c**). Compound **5c** was prepared from *N*-(cyclopropylmethyl)-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 76.2%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.43-7.44 (m, 3H), 7.30-7.36 (m, 4H), 7.13 (d, *J* = 32.0 Hz, 1H), 3.56 (s, 1H), 3.31 (s, 1H), 3.04 (d, *J* = 28.0 Hz, 3H), 2.79 (s, 2H), 2.59 (s, 2H), 2.43 (s, 2H), 2.28 (s, 2H), 2.17 (s, 1H), 1.84 (s, 1H), 1.65 (s, 1H), 1.00 (d, *J* = 76.0 Hz, 1H), 0.48 (d, *J* = 20.0 Hz, 2H), 0.31 (s, 1H), 0.05 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7, 151.3, 137.5, 132.8, 129.1, 128.6, 128.5, 126.5, 126.1, 124.5, 119.7, 55.1, 53.3, 52.6, 51.0, 48.2, 40.2, 10.4, 3.6. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 446.1760, found: 446.1766.

4.1.4.4. *N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)-*N*-(oxetan-3-yl)benzamide (**5d**).

Compound **5d** was prepared from *N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)oxetan-3-amine and benzoic acid, and obtained as yellowish oil. Yield 76.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 4.0 Hz, 3H), 7.30-7.32 (m, 3H), 7.10 (s, 1H), 4.89-4.92 (m, 1H), 4.67 (d, J = 8.0 Hz, 4H), 3.41 (s, 2H), 2.78 (s, 6H), 2.27 (s, 5H), 1.19 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.3, 136.6, 132.8, 129.8, 129.8, 128.6, 128.6, 127.0, 126.1, 124.5, 119.6, 74.9, 55.1, 52.7, 51.0, 40.2, 25.6. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 448.1553, found: 448.1554.

4.1.4.5. *N*-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-*N*-propylbenzamide (**5e**). Compound **5e** was prepared from 3-(4-(2-methoxyphenyl)piperazin-1-yl)-*N*-propylpropan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 73.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (s, 3H), 7.32-7.34 (m, 2H), 6.82-6.92 (m, 4H), 3.76 (d, J = 8.0 Hz, 3H), 3.38-3.43 (m, 2H), 3.13-3.24 (m, 3H), 2.97 (s, 2H), 2.75 (s, 2H), 2.53 (s, 1H), 2.39 (s, 1H), 2.24 (s, 2H), 2.13 (t, J = 8.0 Hz, 1H), 1.79 (s, 1H), 1.58-1.63 (m, 2H), 1.48 (s, 1H), 1.00 (t, J = 8.0 Hz, 1H), 0.91 (t, J = 8.0 Hz, 1H), 0.67 (t, J = 8.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.4, 152.1, 141.4, 137.9, 129.0, 128.6, 126.0, 122.5, 121.0, 118.0, 112.1, 67.4, 55.8, 53.1, 50.2, 40.2, 38.5, 26.2, 20.8. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 396.2646, found: 396.2648.

4.1.4.6. *N*-isopropyl-*N*-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)benzamide (**5f**). Compound **5f** was prepared from *N*-isopropyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as a yellowish oil. Yield 79.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (d, J = 4.0 Hz, 3H), 7.32-7.34 (m, 2H), 6.87-6.93 (m, 4H), 3.77 (s, 3H), 3.29 (s, 2H), 3.16 (s, 1H), 2.97 (s, 3H), 2.73 (s, 1H), 2.55 (s, 2H), 2.40 (s, 2H), 2.11 (d, J = 16.0 Hz, 2H), 1.80 (s, 1H), 1.23-1.24 (m, 2H), 1.10 (d, J = 8.0 Hz, 4H), 0.99-1.02 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6, 152.1, 141.4, 137.6, 129.0, 128.5, 126.4, 122.5, 121.0, 118.0, 112.1, 55.5, 54.8, 52.9, 50.1, 46.8, 25.5, 20.6, 11.4. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 396.2646, found: 396.2647.

4.1.4.7.

N-(cyclopropylmethyl)-*N*-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)benzamide (**5g**). Compound **5g** was prepared from *N*-(cyclopropylmethyl)-3-(4-(2-methoxyphenyl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 68.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (s, 3H), 7.34 (d, J = 4.0 Hz, 2H), 6.82-6.92 (m, 4H), 3.76 (s, 3H), 3.55 (s, 1H), 3.30 (s, 2H), 3.07 (s, 1H), 2.97 (s, 2H), 2.76 (s, 2H), 2.54 (s, 1H), 2.39 (s, 1H), 2.25 (s, 2H), 2.14 (s, 1H), 1.82 (s, 1H), 1.64 (s, 1H), 0.90-1.09 (m, 2H), 0.47 (d, J = 24.0 Hz, 2H), 0.30 (s, 1H), 0.05 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7, 152.1, 141.4, 137.5, 129.1, 128.5, 126.5, 122.5, 121.0, 118.0, 112.1, 55.5, 55.1, 53.2, 50.2, 40.2, 24.6, 9.9, 3.6. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 408.2646, found: 408.2649.

4.1.4.8. *N*-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-*N*-(oxetan-3-yl)benzamide (**5h**). Compound **5h** was prepared from *N*-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)oxetan-3-amine and benzoic acid, and obtained as yellowish oil. Yield 78.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (s, 5H), 6.85-6.92 (m, 4H), 4.90 (t, J = 8.0 Hz, 1H), 4.62-4.68 (m, 4H), 3.75 (s, 3H), 3.57 (s, 1H), 3.42 (s, 1H), 2.74 (s, 5H), 2.17 (d, J = 44.0 Hz, 5H), 1.81 (s, 1H), 1.46 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.8, 141.1, 136.3, 129.4, 128.2, 126.7, 122.1, 120.7, 117.7, 111.8, 74.6, 55.1, 52.6, 49.8, 39.9, 28.9, 26.4, 25.3. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 410.2438, found: 410.2442.

4.1.4.9. *N*-propyl-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)benzamide (**5i**). Compound **5i** was prepared from *N*-propyl-3-(4-(pyridin-2-yl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 65.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.52 (s, 1H), 7.42-7.44 (m, 3H), 7.34 (s, 2H), 6.75-6.83 (dd, J_1 = 20.0 Hz, J_2 = 8.0 Hz, 1H), 6.63 (s, 1H), 3.47 (s, 3H), 3.26 (s, 4H), 3.13 (s, 1H), 2.43 (d, J = 36.0 Hz, 2H), 2.16 (d, J = 24.0 Hz, 4H), 1.80 (s, 1H), 1.62 (s, 2H), 1.48 (s, 1H), 0.92 (d, J = 8.0 Hz, 2H), 0.67 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6, 159.2, 147.7, 137.6, 129.0, 128.5, 126.4, 113.1, 107.2, 54.8, 52.4, 45.9, 44.7, 40.2, 25.4, 20.6, 11.4. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 367.2492, found: 367.2496.

4.1.4.10. *N*-isopropyl-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)benzamide **(5j)**. Compound **5j** was prepared from *N*-isopropyl-3-(4-(pyridin-2-yl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 74.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 3H), 7.34 (d, J = 4.0 Hz, 2H), 6.83 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 3.80 (s, 1H), 3.48 (s, 1H), 3.16-3.30 (m, 4H), 2.40 (s, 2H), 2.08 (s, 2H), 1.54 (s, 1H), 1.24 (s, 1H), 1.09-1.11 (m, 5H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.4, 159.2, 147.7, 137.9, 137.6, 129.0, 128.6, 126.0, 113.1, 107.2, 55.7, 52.6, 50.0, 44.8, 38.4, 26.2, 20.8. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 367.2492, found: 367.2498.

4.1.4.11. *N*-(cyclopropylmethyl)-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)benzamide (**5k**). Compound **5k** was prepared from *N*-(cyclopropylmethyl)-3-(4-(pyridin-2-yl)piperazin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 62.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.50-7.53 (m, 1H), 7.43-7.44 (m, 3H), 7.36 (s, 2H), 6.76-6.84 (dd, J_1 = 24.0 Hz, J_2 = 2.0 Hz, 1H), 6.63 (s, 1H), 3.51 (d, J = 36.0 Hz, 4H), 3.27 (s, 4H), 3.07 (s, 1H), 2.43 (d, J = 36.0 Hz, 2H), 2.16 (d, J = 24.0 Hz, 3H), 1.84 (s, 1H), 1.65 (s, 1H), 1.00 (d, J = 72.0 Hz, 1H), 0.47 (d, J = 24.0 Hz, 2H), 0.31 (s, 1H), 0.05 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7, 159.2, 147.7, 137.6, 137.5, 129.1, 128.5, 126.5, 113.1, 107.2, 55.5, 52.7, 48.2, 44.9, 40.2, 24.6, 10.4, 3.6. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 379.2492, found: 379.2497.

4.1.4.12. *N*-(oxetan-3-yl)-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)benzamide (**5l**). Compound **5l** was prepared from *N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)oxetan-3-amine and benzoic acid, and obtained as yellowish oil. Yield 64.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08-8.10 (m, 1H), 7.46-7.53 (m, 6H), 6.79 (s, 1H), 6.61-6.64 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.89-4.92 (m, 1H), 4.66 (t, J = 8.0 Hz, 4H), 3.44 (s, 2H), 3.25 (s, 3H), 2.42 (s, 2H), 2.16 (s, 5H), 1.47 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.2, 147.7, 137.6, 136.6, 129.8, 128.5, 127.0, 113.1, 107.2, 74.9, 54.8, 52.5, 44.7, 40.2, 25.6. HRMS

(ESI^+) m/z $[M+H]^+$ calculated: 381.2285, found: 381.2292.

4.1.5. General procedures for the preparation of compound **7**.

To a solution of 6-fluoro-3-(piperidin-4-yl)benzo[*d*]isoxazole (45 mmol) and potassium carbonate (91 mmol) in *N*,*N*-dimethylformamide (80 ml) in ice-water bath, a solution of 1-bromo-3-chloropropane in *N*,*N*-dimethylformamide (20 ml) was dropped into the stirred suspension for 30 min. Reaction continued for 17 h at room temperature. After completion of the reaction, the reaction mixture was poured into water and washed with ethyl acetate (5×40 ml). The organic phases were combined and was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by chromatography (petroleum ether: ethyl acetate = 2:1, V/V) to yield compound **7**..

4.1.6. General procedures for the preparation of compound **8**.

To a solution of **7** (2.0 mmol) in acetonitrile (6 ml), potassium carbonate (2.4 mmol) and corresponding amine (2.4 mmol) were added. The reaction mixture was refluxed for 8 h at 85℃. After completion of the reaction, acetonitrile was removed and 40 ml dichloromethane was added and the organic phase was washed with water (3×20 ml), saturated brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane: methanol = 40:1, V/V) to yield compound **8**.

4.1.7. General procedures for the preparation of compound **9a-d**.

To a solution of **8** (1.0 mmol) in dichloromethane (5 ml), benzoic acid (1.2 mmol), HBTU (1.5 mmol), 1-hydroxybenzotriazole (1.5 mmol) and *N*,*N*-diisopropylethylamine (2.0 mmol) were added. The reaction mixture was stirred at room temperature for 6 h. After completion of the reaction, 10 ml dichloromethane was added and the organic phase was washed with water (3×10 ml), saturated brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane: methanol = 40:1, V/V) to yield compounds **9a~9d**.

4.1.7.1.

N-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-propylbenzamide

(**9a**). Compound **9a** was prepared from 3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)-*N*-propylpropan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 60.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, J = 36.0 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.33 (s, 3H), 7.42 (s, 3H), 3.17 (d, J = 32.0 Hz, 3H), 3.03 (s, 2H), 2.67 (s, 1H), 2.39 (s, 1H), 2.08 (d, J = 40.0 Hz, 3H), 1.80-1.88 (m, 4H), 1.61 (s, 4H), 1.48 (s, 1H), 0.91 (s, 2H), 0.66 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6, 164.6, 163.2, 161.5, 137.6, 129.0, 128.4, 126.3, 123.9, 117.4, 112.7, 97.6, 55.0, 52.8, 46.9, 40.2, 33.6, 30.3, 25.6, 20.5, 11.4. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 424.2395 found: 424.2401.

4.1.7.2.

N-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-isopropylbenzamide **(9b**). Compound **9b** was prepared from 3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)-*N*-isopropylpropan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 64.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (d, J = 28.0 Hz, 1H), 7.69-7.22 (dd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H), 7.43 (s, 3H), 7.27-7.34 (m, 3H), 3.80 (s, 1H), 3.29 (s, 2H), 3.10 (d, J = 52.0 Hz, 3H), 2.40 (s, 1H), 2.08 (s, 4H), 1.83 (s, 4H), 1.55 (s, 1H), 1.25 (s, 2H), 1.11 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.4, 164.6, 163.2, 161.5, 138.0, 128.9, 128.6, 126.0, 123.9, 117.4, 112.7, 97.5, 55.9, 53.1, 50.0, 38.5, 33.7, 30.3, 26.4, 20.8. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 424.2395, found: 424.2303.

4.1.7.3.

N-(cyclopropylmethyl)-*N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)benzamide (**9c**). Compound **9c** was prepared from *N*-(cyclopropylmethyl)-3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propan-1-amine and benzoic acid, and obtained as yellowish oil. Yield 61.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 32.0 Hz, 1H), 7.69-7.72 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 3H), 7.36 (d, J = 4.0 Hz, 2H), 7.30 (s, 1H), 3.56 (s, 1H), 3.04-3.16 (m, 4H), 2.68 (s, 1H), 2.40 (s, 1H), 2.11 (d, J = 28.0 Hz, 4H), 1.86 (s, 4H), 1.64 (s, 2H), 0.91-1.10 (m, 1H), 0.48 (d, J = 24.0 Hz, 2H), 0.31 (s, 1H), 0.06 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7, 164.6, 163.2, 161.5, 137.5, 129.0, 128.5,

126.4, 123.9, 117.4, 112.7, 97.6, 55.0, 53.1, 48.2, 40.1, 33.6, 30.3, 25.6, 10.3, 3.5. HRMS (ESI^+) m/z $[M+H]^+$ calculated: 436.2395, found: 436.2400.

4.1.7.4.

N-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)benzamide (**9d**). Compound **9d** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and benzoic acid, and obtained as yellowish solid. Yield 62.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (s, 1H), 7.69-7.72 (dd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H), 7.45 (s, 5H), 7.28-7.33 (m, 1H), 4.91 (p, J = 8.0 Hz, 1H), 4.50-4.69 (m, 4H), 3.43 (s, 1H), 3.04 (s, 2H), 2.64 (s, 2H), 2.11 (s, 3H), 1.87 (s, 4H), 1.52 (d, J = 44.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.6, 163.2, 162.9, 161.4, 136.6, 129.7, 128.5, 127.0, 123.9, 117.3, 112.7, 97.6, 74.9, 55.0, 52.9, 40.2, 33.6, 30.2, 25.8. HRMS (ESI^+) m/z $[M+H]^+$ calculated: 438.2187, found: 438.2191.

4.1.8. General procedure for the preparation of compounds **9e~9i**.

To a solution of **8** (1.0 mmol) in anhydrous dichloromethane (5 ml) in ice-water bath, corresponding benzenesulfonyl chloride derivatives (1.5 mmol) and *N*,*N*-diisopropylethylamine (1.5 mmol) were added. The reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, 10 ml dichloromethane was added and the organic phase was washed with water (3×10 ml), saturated brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane: methanol = 40:1, V/V) to yield compounds **9e~9i**.

4.1.8.1

N-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)benzenesulfonamide (**9e**). Compound **9e** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and benzenesulfonyl chloride, and obtained as yellowish solid. Yield 54.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99-8.02 (dd, J_1 =8.0 Hz, J_2 = 4.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.69-7.75 (m, 2H), 7.64 (t, J = 8.0 Hz, 2H), 7.26-7.31 (td, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.80 (p, J = 4.0 Hz, 1H), 4.59-4.62 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H),

4.49-4.53 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 3.11-3.20 (m, 3H), 2.93 (d, J = 12.0 Hz, 2H), 2.34 (t, J = 8.0 Hz, 2H), 2.02-2.09 (m, 4H), 1.71-1.84 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.6, 163.2, 162.9, 161.5, 138.0, 133.4, 129.8, 127.1, 123.9, 117.4, 112.7, 97.6, 75.0, 54.9, 53.0, 51.9, 43.6, 40.2, 33.6, 30.3, 27.4. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 474.1857, found: 474.1863.

4.1.8.2.

4-Fluoro-*N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)benzenesulfonamide (**9f**). Compound **9f** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and 4-fluorobenzenesulfonyl chloride, and obtained as yellowish solid. Yield 63.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99-8.03 (dd, J_1 =8.0 Hz, J_2 = 4.0 Hz, 1H), 7.87-7.90 (m, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.46-7.50 (m, 2H), 7.29 (t, J = 8.0 Hz, 1H), 4.79 (p, J = 8.0 Hz, 1H), 4.61 (t, J = 8.0 Hz, 2H), 4.53 (t, J = 8.0 Hz, 2H), 3.12-3.21 (m, 3H), 2.94 (d, J = 8.0 Hz, 2H), 2.36 (t, J = 8.0 Hz, 2H), 2.03-2.10 (m, 4H), 1.74-1.85 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.3, 164.3, 162.9, 162.6, 161.2, 134.1, 130.0, 123.6, 117.1, 116.7, 112.4, 97.2, 74.7, 54.6, 52.7, 51.5, 43.3, 33.3, 30.0, 27.1. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 492.1763, found: 492.1767.

4.1.8.3.

4-Chloro-*N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)benzenesulfonamide (**9g**). Compound **9g** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and 4-chlorobenzenesulfonyl chloride, and obtained as yellowish solid. Yield 63.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99-8.02 (dd, J_1 =8.0 Hz, J_2 = 4.0 Hz, 1H), 7.81-7.83 (dd, J_1 =8.0 Hz, J_2 = 4.0 Hz, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.46-7.50 (m, 2H), 7.29 (t, J = 8.0 Hz, 1H), 4.79 (p, J = 8.0 Hz, 1H), 4.61 (t, J = 8.0 Hz, 2H), 4.53 (t, J = 8.0 Hz, 2H), 3.12-3.21 (m, 3H), 2.94 (d, J = 8.0 Hz, 2H), 2.36 (t, J = 8.0 Hz, 2H), 2.03-2.10 (m, 4H), 1.74-1.85 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.3, 162.9, 162.6, 161.2, 138.1, 136.6, 129.5, 128.8, 123.6, 117.1, 112.4, 97.2, 74.7, 54.6, 52.7, 51.5, 43.4, 33.3, 30.0, 27.1. HRMS (ESI$^+$) m/z $[M+H]^+$ calculated: 508.1468, found: 508.1471.

4.1.8.4.

N-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)-4-(trifluoromethyl)benzenesulfonamide (**9h**). Compound **9h** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and 4-(trifluoromethyl)benzenesulfonyl chloride, and obtained as yellowish solid. Yield 64.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99-8.05 (m, 5H), 7.69-7.72 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 2H), 7.69-7.72 (dt, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 3H), 7.26-7.31 (td, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.81 (p, J = 8.0 Hz, 1H), 4.61 (t, J = 8.0 Hz, 2H), 4.54 (t, J = 8.0 Hz, 2H), 3.20 (t, J = 8.0 Hz, 2H), 3.11-3.14 (m, 1H), 2.93 (d, J = 12.0 Hz, 2H), 2.35 (t, J = 8.0 Hz, 2H), 2.02-2.10 (m, 4H), 1.72-1.85 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.3, 162.9, 162.6, 161.2, 141.7, 132.8, 127.9, 126.6, 123.6, 117.1, 112.4, 97.2, 74.6, 54.6, 52.7, 51.5, 43.5, 33.3, 30.0, 27.1. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 542.1731, found: 542.1730.

4.1.8.5.

4-Cyano-*N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)-*N*-(oxetan-3-yl)benzenesulfonamide (**9i**). Compound **9i** was prepared from *N*-(3-(4-(6-fluorobenzo[*d*]isoxazol-3-yl)piperidin-1-yl)propyl)oxetan-3-amine and 4-cyanobenzenesulfonyl chloride, and obtained as yellowish solid. Yield 66.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, J = 8.0 Hz, 2H), 7.98-8.02 (m, 3H), 7.69-7.72 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 7.26-7.31 (td, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H), 4.87 (p, J = 8.0 Hz, 1H), 4.62 (t, J = 8.0 Hz, 2H), 4.54 (t, J = 8.0 Hz, 2H), 3.25 (t, J = 8.0 Hz, 2H), 3.14 (t, J = 12.0 Hz, 1H), 2.94 (d, J = 12.0 Hz, 2H), 2.35 (t, J = 8.0 Hz, 2H), 2.02-2.10 (m, 4H), 1.73-1.85 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.3, 162.9, 162.6, 161.2, 141.9, 133.6, 127.6, 123.6, 117.1, 115.5, 112.4, 97.3, 74.6, 54.5, 52.7, 51.5, 43.4, 33.3, 30.0, 27.2. HRMS (ESI$^+$) m/z [M+H]$^+$ calculated: 499.1810, found: 499.1812.

4.2. Biological Evaluation

4.2.1. Ultra Lance cAMP assay

The Ultra Lance cAMP assay was performed to evaluate the function of synthesized compounds to D_2 receptor and 5-HT_{1A} receptor, using HEK-293 cells expressing the

human D_2 receptor and 5-HT_{1A} receptor. Reference compounds for D_2 receptor and 5-HT_{1A} receptor were risperidone, and 8-OH-DPAT, respectively. Procedures for Ultra Lance cAMP assay are: (1) Transfer compound to assay plate by Echo (compound total volume 100 μl; (2) Collect cells with stimulation buffer; (3) Transfer 10 μl of cell solution to assay plate; (4) Centrifuge at 600 rpm for 3 min and incubate 60 min at room temperature; (5) Add 5 μl 4X Eu-cAMP tracer solution and 5 μl 4X ULight™-anti-cAMP solution to assay plate; (6) Centrifuge at 600 rpm for 3 min and incubate 60 min at room temperature; (7) Read plate on EnVision. The IC_{50} values were calculated by nonlinear regression using a sigmoidal function.

4.2.2. Fluorometric Imaging Plate Reader (FLIPR) assay

Using CHO-K1 cells expressing human 5-HT_{2A} receptor, The FLIPR assay was performed to evaluate the function of synthesized compounds to 5-HT_{2A} receptor. Reference compounds for 5-HT_{2A} receptor was risperidone. Procedures for FLIPR assay are : (1) Add 50 μl of the cell suspension to each well on the assay plate and place the assay plate in 37□, 5% CO_2 incubate for 16-24 h; (2) Remove cell plates from the incubator or centrifuge, remove the supernatant and add 30 μl 1* dye; (3) Place the assay plates in the 37□, 5% CO_2 incubator for 1 h; (4) add 30 μl assay buffer into the assay plate and then shake for 20-40 min; (5) Place the plates on the FLIPR and 15 μl /well of test compound is added and calcium flux signal is measured. After 15 min add 22.5 μl /well of inducer and calcium flux signal is measured. The IC_{50} values were calculated by nonlinear regression using a sigmoidal function.

4.2.3. *In vitro* metabolic stability assay

The *in vitro* metabolic stability assay was performed to evaluate metabolic stability of these newly discovered compounds in incubation with mouse liver microsomes. Compounds were first dissolved in methanol to obtain the 1 mM stock solution. The working solution was prepared by diluting 10 μl from stock solution with 90 μl methanol and 900 μl water. The incubation system was consisted of 40 μl working solution, 10 μl 20 mg/ml mouse liver microsomes and 310 μl 0.1 M PBS (pH = 7.4) and incubated at 37□. Add 40 μl 10 mM NADPH to start the reaction and timing. 50

μl incubation medium was collected at 0 min, 5 min, 10 min, 20 min, 40 min, 60 min and then added to 100 μl cold methanol, centrifuge at 4000 rpm for 15 min under 4□. The supernatant was analyzed by liquid chromatography tandem with spectrometry (LC-MS/MS).

4.3. Molecular Modeling

4.3.1. Homology Modeling

The amino acid sequence of 5-HT_{1A} receptor were downloaded from the UniProtKB database (Entry code: P08908), and sequence similarity search was performed using NCBI BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The structure (PDB code: 4IAR) of 5-HT_{1B} receptor was selected as the template to construct the agonistic conformation of 5-HT_{1A} receptor. Sequence alignment of 5-HT_{1B} receptor and 5-HT_{1A} receptor was carried out using Discovery Studio 2016 (hereafter abbreviated to DS). Homology modeling was performed with DS. Ten models were generated after loop refinement and the one with the lowest Discrete Optimized Protein Energy (DOPE) score was submitted to energy minimization (100 steps steepest descent with backbone constrained). The PROCHECK program (http://servicesn.mbi.ucla.edu/PROCHECK) was used to evaluate the stereochemical quality of 5-HT_{1A} receptor.

4.3.2. Molecular Docking Operations

Molecular docking was carried out using GOLD 5.0.1. The binding site was defined to include all residues within a 15.0 Å radius of the conserved D3.32 Cγ carbon atom of D_2, 5-HT_{1A} and 5-HT_{2A} receptors. A hydrogen-bond constraint was set between the protonated nitrogen atom (N1) of ligand and D3.32 of D_2, 5-HT_{1A} and 5-HT_{2A} receptor. Ten conformations were produced for each ligand, and Gold-Score was used as scoring function. Other parameters were set as standard default. High-scoring complexes were inspected visually to identify the most reasonable solution. **FW01** was docked with 5-HT_{1A} and D_2 receptors, respectively. Compound **1** and iloperidone was docked with D_2 receptor, respectively. Compounds **5c**, **5g**, **5k** and **9f** were docked with D_2, 5-HT_{2A} and 5-HT_{1A} receptors respectively.

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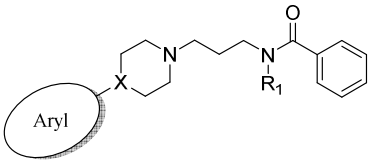
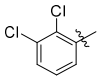
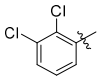
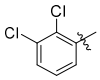
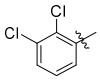
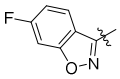
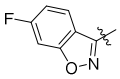
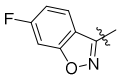
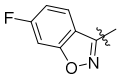
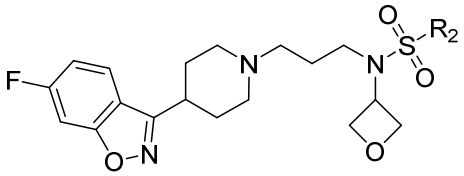
Table 1 Biological activities of compounds **5a~5l**, and **9a~9d** for D_2, 5-HT_{1A} and 5-HT_{2A} receptors.

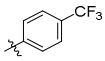


Compounds	Aryl	X	R_1	D_2 IC_{50} (nM)	5-HT_{1A} EC_{50} (nM)	5-HT_{2A} IC_{50} (nM)	in *vitro* $t_{1/2}$
FW01	--	--	--	2161.35±25.55 [a]	7	206.71±7.46 [a]	--
5a		N		484.7	451.6	3249	--
5b		N		528	**46.1**	2008	--
5c		N		**459.2**	**175.1**	768.1	--
5d		N		1878	583.2	2177	--
5e		N		466.9	NA	NA	--
5f		N		823.5	NA	NA	--
5g		N		**288.7**	NA	NA	--
5h		N		3765	NA	NA	--
5i		N		6935	54.5	7549	--
5j		N		NA	22.8	NA	--
5k		N		NA	24.9	7719	--
5l		N		NA	373.8	NA	--
9a		C		18.5	NA	73.1	6.2
9b		C		59.3	NA	11.6	31.5
9c		C		10.8	NA	40.8	6.9
9d		C		156.0	NA	18.8	173.2

a: Ki value; NA: no activity.

Table 2 Biological activities of compounds **9e~9i** for D_2, 5-HT_{1A} and 5-HT_{2A} receptors.



Compounds	R_2	D_2 IC_{50} (nM)	5-HT_{1A} EC_{50} (nM)	5-HT_{2A} IC_{50} (nM)
9e		1.9	NA	9.3
9f		**3.0**	**NA**	**15.1**
9g		2.0	NA	34.6
9h		2.3	NA	92.7
9i		3.6	NA	11.3

NA: no activity

Table 3. Pharmacokinetics data of compound **9f** measured in ICR mice (n = 6/group).

Dose (mg/kg)	AUC_{0-24h} (ng×h/ml)	$t_{1/2}$ (h)	C_{max} (ng/ml)	T_{max} (h)	F (%)
5 (i.v.)	829.1	0.54	-	-	-
25 (p.o.)	259.4	2.00	141.7	0.25	6.3

Table 4. Brain-plasma ratio of **9f** measured in ICR mice after (n = 3/group).

Dose (mg/kg)	Time point (h)	C_{Plasma} (ng/ml)	C_{CSF} (ng/ml)	C_{Brain} (h)	K_p
1 (iv)	1	75.4	2.0	129.1	1.72
	4	2.7	0.0	10.7	4.03

Research highlights

- A series of aryl-piperazine/piperidine compounds towards both the D_2 receptor and 5-HT_{2A} receptor
- **9f** possesses high antagonist activity on D_2 receptor and 5-HT_{2A} receptor
- **9f** is able to permeate the blood-brain barrier and the in vivo $t_{1/2}$ of 9f is up to 2 h after oral administration
- The binding modes of the **9f** with D_2 receptor and 5-HT_{2A} receptor are discussed

Declaration of interests

☐ ☑ 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.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: