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Research paper

Synthesis and pharmacological characterization of novel *N*-(*trans*-4-(2-(4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl) amides as potential multireceptor atypical antipsychotics

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ABSTRACT

A series of novel benzisothiazolylpiperazine derivatives combining potent dopamine D_2 and D_3, and serotonin 5-HT_{1A} and 5-HT_{2A} receptor properties were synthesized and evaluated for their potential antipsychotic properties. The most-promising derivative was **9j**. The unique pharmacological features of **9j** were a high affinity for D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors, together with a 20-fold selectivity for the D_3 versus D_2 subtype, and a low affinity for muscarinic M_1 (reducing the risk of anticholinergic side effects), and for hERG channels (reducing incidence of QT interval prolongation). In animal behavioral models, **9j** inhibited the locomotor-stimulating effects of phencyclidine, blocked conditioned avoidance response, and improved the cognitive deficit in the novel object recognition tests in rats. **9j** exhibited a low potential for catalepsy, consistent with results with risperidone. In addition, favorable brain penetration of **9j** in rats was detected. These studies have demonstrated that **9j** is a potential atypical antipsychotic candidate.

1. Introduction

Schizophrenia is a chronic, severe, and often disabling mental disorder that affects ~1% of the global population [1,2]. Regarded as a spectrum disorder [3], schizophrenia is categorized by positive, negative, and cognitive symptoms. First generation antipsychotic agents (FGAs), such as dopamine D_2 receptor antagonists, effectively treat positive symptoms, but are ineffective in alleviating negative symptoms or cognitive impairment. FGAs can also cause serious side effects such as extrapyramidal symptoms (EPS) [4,5]. Second-generation agents (SGAs) (i.e., lurasidone, ziprasidone and risperidone, Fig. 1) are characterized by multi-receptor affinity and offer a variety of therapeutic advantages, while causing fewer EPSs than FGAs. However, SGAs cause adverse metabolic, anticholinergic and cardiovascular effects such as weight gain, constipation and prolonged heart rate-corrected QT intervals [6–8]. Inadequacies in treating these negative side effects and cognitive impairments create an unmet clinical need for patients suffering from schizophrenia. Therefore, innovative antipsychotic agents that provide superior therapeutic and decreased side effect profiles are urgently needed.

Drugs that have multiple targets are generally accepted to be better antipsychotic agents [9–12]. SGAs studies have confirmed that simultaneous action on both 5-HT_{1A} and 5-HT_{2A} receptors play a major role in managing nonpsychotic schizophrenia symptoms and reducing incidents of EPS [13,14]. Studies have also indicated that the D_2 receptor is an indispensable target for drug action, as all current antipsychotics block the D_2 receptor; aripiprazole, brexpiprazole and cariprazine (Fig. 1) are D_2 partial agonists. Dopamine D_3 receptors, which belong to a subfamily of D_2-like receptors [15], are mainly expressed in the limbic system and are engaged in regulating emotional and cognitive functions [16,17]. Selective D_3 versus D_2 receptor (D_3 vs D_2R) antagonists enhance frontocortical cholinergic transmission and improve social cognition, which may be beneficial in managing of cognitive impairment in schizophrenia [18]. Early studies have demonstrated that ligands with high preference for D_3 receptors (>60-fold selectivity for D_3 vs D_2R) were inactive in animal models with potential antipsychotic activity, such as those with conditioned avoidance behavior [19] or

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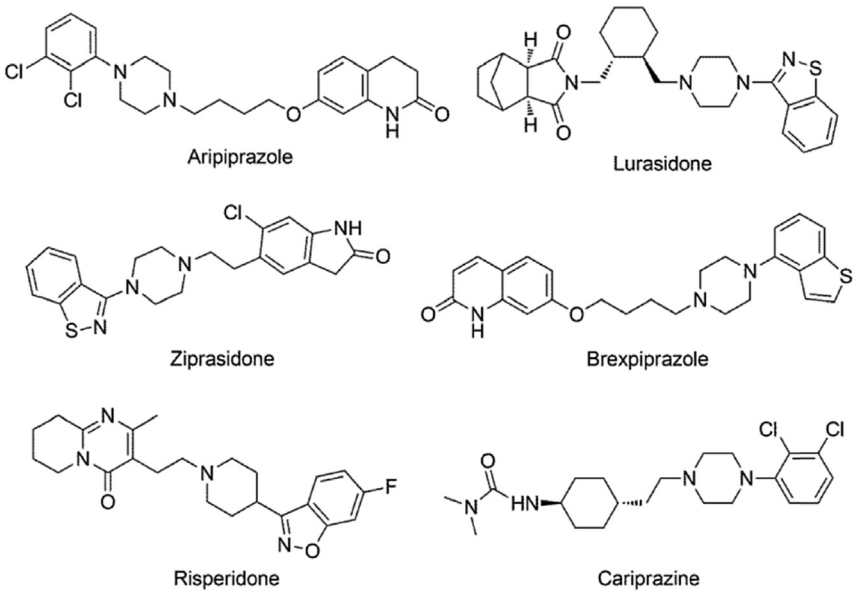


Fig. 1. Structures of reference compounds.

locomotor response to phencyclidine (PCP) in rats [20]. Recent efforts developing selective D_3 vs D_2R antagonists as potential antipsychotic agents have been focused on achieving compounds with a minimum of 10-fold D_3/D_2 selectivity, as well as a potent degree (subnanomolar) of D_3 antagonism coupled to a moderate degree (nanomolar) of D_2 antagonism to achieve simultaneous D_2 and D_3 antagonism [21–25]. Cariprazine was recently released in America as the first orally active dopamine D_3-preferring D_3/D_2 receptor partial agonist that has ~10-fold selectivity for the D_3 versus D_2 subtype and subnanomolar affinity for D_2 and D_3 receptors, and this drug exhibited good antipsychotic activity with favorable safety and tolerability [26,27].

Previously, Meltzer et al. studied the biological profiles of a series of neuroleptic agents and postulated that mixed D_2/5-HT_2 antagonists (higher affinity ratio for 5-HT_{2A} receptors relative to D_2 receptors) should possess atypical antipsychotic properties [28]. Based on this 5-HT_2/D_2 receptor antagonism concept, several atypical antipsychotic agents have been developed including risperidone [29], which has been widely used in clinical therapies.

Based on these findings, efforts were focused on obtaining new atypical antipsychotic agents that possess the following multireceptor affinity profiles: (a) high affinity for D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors (K_i < 10 nM); (b) 10–60 fold selectivity for the D_3 versus D_2 subtype as well as potent D_3 antagonism coupled to moderate D_2 antagonism; (c) a higher receptor affinity for 5-HT_{2A} than D_2 (K_i ratio > 1) [28]; (d) low affinity for muscarinic M_1 receptors (to reduce the risk of anticholinergic side effects) and for hERG channels (to reduce incidence of QT interval prolongation).

The main hurdle in discovering novel multireceptor antipsychotics lies in balancing desired receptor affinity with receptor subtype selectivity. Subtype selectivity is further complicated due to the highly similar nature of the D_2 and D_3 dopamine receptors which share approximately 50% sequence homology [30]. Studies have revealed that most D_3-specific ligands share a common structure including an amide moiety, an arylpiperazine system, and a flexible/partially rigid-spacer (e.g., cyclohexyl, cyclopropyl, alkenyl or alkynyl). Structural variations in amide moiety influence both the D_3 receptor affinity and D_3/D_2 receptor selectivity ratio [31,32], and this ratio is also altered by the spacer group [33–36]. Ligands that preferentially bind to dopamine and serotonin receptors share common arylpiperazine fragments [37–39]. 3-Benzisothiazolyl-piperazine, a known serotonin pharmacophore, was combined with dopamine activity-related pharmacophores to achieve potential antipsychotic agents that incorporate high affinity and antagonism for both 5-HT_{2A} and D_2 receptors [40–43]. In order to validate the proposed multireceptor affinity profile approach to developing novel antipsychotics, while achieving an optimum interaction with dopamine and serotonin receptors, a new set of compounds with a cyclohexylamide system linked to the benzisothiazolylpiperazine moiety was designed (Fig. 2).

Herein, we report the synthesis and pharmacological evaluation of benzisothiazolylpiperazine derivatives as potential novel multitarget antipsychotics. The structure-activity relationships of target compounds for the D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors were investigated as a function of various substituents in the amide moiety and spacer groups. The effect on receptor affinity of various substitutions at the 6-position of the benzo[*d*]isothiazole moiety was further studied, which has been seldom reported [44]. The selected compounds balanced for optimum dopamine to serotonin receptor affinity, were further subjected to behavioral study. Among these compounds, compound **9j** was chosen for further pharmacological investigation including affinity for crucial antitargets (muscarinic M_1 receptor and hERG channels) and potential atypicality (intrinsic activity at selected receptors and cataleptogenic propensity). Moreover, brain and blood pharmacokinetics of **9j** in rats were further explored as druggable properties.

2. Chemistry

The synthesis of target compounds **6**–**9**, **12**, **18**, **35**–**37**, **41** and **45** is depicted in Schemes 1–5. Scheme 1 outlines the synthesis of **6**–**9** and **12**. The starting material *N*-(4-oxocyclohexyl)acetamide was converted into the ethyl ester **1** via Horner Wittig reaction and hydrogenation, which was then hydrolyzed and recrystallized in DMF to give a *trans* carboxylic acid derivative (**2**) (57.6% yield). After an esterification reaction and $NaBH_4$/MeOH reduction, the alcohol derivative **4** was prepared. Intermediate **4** was reacted with mesyl chloride to give an activated-ester **5**, which was coupled with 1-(1,2-benzisothiazol-3-yl)piperazine to give compound **6**. Then, **6** was refluxed in 5% aqueous hydrochloric acid solution to yield the

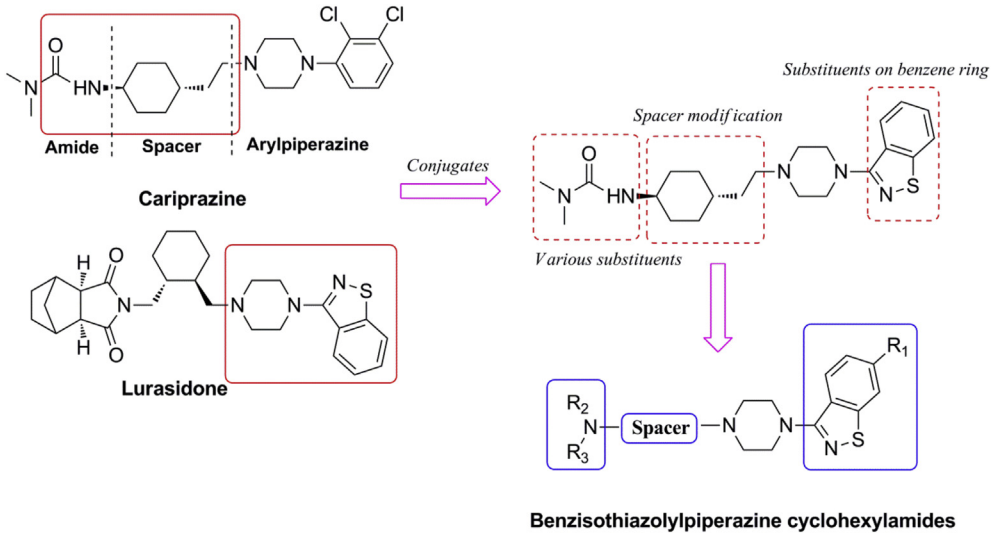
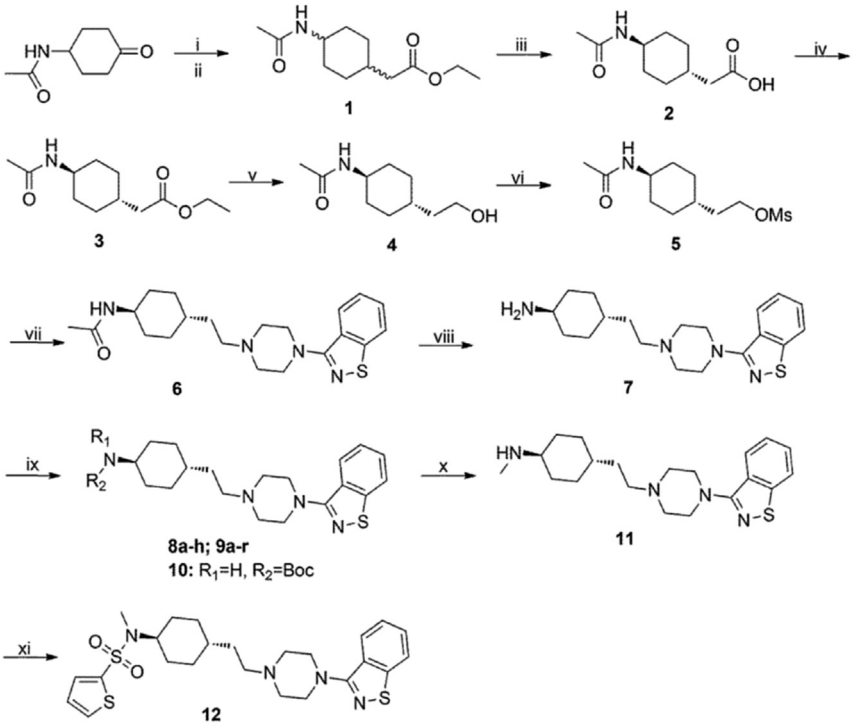


Fig. 2. Design of benzisothiazolylpiperazine cyclohexylamides.

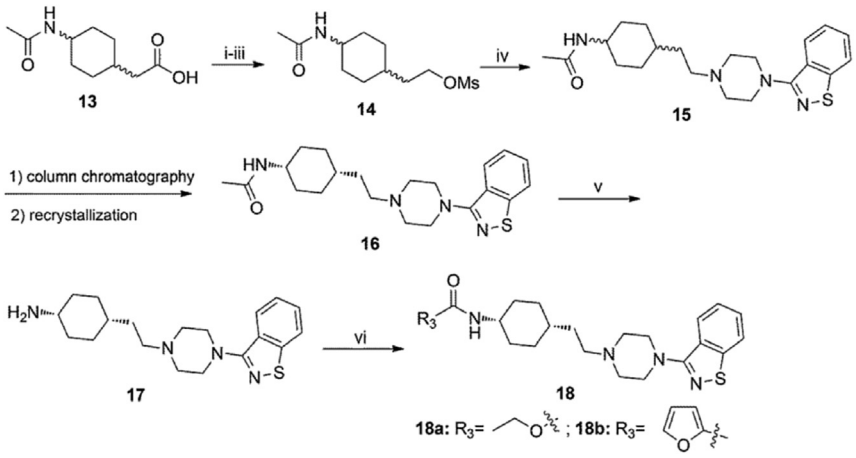


Scheme 1. Reagents and conditions: (i) Triethyl phosphonoacetate, *t*BuOK, THF, 0–5 °C; (ii) H_2, 10%Pd/C, EA, rt; (iii) LiOH·H_2O, THF, H_2O, DMF, recrystallization; (iv) EtOH, conc. H_2SO_4, reflux; (v) $NaBH_4$, MeOH, THF, reflux; (vi) Et_3N, MsCl, CH_2Cl_2, 0–5 °C; (vii) Na_2CO_3, KI, Acetonitrile, reflux; (viii) 5%HCl, reflux; (ix) for **8**: BTC, Et_3N, IPA, CH_2Cl_2; for **9a-q**: Et_3N, CH_2Cl_2, 0–5 °C; for **9r**: EDC.HCl, DMAP, CH_2Cl_2; for **10**: DIBOC, Et_3N, CH_2Cl_2; (x) $LiAlH_4$, THF, reflux; (xi) Et_3N, CH_2Cl_2, 0–5 °C.

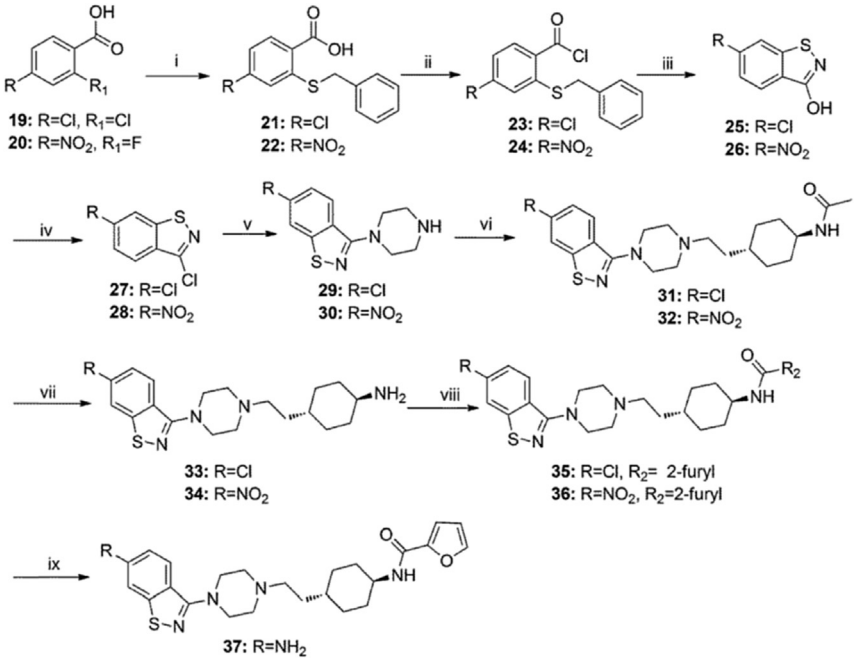
key intermediate **7**. Condensation of **7** with various amines afforded compound **8**. Compounds **9a-q** were obtained via acylation of 7 with acyl chlorides. **7** reacted with indole-2-carboxylic acid to give compound **9r**. Reduction of intermediate **10** using $LiAlH_4$ in THF afforded compound **11**, which reacted with 2-thiophenesulfonyl chloride to give target compound **12**.

Scheme 2 illustrates the synthesis of compound **18**. Using a *cis*/*trans* mixture **13** (obtained from the mother liquor of **2**) as a starting material, *cis*/*trans* mixture **15** was achieved by esterification, reduction, activation, and condensation with 1-(1,2-benzisothiazol-3-yl)piperazine. The *cis* stereoisomer **16** was obtained via column chromatography and recrystallization from **15**. Finally, compound **18** was achieved by hydrolysis and acylation.

Scheme 3 depicts the synthesis of compounds **35**–**37**. First, intermediate **23** was prepared as previously described [44]. Then, **23** was treated with sulfuryl chloride in chloroform and ammonia hydrate to provide **25**, which was chlorinated and condensed with piperazine to afford **29** [44]. In synthesizing of 3-piperazinyl derivative **30**, first benzyl mercaptan and 2-fluoro-4-nitrobenzoic **20** were condensed in the presence of DIPEA in DMF to provide compound **22**. Then, **22** was converted into the corresponding acyl chloride **24**, which was then treated with sulfuryl chloride in



Scheme 2. Reagents and conditions: (i) EtOH, conc. H_2SO_4, reflux; (ii) $NaBH_4$, MeOH, THF, reflux; (iii) Et_3N, MsCl, CH_2Cl_2, 0–5 °C; (iv) Na_2CO_3, KI, Acetonitrile, reflux; (v) 5%HCl, reflux; (vi) Et_3N, CH_2Cl_2, 0–5 °C.



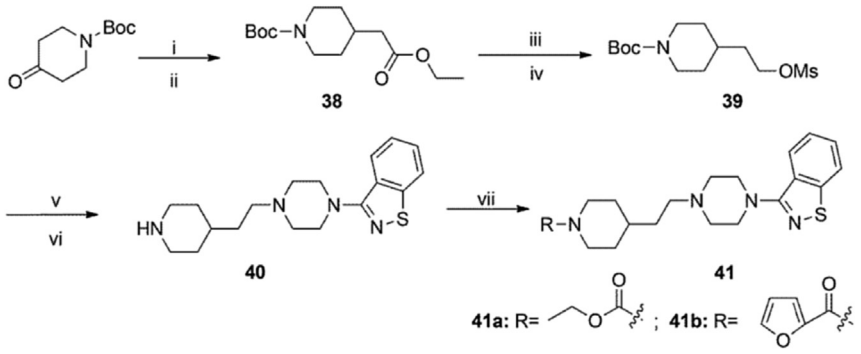
Scheme 3. Reagents and conditions: (i) for **21**: Benzyl mercaptan, CuCl, K_2CO_3, DMF, 120 °C, Nitrogen ambience; for **22**: Benzyl mercaptan, DIPEA, DMF, 65 °C; (ii) for **23**: $SOCl_2$, DMF, Toluene, reflux; for **24**: $(COCl)_2$, DMF, $CHCl_3$, reflux; (iii) SO_2Cl_2, $CHCl_3$, $NH_3 \cdot H_2O$, rt; (iv): $POCl_3$, PCl_5, DIPEA, reflux; (v) for **29**: Piperazine, Chlorobenzene, reflux; for **30**: Piperazine, Chlorobenzene, K_2CO_3, DMF, 80 °C, Nitrogen ambience; (vi) Na_2CO_3, KI, Acetonitrile, reflux; (vii) 5%HCl, reflux; (viii) Et_3N, CH_2Cl_2, 0–5 °C; (ix) R–Ni, 85% Hydrazine monohydrate, MeOH, rt.

chloroform and ammonia hydrate to provide **26**. Compound **26** was chlorinated and subsequently condensed with piperazine to afford **30**. With **29** and **30** in hand, compounds **35**–**36** were achieved by condensation of **29** and **30** with **5**, and subsequent hydrolysis, and acylation, respectively. Finally, nitro reduction of **36** by nickel Raney afforded **37**.

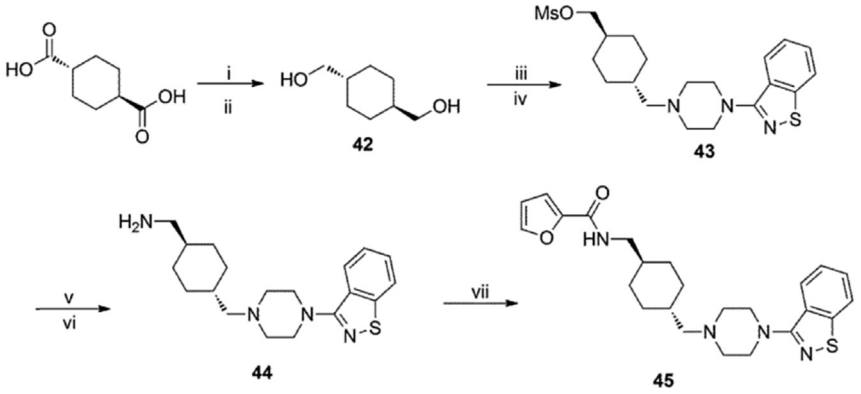
Scheme 4 describes the synthesis of compound **41**. Intermediate **39** was obtained by Horner Wittig reaction, hydrogenation, and subsequent reduction and activation with mesyl chloride. The key intermediate **40** was obtained by deprotecting the *tert*-butoxycarbonyl group. Finally, acylation of **40** with acyl chlorides furnished **41**.

Scheme 5 depicts the synthesis of compound **45**. The methyl esterification of *trans*-cyclohexane-1,4-dicarboxylic acid and corresponding $NaBH_4$/MeOH reduction yielded *trans*-cyclohexane-1,4-diyldimethanol **42**. Subsequent treatment with mesyl chloride and condensation with 3-(piperazin-1-yl)benzo[*d*]isothiazole afforded **43**. The key intermediate **44** was obtained using Gabriel reaction from **43**. Finally, **44** was condensed with 2-furoyl chloride to furnish **45**.

In order to assign the *cis*/*trans* stereochemistry, a single X-ray crystal structure was obtained for compound **6**. The single crystal image for compound **6** was numbered by atom, demonstrating that the cyclohexane ring was in the *trans* conformation (Fig. 3). It is worth noting that the corresponding compounds **7**–**9**, **12** and **35**–**37** were preferentially in the *trans* conformation of cyclohexane ring as observed in the crystal structure of **6**.



Scheme 4. Reagents and conditions: (i) Triethyl phosphonoacetate, *t*BuOK, THF, 0–5 °C; (ii) H_2, 10%Pd/C, EtOH, rt; (iii) $NaBH_4$, MeOH, reflux; (iv) Et_3N, MsCl, CH_2Cl_2, 0–5 °C; (v) Na_2CO_3, KI, Acetonitrile, reflux; (vi) TFA, CH_2Cl_2, rt; (vii) Et_3N, CH_2Cl_2, 0–5 °C.



Scheme 5. Reagents and conditions: (i) $SOCl_2$, CH_3OH, reflux; (ii) $NaBH_4$, CH_3OH, THF; (iii) Et_3N, MsCl, CH_2Cl_2, 0–5 °C; (iv) Na_2CO_3, KI, Acetonitrile, reflux; (v) Potassium phthalimide, DMF, 100 °C; (vi) 85% Hydrazine monohydrate, EtOH, reflux; (vii) 2-Furoyl chloride, Et_3N, CH_2Cl_2, 0–5 °C.

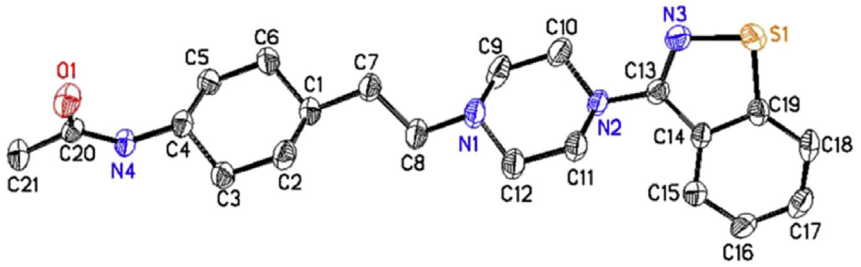


Fig. 3. ORTEP diagram of **6** with 30% thermal ellipsoid plot for non-hydrogen atoms with atom labeling. Details about X-ray crystallographic data of **6** was reported in the Supplementary Material (Table S1 and Fig. S1; CCDC 1414993).

3. Pharmacology

3.1. In vitro binding studies

The affinities (K_i values) of the synthesized compounds to human D_{2L}, D_3, 5-HT_{1A}, 5-HT_{2A} and M_1 receptors were evaluated by *in vitro* binding assays. The following specific radioligands and tissue sources were used: (a) D_{2L} receptors, [^{3}H]spiperone, human recombinant (CHO cells); (b) D_3 receptors, [^{3}H]methyl-spiperone, human recombinant (CHO cells); (c) 5-HT_{1A} receptors, [^{3}H]8-OH-DPAT, human recombinant (HEK-293 cells); (d) 5-HT_{2A} receptors, [^{3}H]ketanserin, human recombinant (HEK-293 cells); (e) M_1 receptors, [^{3}H]pirenzepine, human recombinant (CHO cells). Binding studies were conducted by Eurofins Cerep SA, Celle l'Evescault, France. Cariprazine was used as a reference.

The IC_{50} values and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting. The inhibition constants (K_i) were calculated using the Cheng Prusoff equation: $K_i = IC_{50}/(1 + L/K_D)$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor. A scatchard plot was used to determine the K_D.

The ability to block hERG potassium channels was determined using an electrophysiological method and cloned hERG potassium channels (expressed in CHO-K1 cells).

3.2. In vivo studies

The selected compounds were further subjected to preliminary pharmacological evaluation to determine their antipsychotic

activity *in vivo* animal models, including PCP-induced locomotive activity, conditioned avoidance response (CAR), novel object recognition (NOR) task, and catalepsy models.

3.3. Intrinsic activity studies

The most-promising compound was subjected to intrinsic activity studies at D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors.

3.4. In vivo pharmacokinetic studies

The selected compound was further subjected to pharmacokinetic studies in rats.

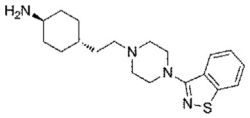
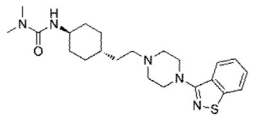
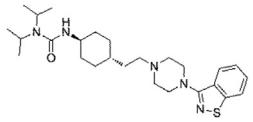
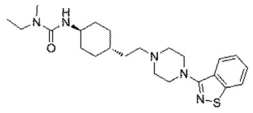
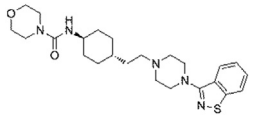
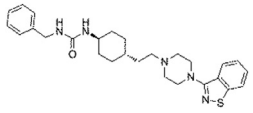
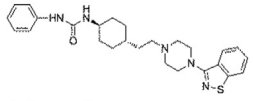
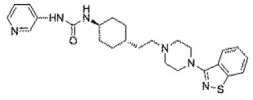
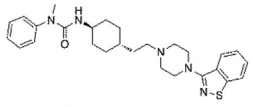
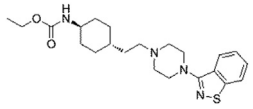
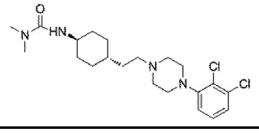
4. Results and discussion

4.1. In vitro studies of new compounds

4.1.1. Effect of various substituents in amide moiety

4.1.1.1. Effect of different urea moieties. Initially, the affinity profile of compound **7** bearing unsubstituted amine moiety at the cyclohexyl side of the molecule was investigated (Table 1). Nanomolar affinities for D_2, D_3 and 5-HT_{1A} receptors (K_i = 4.60 nM, 9.60 nM and 7.20 nM respectively), and subnanomolar affinity for 5-HT_{2A}

Table 1
Binding affinities for recombinant human D_{2L}, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with compounds **7**, **8a-h**, **9p**, and reference antipsychotic.[a]

Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
7		4.60	9.60	7.20	0.21	0.48
8a		0.53	0.40	1.20	0.60	1.33
8b		0.72	1.40	2.30	0.38	0.51
8c		0.51	0.78	1.40	0.50	0.65
8d		2.20	1.60	15.00	3.20	1.38
8e		1.40	0.18	5.30	0.91	7.78
8f		0.63	0.043	1.40	0.21	14.65
8g		0.51	0.055	12.00	0.14	9.27
8h		2.40	1.20	5.20	0.35	2.00
9p		0.71	0.058	0.20	0.53	12.24
cariprazine		1.30	0.096	3.10	23.00	13.54

[a] Shown are the mean K_i values from two independent experiments with eight concentrations in duplicates (Binding assays were conducted by Eurofins Cerep SA, Celle l'Evescault, France).

Table 2
Binding affinities for recombinant human D_{2L}, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with compounds **6**, **9a-o**, **9q-r**, and **12**.[a]

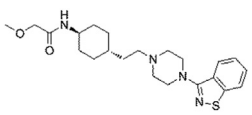
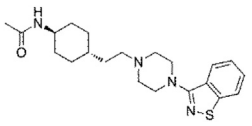
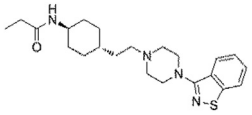
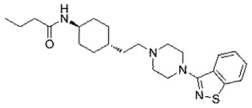
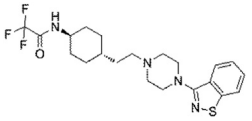
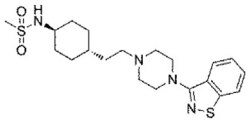
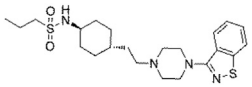
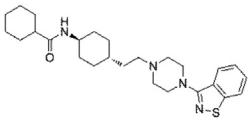
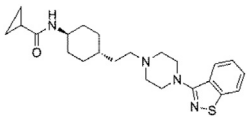
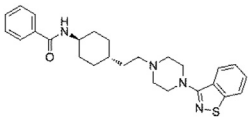
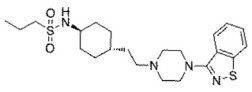
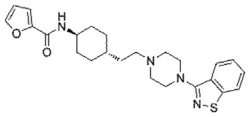
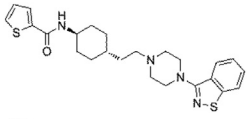
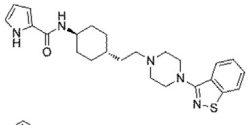
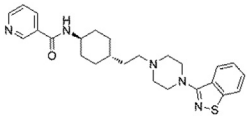
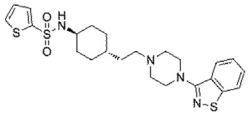
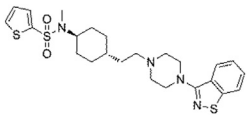
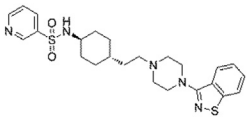
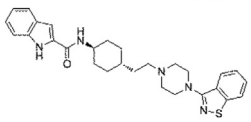
Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
9q		9.60	0.31	3.0	0.24	30.97
6		25.00	0.16	0.69	5.80	156.25
9a		18.00	0.052	1.50	1.10	346.15
9b		2.80	0.14	2.10	0.87	20.00
9c		1.70	0.27	1.10	0.63	6.30
9d		8.9	0.095	1.30	0.28	93.68
9e		1.90	0.22	1.10	0.34	8.64
9f		0.94	0.19	5.60	0.36	4.95
9g		0.85	0.21	0.92	0.35	4.05
9h		0.33	0.047	1.70	0.24	7.02
9i		0.42	0.062	3.90	0.26	6.77
9j		2.90	0.13	1.30	0.23	22.31
9k		0.19	0.056	1.10	0.15	3.40
9l		0.21	0.31	0.52	0.29	0.68
9m		0.85	0.090	2.10	0.29	9.44

Table 2 (*continued*)

Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
9n		0.43	0.043	1.1	0.30	10.00
12		0.30	0.046	2.9	1.2	6.52
9o		0.35	0.058	11.00	8.10	6.03
9r		0.43	0.029	7.60	0.64	14.83

[a] Shown are the mean K_i values from two independent experiments with eight concentrations in duplicates (Binding assays were conducted by Eurofins Cerep SA, Celle l'Evescault, France).

receptor (K_i = 0.21 nM) were observed. These encouraging results prompted the introduction of urea moieties to further increase affinity for the D_3 receptor duo to small amide-like functionalities at the cyclohexyl side that are important structural elements of the D_3 receptor pharmacophore [21]. Affinity for the D_3 receptor, as well as the D_2 and 5-HT_{1A} receptors was increased to ~1 nM after introducing the alkyl urea; affinity for 5-HT_{2A} receptor remained within subnanomolar range (**8a-c**). Analogues with increased affinity for the D_3 and 5-HT_{1A} receptors displayed the following order of affinity: methyl > ethyl > isopropyl substitution. Further work was focused on improving affinity at the D_3 receptor to obtain appropriate selectivity for the D_3 versus D_2 subtype. Aromatic or heteroaromatic ureas, such as phenyl and pyridyl ureas, were introduced and resulted in picomolar affinities for the D_3 receptor (**8f**, K_i = 0.043 nM; **8g**, Ki = 0.055 nM). After addition of methyl or introduction of benzyl, **8h** and **8e** reduced both D_2 and D_3 receptor affinity compared to **8f**. Aryl substituents (except for **8f**) reduced the 5-HT_{1A} receptor affinity in contrast with alkyl substituents, while the affinity for 5-HT_{2A} receptor stayed within the subnanomolar range (**8e-h**). **8d** bearing the morpholine ring in the urea moiety displayed lower affinities than other derivatives for these four receptors, overall indicating that alkyl and aryl substituents had greater influence on affinity to the D_3 and 5-HT_{1A} receptors than the D_2 and 5-HT_{2A} receptors. Among these derivatives, **8f** had both high affinities for D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with approximately 15-fold selectivity for D_3 vs D_2R (K_i ratio D_2/D_3 = 14.65).

4.1.1.2. Effect of carbamate. The effect of replacing the alkyl urea moiety at the cyclohexyl side of the molecule with carbamate moiety was investigated (Table 1, compound **9p**). Unlike alkyl urea derivatives (**8a-c**), the ethyl carbamate **9p** demonstrated subnanomolar affinities for D_2, 5-HT_{1A}, and 5-HT_{2A} receptors, picomolar affinity for D_3 receptor, and 12-fold higher affinity for D_3 vs D_2R (K_i ratio D_2/D_3 = 12.24). These results suggested that carbamate modifications were important for designing ligands with both high D_2, D_3, 5-HT_{1A}, 5-HT_{2A} receptor affinity and good selectivity for D_3 vs D_2R.

4.1.1.3. Effect of different alkyl amides. Considering the possible instability of **9p**, the 2-methoxyacetamide compound **9q** (Table 2) was explored and the affinities for D_2, D_3, and 5-HT_{1A} receptors were decreased in comparison to **9p**. A butyryl moiety (**9b**) was introduced to obtain an isostere of **9p** causing a decrease in potency for the D_2, D_3, and 5-HT_{1A} receptors was observed in contrast with **9p**. Affinities for the D_2 and 5-HT_{2A} receptors were further reduced by shortening the carbon length (affinity order: **6** <**9a** <**9b**). An inverse trend was observed in regard to the 5-HT_{1A} receptor (affinity order: **6** >**9a** >**9b**). Moreover, introduction of sulfonamide (**9d-e**) brought negative effects to affinity with the D_2, D_3 and 5-HT_{1A} receptors compared to **9p**. The D_2 and 5-HT_{2A} receptors maintained affinity with either fluoro-substitution (**9c**) or introduction of a cycloalkyl (**9f-g**), but affinities for the D_3 and 5-HT_{1A} receptors were moderately reduced. Based on these results, structural modification with alkyl amides did not yield ideal ligands that possessed both high affinity for D_2, D_3, 5-HT_{1A}, 5-HT_{2A} receptors and good D_3 receptor subtype preference.

4.1.1.4. Effect of different aryl amides. Considering the positive effect on affinity when an aryl group was introduced (**8f-g**) in the urea derivatives, phenyl and heteroaryl were introduced to the cyclohexyl amide. Compounds **9h-o**, **9r** and **12** exhibited subnanomolar and picomolar affinities for the D_2 and D_3 receptors, respectively (Table 2). All of the aryl carbamides (**9h**, **9j-m** and **9r**) had subnanomolar affinities for the 5-HT_{2A} receptor and ~1 nM affinity for the 5-HT_{1A} receptor (except **9m** and **9r**). In contrast, aryl sulfonamides (**9i**, **9o** and **12**, excepting **9n**) displayed lower affinities for the 5-HT_{1A} receptor, and **9o** (K_i = 8.10 nM) had the lowest affinity for the 5-HT_{2A} receptor.

In regards to the selectivity of the D_3 versus D_2 subtype, **9j** demonstrated the highest K_i ratio (D_2/D_3 = 22.31). **9j** analogues displayed the following ascending order of D_3 receptor preference: 1*H*-indole-2-yl (**9r**, K_i ratio D_2/D_3 = 14.83), pyridine-3-yl (**9m**, K_i ratio D_2/D_3 = 9.44), phenyl (**9h**, K_i ratio D_2/D_3 = 7.02), thiophene-2-yl (**9k**, K_i ratio D_2/D_3 = 3.40), and pyrrole-2-yl (**9l**, K_i ratio D_2/D_3 = 0.68) substitution. Aryl sulfonamides displayed the following ascending order of D_3 receptor preference: thiophene-2-yl (**9n**, K_i ratio D_2/D_3 = 10.00) > 4-methylphenyl (**9i**, K_i ratio D_2/D_3 = 6.77) > pyridine-3-yl (**9o**, K_i ratio D_2/D_3 = 6.03).

These results suggested that structural modification by introducing aryl carbamides was more favorable than introduction of alkyl amides in designing multireceptor ligands for D_2, D_3, 5-HT_{1A},

and 5-HT_{2A} receptors. Introduction of the furyl (**9j**) was more likely to improve D_3 vs D_2R selectivity.

4.1.1.5. Effect of conformational changes of the amide nitrogen. Compounds **41a-b** (Table 3) were prepared in order to investigate the effect of conformational changes of the amide nitrogen. Increasing the rigidity of the nitrogen via piperidine ring resulted in a decrease of D_2 and D_3 receptor affinity (**41a** vs **9j** and **41b** vs **9p**). Affinities were only slightly affected for the 5-HT_{1A} and 5-HT_{2A} receptors.

4.1.2. Effect of various spacers

The effect of spacer identity on receptor affinity was further studied following specific structural modifications at the amide moiety (Table 4, compounds **18a-b** and **45**). The *cis* isomers **18a-b** (D_2, K_i = 6.30, 4.00; D_3, K_i = 2.30, 2.00, respectively) displayed lower affinities for the D_2 and D_3 receptors compared with the *trans* isomers (**9j** and **9p**). The cis-configuration had virtually no influence on 5-HT_{1A} and 5-HT_{2A} receptor affinity. Shifting the position of the cyclohexyl by one carbon (**45**) diminished the affinities for the D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors. These results indicated that the *trans* configuration of the cyclohexyl was important in obtaining high affinity for D_2 and D_3 receptors, while also suggesting that the cyclohexylethyl spacer allowed for high affinity towards the D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors.

4.1.3. Effect of substitution on the 1,2-benzisothiazole moiety

The effect of substitution with Cl, NO_2, and NH_2 at the 6-position of benzo[*d*]isothiazole on receptor affinity was investigated (Table 5). Both electron-withdrawing NO_2 and electron-donating NH_2 decreased the affinity of the D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors (**36** vs **9j**, **37** vs **9j**). Cl substitution (**35**) increased D_3 receptor affinity (K_i = 0.051 nM), maintained affinities for the D_2 and 5-HT_{2A} receptors (K_i = 2.10 nM, 0.32 nM respectively), and reduced affinity for the 5-HT_{1A} receptor (K_i = 15.00 nM). Therefore, the 5-HT_{1A} receptor affinity is sensitive to substitutions at the 6-position of benzo[*d*]isothiazole.

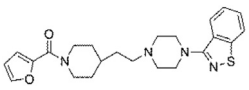
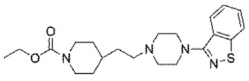
Through specific investigation of amide moiety substituents, different spacer groups and substitutions at the 6-position of benzo[*d*]isothiazole, the optimal dopamine/serotonin receptors' affinity balance was obtained for compounds **8f**, **9j**, **9n** and **9p**. The new compounds were characterized by nanomolar or picomolar range affinities for the D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors, a 10–20-fold increase in selectivity for the D_3 over the D_2 receptor, and a higher ratio of affinity for 5-HT_{2A} receptor relative to D_2 receptor (K_i ratio, 1.3–12.6). Specifically, **8f**, **9j**, **9n** and **9p** (K_i < 1.00 nM) demonstrated subnanomolar affinities for the 5-HT_{2A} receptor, which was higher in comparison to cariprazine (K_i = 23.00 nM). Therefore, **8f**, **9j**, and **9n** were selected for further *in vivo* experiments concerning the possibly unstable carbamate **9p**.

4.2. Behavioral studies

Compounds **8f**, **9j**, and **9n** were selected as potential atypical antipsychotic agents and evaluated in animal models for behavioral studies due to their prominent effect on serotonergic and dopaminergic receptors.

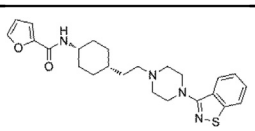
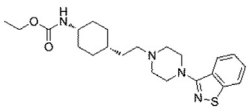
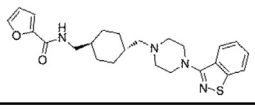
Since the 1990s, PCP has been used to simulate schizophrenia symptoms in animals as a non-competitive *N*-methyl-D-aspartate receptor antagonist. While acute PCP can imitate positive

Table 3
Binding affinities for recombinant human D_{2L}, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with compounds **41a-b**.[a]

Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
41a		15	7.3	0.72	2.2	2.2
41b		10	8.7	1.3	3.3	1.15

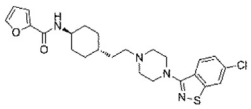
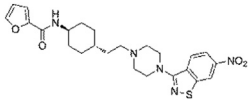
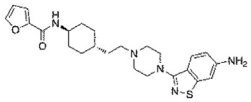
[a] Shown are the mean K_i values from two independent experiments with eight concentrations in duplicates (Binding assays were conducted by Eurofins Cerep SA, Celle l'Evescault, France).

Table 4
Binding affinities for recombinant human D_{2L}, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with compounds **18** and **45**.[a]

Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
18a		6.30	2.30	2.20	0.19	2.74
18b		4.00	2.00	0.57	0.18	2.0
45		8.9	1.0	5.3	16	8.9

[a] Shown are the mean K_i values from two independent experiments with eight concentrations in duplicates (Binding assays were conducted by Eurofins Cerep SA, Celle l'Evescault, France).

Table 5
Binding affinities for recombinant human D_{2L}, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors with compounds **35**–**37**.[a]

Compound	Structure	Receptor affinity K_i (nM)				
		hD_{2L}	hD_3	h5-HT_{1A}	h5-HT_{2A}	K_i ratio D_2/D_3
35		2.10	0.051	15.00	0.32	41.18
36		26.00	0.24	39.00	1.80	108.33
37		14.00	1.10	33.00	4.30	12.73

[a] Shown are the mean K_i values from two independent experiments with eight concentrations in duplicates (Binding assays were conducted by Eurofins Cerep SA, Celle l'Evescault, France).

symptoms [45]. Moreover, PCP-induced hyperlocomotion can be reversed through atypical antipsychotic administration [45]. **9j** and cariprazine produced significant dose-dependent responses (0.1, 0.2, and 0.4 mg/kg, i.p.) in this model compared with PCP-treated group (Fig. 4). In comparison, treatment with **8f** (0.1, 0.2 and 0.4 mg/kg, i.p.) and **9n** (0.1 and 0.2 mg/kg, i.p.) did not significantly cause inhibition of the PCP-induced locomotion.

Previously, almost all antipsychotics have been demonstrated to inhibit CAR in rats; CAR is used to assess the antipsychotic activity for potential agents that display high affinities for dopamine receptors [46,47]. In this model, the tested compound **9j** and cariprazine effectively inhibited CAR in the shuttle-box in a dose range of 1.0–2.0 mg/kg i.p. (Fig. 5). **8f** and **9n** displayed weak efficacies at the dose tested.

These results implied that **9j** is more effective as a potential antipsychotic agent than **8f** and **9n**, and chosen for further studies.

Improving cognitive impairment in schizophrenia is critically important for efficiently treating schizophrenia. NOR is commonly used in research to investigate memory performance. Repeated PCP administration can effectively produce cognitive deficits in the NOR paradigm in rodents, and this PCP-based model has been widely used to evaluate the efficacy of atypical antipsychotics in attenuating cognitive impairment [48,49]. In this model, there were no significant differences in the exploration time of two identical objects for any group in the training session (Fig. 6A). In the retention session, saline treated rats spent significantly more time exploring the novel object in comparison to the familiar object ($P < 0.05$), whereas rats treated with chronic PCP exhibited deficits discriminating between novel and familiar objects (Fig. 6B). The chronic PCP-induced impairment in NOR was reversed by **9j** 0.1 mg/kg (p.o.) ($P < 0.05$), **9j** 0.2 mg/kg (p.o.) ($P < 0.05$), and cariprazine 0.1 mg/kg (p.o.) ($P < 0.05$) (Fig. 6B).

4.3. Affinity for M_1 receptor and hERG channels

Affinity with muscarinic M_1 receptors is linked to anticholinergic side effects such as dry mouth, constipation and blurred vision [50]; atypical antipsychotics like ziprasidone, aripiprazole, risperidone and cariprazine show low M_1 receptor affinity [51]. Interestingly, **9j** exhibited weak affinities for M_1 receptors (Table 6).

Many antipsychotic drugs produce QT interval prolongation on the electrocardiogram, and hERG channel blockade is an important indicator of QT interval prolongation liability. **9j** was subject to the hERG channel binding assay, and **9j** ($IC_{50} = 2.11$ μM) and cariprazine ($IC_{50} = 20.72$ μM) displayed micromolar affinities for hERG channel and over 700-fold selectivity for D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptor binding compared to their hERG channel IC_{50} values (Table 6).

Based on these results, **9j** showed high selectivity for the affinity profile compared to M_1 receptor binding and hERG channel IC_{50}.

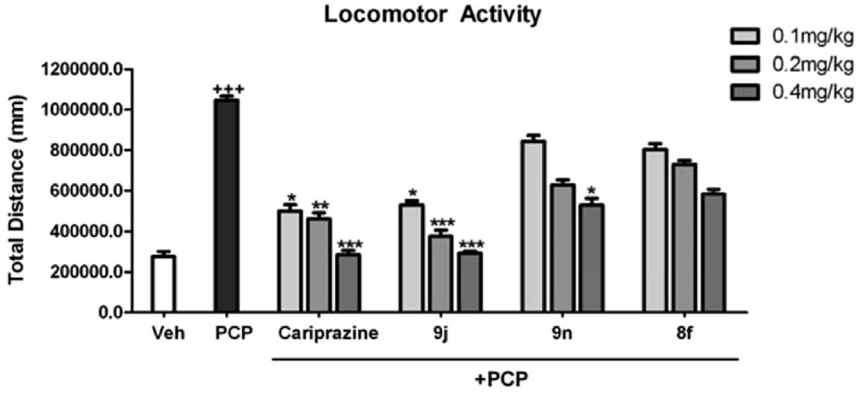


Fig. 4. Inhibition of PCP induced hyperactivity by tested compounds and reference antipsychotic drug cariprazine in male Sprague-Dawley rats. Compounds were administered 10 min before PCP injection. Locomotor activities were measured for a 1.5 h duration after PCP administration and the total travel distance was expressed as Mean ± SEM (n = 8–10). +++, $P < 0.001$ versus vehicle treated control group (Veh). *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$ versus PCP treated group.

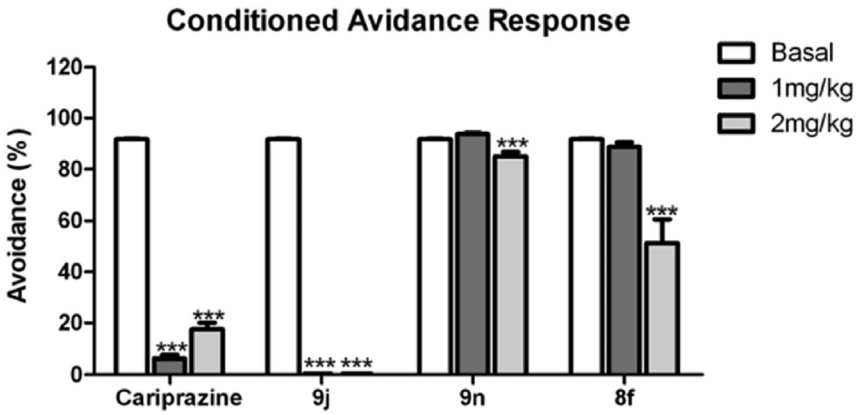


Fig. 5. Inhibition of CAR by tested compounds and reference antipsychotic drug cariprazine in male Sprague-Dawley rats. Compounds were administered 1 h before CAR testing via single intraperitoneal injection. Results were expressed as percentage of avoidance. Data was expressed as Mean ± SEM (n = 6). ***, P < 0.001 versus vehicle treated control group (Basal).

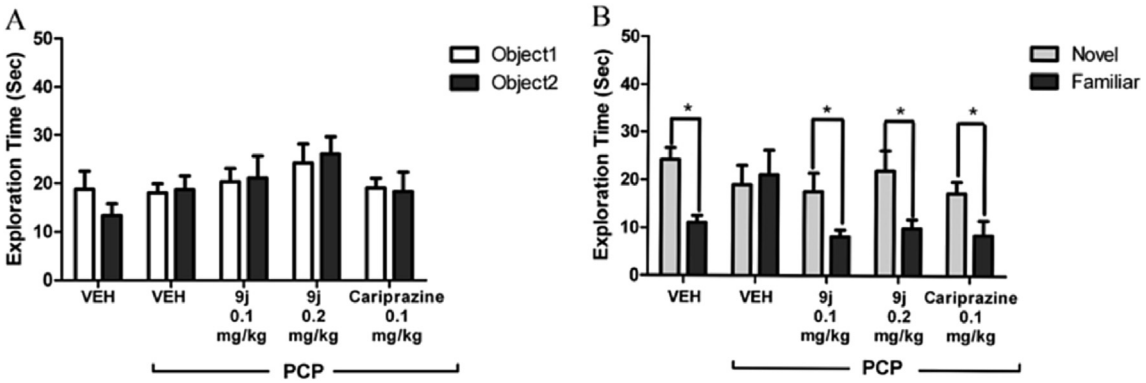


Fig. 6. Mean exploration time ± SEM for (A) Two identical objects in the training phase and (B) One familiar and one novel object in the retention phase of the novel recognition test following acute treatment with saline, **9j**, or cariprazine in chronic PCP-treated rats (n = 10). Data were analyzed by post-hoc Student's t-test; *P < 0.05 for time spent exploring the familiar versus the novel object.

Table 6
Binding affinities for the M_1 receptor and hERG channels with **9j** and reference antipsychotics.

Compound	M_1 K_i, nM	hERG IC_{50}, μM[a]	IC_{50}/Ki			
			D_2	D_3	5-HT_{1A}	5-HT_{2A}
9j	50.6%[b]	2.11	727.6	16230.8	1623.1	9173.9
Cariprazine	<20%[b,c]	20.72	15938.5	215833.3	6683.9	900.9

[a] Shown are the mean IC_{50} values from two independent experiments with six concentrations.
[b] Percent inhibition measured at a concentration of 1 μM.
[c] See ref [27].

Table 7
Activities of **9j** and reference compounds to D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors.[a]

Receptor	Compound	Activation (10 μM, %)	EC_{50} (nM)	Inhibition (10 μM, %)	IC_{50} (nM)
D_2	Dopamine	103	0.69		
	Amisulpride			102.17	3.04
	9j	−2.1	>10,000	99	61.45
D_3	Quinpirole	90.8	186.30		
	9j	25.04	>10,000	98.63	1.39
5-HT_{1A}	5-HT	99.7	15.33		
	Methiothepin mesylate			108	71
	9j	−5.0	>10,000	115	46.12
5-HT_{2A}	5-HT	100	47		
	Methiothepin mesylate			100	56
	9j	−1.4	>10,000	97	31.75

[a] Shown are the mean EC_{50} or IC_{50} values from two independent experiments with eight concentrations in duplicates.

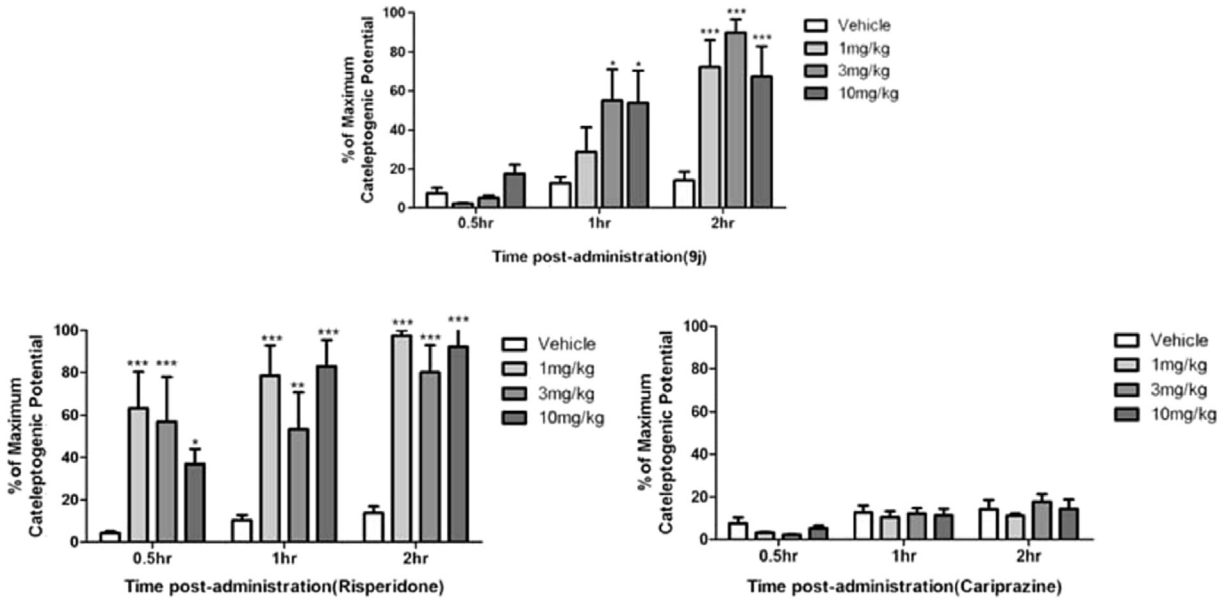


Fig. 7. Cataleptogenic effects of compound **9j**, risperidone, and cariprazine in rats. **9j**, cariprazine and risperidone (1–10 mg/kg, p.o.) were individually evaluated for cataleptic potential in SD rat (n = 7). Catalepsy was measured at 0.5, 1, and 2 h after dosing, with the mean time ± S.E.M. spent in the catalepsy position for each dose at each time point expressed as a percentage of maximal catalepsy possible (60 s). Statistically significant effect at post-administration time: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 8
Pharmacokinetic parameters for **9j** in male rats following i.v. and p.o. administration.

Dose (mg/kg)	Region	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	AUC_{0-t} (ng/mL*h)	CL_Z (L/h/kg)	*F* (%)
0.5 (iv)	Plasma	135.0	0.8		105.8	4.6	
	Brain	259.4	15.8		1066.7	0.3	
2.0 (op)	Plasma	18.4	1.7	0.5	55.0	34.9	13.0
	Brain	52.8	16.3	2.0	883.9	1.5	20.7

4.4. Intrinsic activity and cataleptogenic effect of **9j**

4.4.1. Intrinsic activity of **9j** at selected receptors

The intrinsic activity of **9j** was selected for further investigation. **9j** displayed no agonist activity against D_2, D_3, 5-HT_{1A}, and 5-HT_{2A} receptors (Table 7). In antagonist assays, **9j** had potent D_3 (IC_{50} = 1.39 nM) antagonism, which was coupled to moderate D_2 (IC_{50} = 61.45 nM), 5-HT_{1A} (IC_{50} = 46.12 nM), and 5-HT_{2A} (IC_{50} = 31.75 nM) antagonism.

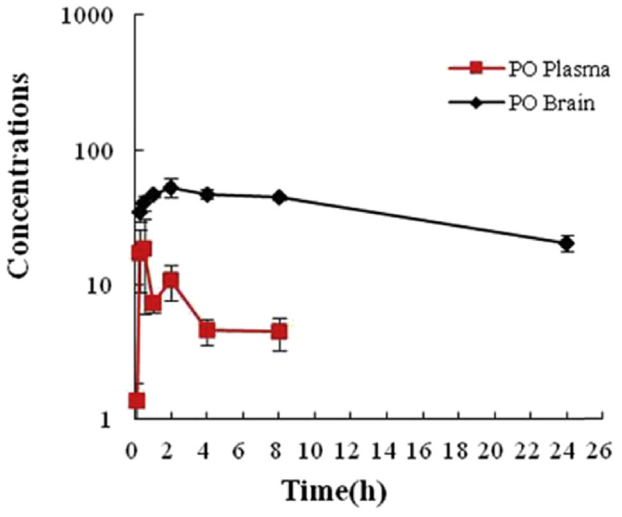


Fig. 8. Plasma concentrations (ng/mL) and brain concentrations (ng/g) of **9j** in 2.0 mg/kg po dosed SD male rats.

4.4.2. Cataleptogenic effect of **9j**

Catalepsy is widely used to predict the incidence of EPS caused by antipsychotic agents in humans [52]. In this model, **9j** exhibited a low potential for catalepsy, consistent with the marketed atypical antipsychotic risperidone, and had a higher propensity to induce catalepsy compared with cariprazine in rats (1.0, 3.0 and 10.0 mg/kg, p.o.; Fig. 7).

Taken together, the antagonistic effect at four receptors (D_2, D_3, 5-HT_{1A} and 5-HT_{2A}) and cataleptogenic effect of **9j** indicated its potential atypicality.

4.5. In vivo pharmacokinetics of **9j** in rats

The pharmacokinetics of **9j**, including plasma and brain, were measured in rats (Table 8). The area under the curve (AUC) values of **9j** were 105.8 ng/mL*h for plasma and 1066.7 ng/mL*h for the brain after intravenous administration. After oral administration of **9j**, AUC values of **9j** were 55.0 ng/mL*h for plasma and 883.9 ng/mL*h for the brain. Furthermore, the half-life of the brain samples was >15 h regardless of intravenous administration or oral administration. **9j** had high brain penetration with a brain-to-plasma AUC ratio of 16.1:1 (Fig. 8) in spite of low orally bioavailable (*F* = 13.0%) in rats.

5. Conclusion

In this work, a series of novel benzisothiazolylpiperazine derivatives were designed and synthesized in order to discover novel multitarget antipsychotics. A detailed structure-activity relationship investigation of these derivatives revealed that multiple factors, such as substituents on the amide, the configuration of the spacer, and substitutions at the 6-position of benzo[*d*]isothiazole, had influence on the affinity for the D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors both individually and collectively. Among these derivatives, compound **9j** showed unique affinities for the D_2, D_3, 5-HT_{1A} and 5-HT_{2A} receptors, along with a 20-fold selectivity for the D_3 vs D_2R and low affinities for M_1 receptors and hERG channels. Further *in vivo* animal model tests displayed that **9j** was a promising multireceptor antipsychotic. **9j** exhibited a low potential for catalepsy, consistent with the atypical antipsychotic risperidone. Moreover, **9j** showed high brain penetration in rats.

This study sheds light on the development of novel benzisothiazolylpiperazine derivatives as potential multitarget atypical antipsychotics for the treatment of schizophrenia.

6. Experimental

6.1. Chemistry

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE II 400 (400 MHz) with TMS in DMSO or $CDCl_3$ solution as an internal standard. Chemical shifts were given in δ values (ppm) and coupling constants (J) were given in Hz. Signal multiplicities were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). ESI mass spectra was performed on a Waters ZQ2000 spectrometer. Reagents were all of analytical grade or of chemical purity and used without any pretreatment. TLC on silica gel GF254 was used to monitor the progress of all reactions. Silica gel column chromatography was carried out with Silica gel 60G. Aluminum oxide column chromatography was carried out with Aluminum oxide 70G. All solvent evaporation was performed under vacuum. Compound purity was determined by high performance liquid chromatography (HPLC), and all final test compounds were >95% purity. The HPLC methods used a Waters Symmetry column C18 (5 μm, 250 mm × 4.6 mm); with a mobile phase A (CH_3CN) and B (0.05% phosphoric acid solution), 10:90–90:10 v/v; detection at 233 nm; flow: 1.0 mL/min; temp 30 °C.

6.1.1. Procedure A. trans/cis-Ethyl-2-(4-acetamidocyclohexyl) acetate (*1*)

A solution of triethyl phosphonoacetate (52.0 g, 232.0 mmol) in THF (200 mL) was added dropwise while stirring to a solution of potassium *tert*-butanolate (43.4 g, 386.6 mmol) in THF (400 mL) at 0–5 °C and continuously stirred for 1 h. Then a suspension of *N*-(4-oxocyclohexy)acetamide (30.0 g, 193.3 mmol) in THF (150 mL) was added dropwise to the solution at 0–5 °C. The resulting solution was stirred for another 2 h at room temperature and subsequently quenched with water (100 mL). The resultant mixture was extracted with acetic ether (2 × 20 mL) after separation. Palladium 10% on carbon (1.5 g) was then added to the organic layer and hydrogenation was conducted at ambient temperature and pressure conditions. The resulting solution was stirred for 24 h and then filtered. The filtrate evaporated *in vacuo* to give a colorless oil. The residue was partitioned between water (100 mL) and dichloromethane (2 × 100 mL), and the combined extracts were dried ($MgSO_4$) and evaporated *in vacuo* to afford **1** (40.7 g, 92.6%) as a colorless semisolid. MS (ESI) *m*/*z*: 228.2 ($[M + H]^+$).

6.1.2. trans-4-Acetamidocyclohexyl acetic acid (*2*)

A mixture of **1** (40.0 g, 176.0 mmol), Lithium hydroxide monohydrate (14.8 g, 352.0 mmol), water (100 mL), and THF (400 mL) was stirred at 45 °C for 16 h. The resulting solution was evaporated *in vacuo* to remove most of the THF and acidified by hydrochloric acid (6.0 mol/L, 176 mL) in an ice bath and stirred for 1 h. The resulting mixture was filtered and washed with water (50 mL) to give a colorless solid (31.6 g, 158.6 mmol). The solid was recrystallized from DMF to yield **2** (18.2 g, 57.6%). Mp: 237–239 °C. ^{1}H NMR ($CDCl_3$): δ 1.14–1.30 (m, 5H), 1.64–1.71 (m, 4H), 1.99 (s, 3H), 2.32 (d, 2H, J = 4.0 Hz), 4.02–4.03 (m, 1H), 5.42 (d, 1H, J = 4.0 Hz).

6.1.3. Procedure B. trans-Ethyl-2-(4-acetamidocyclohexyl)acetate (*3*)

A mixture of **2** (16.5 g, 82.8 mmol), concentrated H_2SO_4 (1.0 mL) and ethanol (300 mL) was stirred at reflux for 7 h. The resulting solution was evaporated *in vacuo* to give a colorless solid (19.6 g). The crude residue was extracted with dichloromethane (100 mL) and water (50 mL). The organic layer was washed with brine (50 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed *in vacuo* to afford **3** as a white solid (18.2 g, 96.7%). Mp: 113–115 °C. ^{1}H NMR ($CDCl_3$): δ 1.06–1.17 (m, 4H), 1.20–1.27 (m, 3H), 1.72–1.79 (m, 3H), 1.95–1.99 (m, 5H), 2.19 (d, 2H, J = 6.8 Hz), 3.67–3.72 (m, 1H), 4.11 (q, 2H, J = 4.0 Hz), 5.51 (d, 1H, J = 7.2 Hz).

6.1.4. Procedure C. trans-4-(2-Hydroxyethyl)acetamide (*4*)

A mixture of **3** (18.2 g, 80.1 mmol), sodium borohydride (15.1 g, 400.4 mmol), and THF (182 mL) was stirred at reflux for 0.5 h. Methanol (30 mL) was then added dropwise to this mixture at room temperature. The resulting solution was stirred at reflux for 20 h and quenched with 30% hydrochloric acid (50 mL) in an ice bath. The resulting mixture was stirred for 1 h and alkalized with 50% sodium hydroxide solution (60 mL). The resulting mixture was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give **4** (13.2 g, 89.0%) as a colorless solid. Mp: 120–122 °C. ^{1}H NMR ($CDCl_3$): δ 1.06–1.14 (m, 4H), 1.29–1.41 (m, 2H), 1.47–1.50 (m, 2H), 1.78–1.80 (m, 2H), 1.95 (s, 3H), 1.98–2.04 (m, 2H), 3.67–3.73 (m, 3H), 5.29 (d, 1H, J = 4.0 Hz).

6.1.5. Procedure D. trans-4-(Acetamidocyclohexyl)ethyl methanesulfonate (*5*)

Methylsulfonyl chloride (8.9 g, 77.8 mmol) in dichloromethane (10 mL) was added dropwise to a solution of **4** (12.0 g, 64.8 mmol) and triethylamine (22.6 mL, 162.0 mmol) in dichloromethane (30 mL) at 0–5 °C. The resulting mixture was stirred for 3 h at ambient temperature and quenched with a saturated sodium bicarbonate solution (50 mL). The resultant mixture was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were washed with 5% HCl (2 × 20 mL), water (1 × 50 mL) and brine (1 × 50 mL), dried over anhydrous sodium sulfate. The solvent was then removed under reduced pressure to give **5** (13.9 g, 81.7%) as a light yellow solid. Mp: 79–81 °C. ^{1}H NMR ($CDCl_3$): δ 1.17–1.19 (m, 5H), 1.46–1.48 (m, 2H), 1.64–1.68 (m, 2H), 1.79–1.82 (m, 2H), 1.99 (s, 3H), 3.57–3.58 (m, 1H), 3.68 (s, 3H), 4.26 (t, 2H, J = 4.0 Hz)6.16 (d, 1H, J = 4.0 Hz).

6.1.6. Procedure E. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl) piperazin-1-yl)ethyl)cyclohexyl)acetamide (*6*)

A mixture of **5** (12.0 g, 45.6 mmol), 3-(piperazin-1-yl)benzo[*d*] isothiazole (11.0 g, 50.2 mmol), sodium carbonate (14.5 g, 136.8 mmol), potassium iodide (0.01 g, 0.05 mmol), and acetonitrile (180 mL) was stirred at reflux for 36 h. The resulting solution was cooled to ambient temperature, filtered and washed with water

(100 mL) and further recrystallized from ethanol (120 mL) to afford **6** (12.0 g, 68.3%) as a colorless solid. Mp: 222–223 °C. HPLC t_R = 8.40 min, 99.9% purity. ^{1}H NMR ($CDCl_3$): δ 1.06–1.12 (m, 3H), 1.21–1.29 (m, 2H), 1.46–1.53 (m, 2H), 1.81–1.85 (m, 2H), 2.06–2.10 (m, 2H), 2.27 (s, 3H), 2.46 (t, 2H, *J* = 7.6 Hz), 2.64–2.67 (m, 4H), 3.50–3.53 (m, 4H), 4.07–4.09 (m, 1H), 5.52 (d, 1H, *J* = 7.6 Hz), 7.32 (t, 1H, *J* = 8.4 Hz), 7.44 (t, 1H, *J* = 8.4 Hz), 7.79 (d, 1H, *J* = 8.4 Hz), 7.93 (d, 1H, *J* = 8.0 Hz). MS (ESI) *m*/*z*: 387.3 ([M+H] $^+$).

*6.1.7. Procedure F. trans-4-(2-(4-(Benzo[d]isothiazol-3-yl) piperazin-1-yl)ethyl)cyclohexanamine (**7**)*

A mixture of **6** (9.8 g, 25.4 mmol) and 5%HCl (130 mL) was stirred at reflux for 30 h. The resulting solution was cooled to ambient temperature and 20% sodium hydroxide solution (65 mL) was added and stirred for another 1 h. The resulting solution was filtered and the filtrate was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with water (1 × 50 mL) and brine (1 × 50 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to yield **7** (7.5 g, 86.1%) as a colorless solid. Mp: 66–68 °C. HPLC t_R = 6.23 min, 99.2% purity. ^{1}H NMR ($CDCl_3$): δ 0.97–1.12 (m, 4H), 1.23–1.26 (m, 1H), 1.43–1.47 (m, 2H), 1.76–1.87 (m, 6H), 2.45 (t, 2H, *J* = 4.0 Hz), 2.59–2.63 (m, 1H), 2.66–2.68 (m, 4H), 3.56–3.58 (m, 4H), 7.35 (t, 1H, *J* = 8.0 Hz), 7.46 (t, 1H, *J* = 8.0 Hz), 7.81 (d, 1H, *J* = 4.0 Hz), 7.91 (d, 1H, *J* = 4.0 Hz). MS (ESI) *m*/*z*: 345.2 ([M+H]$^+$).

*6.1.8. General procedure for the preparation of compounds **8a-h***

Compound **7** (0.5 g, 1.5 mmol) in dichloromethane (2 mL) was added dropwise to a solution of bis(trichloromethyl)carbonate (0.5 g, 1.7 mmol) and triethylamine (0.4 mL, 3.0 mmol) in dichloromethane (5 mL) at −5to−10 °C and stirred for 1 h. The reaction mixture obtained was added to a solution of substituted amines (13.5 mmol) in isopropyl alcohol (10 mL) and cooled at −10~−0 °C which kept the temperature of the reaction mixture under 0 °C. After stirring at −5~0 °C for 6 h a saturated ammonium chloride aqueous solution (10 mL) was added to the reaction mixture. The resulting mixture was extracted with dichloromethane (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by recrystallization from ethanol to yield **8a-h**.

*6.1.8.1. 3-(trans-4-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1,1-dimethylurea (**8a**).* Yield 86.1%; mp: 137–139 °C. HPLC t_R = 8.90 min, 96.3% purity. ^{1}H NMR (DMSO-d_6): δ 1.08–1.24 (m, 3H), 1.27–1.28 (m, 2H), 1.44–1.50 (m, 2H), 1.80–1.81 (m, 2H), 2.05–2.06 (m, 2H), 2.46 (t, 2H, *J* = 7.6 Hz), 2.66–2.69 (m, 4H), 2.89 (s, 6H), 3.57–3.59 (m, 4H), 4.11–4.13 (m, 1H), 5.54 (d, 1H, *J* = 8.0 Hz), 7.35 (t, 1H, *J* = 8.4 Hz), 7.46 (t, 1H, *J* = 8.4 Hz), 7.81 (d, 1H, *J* = 8.4 Hz), 7.91 (d, 1H, *J* = 8.0 Hz). MS (ESI) *m*/*z*: 416.2 ([M+H] $^+$).

*6.1.8.2. 3-(trans-4-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1,1-diisopropylurea (**8b**).* Yield 89.7%; mp: 142–143 °C. HPLC t_R = 10.69 min, 97.3% purity. ^{1}H NMR (DMSO-d_6): δ 0.84–1.00 (m, 2H), 1.14–1.16 (m, 12H),1.19–1.24 (m, 3H), 1.35–1.41 (m, 2H), 1.64–1.77 (m, 4H), 2.38 (t, 2H, *J* = 8.0 Hz), 2.58–2.60 (m, 4H), 3.36–3.40 (m, 1H), 3.44–3.46 (m, 4H), 3.62–3.72 (m, 2H), 5.46 (d, 1H, *J* = 8.0 Hz), 7.44 (t, 1H, *J* = 8.0 Hz), 7.56 (t, 1H, *J* = 8.0 Hz), 8.04–8.12 (m, 2H). MS (ESI) *m*/*z*: 472.2([M+H] $^+$).

*6.1.8.3. 3-(trans-4-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1-ethyl-1-methylurea (**8c**).* Yield 90.3%; mp: 108–110 °C. HPLC t_R = 9.20 min, 96.7% purity. ^{1}H NMR (DMSO-d_6): δ 0.96–1.05 (m, 4H), 1.19–1.24 (m, 4H), 1.38 (m, 2H), 1.74–1.76 (m, 4H), 2.38 (t, 2H, *J* = 8.0 Hz), 2.51–2.53 (m, 4H), 2.73 (s, 3H), 3.32–3.36 (m, 1H), 3.19 (q, 2H, *J* = 8.0 Hz), 3.44–3.46 (m, 4H), 5.81 (d, 1H, *J* = 8.0 Hz), 7.44 (t, 1H, *J* = 8.0 Hz), 7.56 (t, 1H, *J* = 8.0 Hz), 8.04–8.07 (m, 2H). MS (ESI) *m*/*z*: 430.1 ([M+H] $^+$).

*6.1.8.4. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)morpholine-4-carboxamide (**8d**).* Yield 79.8%; mp: 137–139 °C. HPLC t_R = 6.90 min, 95.8% purity. ^{1}H NMR (DMSO-d_6): δ 1.11–1.25 (m, 3H), 1.30–1.32 (m, 2H), 1.48–1.54 (m, 2H), 1.85–1.86 (m, 2H), 2.09–2.11 (m, 2H), 2.51 (t, 2H, *J* = 7.6 Hz), 2.69–2.73 (m, 4H), 3.44–3.46 (m, 4H), 3.61–3.65 (m, 4H), 4.01–4.02 (m, 4H), 4.17–4.19 (m, 1H), 5.62 (d, 1H, *J* = 8.0 Hz), 7.38 (t, 1H, *J* = 8.0 Hz), 7.49 (t, 1H, *J* = 8.0 Hz), 7.85–7.92 (m, 2H). MS (ESI) *m*/*z*: 458.3 ([M+H] $^+$).

*6.1.8.5. 1-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-3-benzylurea (**8e**).* Yield 85.4%; mp: 158–160 °C. HPLC t_R = 10.10 min, 97.3% purity. ^{1}H NMR (DMSO-d_6): δ 0.94–1.09 (m, 4H), 1.23–1.24 (m, 1H), 1.38–1.39 (m, 2H), 1.73–1.85 (m, 4H), 2.38 (t, 2H, *J* = 7.6 Hz), 2.59–2.62 (m, 4H), 3.44–3.47 (m, 4H), 3.62–3.64 (m, 1H), 4.07 (d, 2H, *J* = 8.0 Hz), 5.78 (d, 1H, *J* = 8.0 Hz), 6.43–6.46 (m, 5H), 7.44 (t, 1H, *J* = 8.0 Hz),7.57 (t, 1H, *J* = 8.0 Hz), 8.04–8.07 (m, 2H). MS (ESI) *m*/*z*: 477.8 ([M+H] $^+$).

*6.1.8.6. 1-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-3-phenylurea (**8f**).* Yield 88.6%; mp: 234–236 °C. HPLC t_R = 10.18 min, 98.6% purity. ^{1}H NMR (DMSO-d_6): δ 0.97–1.14 (m, 4H), 1.24–1.25 (m, 1H), 1.37–1.42 (m, 2H), 1.76–1.78 (m, 2H), 1.87–1.90 (m, 2H), 2.38 (t, 2H, *J* = 7.2 Hz), 2.58–2.61 (m, 4H), 3.44–3.47 (m, 4H), 3.61–3.63 (m, 1H), 6.00 (d, 1H, *J* = 7.6 Hz), 6.87 (t, 1H, *J* = 7.6 Hz), 7.19–7.23 (m, 2H), 7.36 (d, 1H, *J* = 8.0 Hz), 7.44 (t, 1H, *J* = 7.6 Hz), 7.56 (t, 1H, *J* = 7.6 Hz), 8.04–8.07 (m, 2H). MS (ESI) *m*/*z*: 464.3 ([M+H] $^+$).

*6.1.8.7. 1-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-3-(pyridin-3-yl)urea (**8g**).* Yield 80.5%; mp: 226–229 °C. HPLC t_R = 7.15 min, 99.1% purity. ^{1}H NMR (DMSO-d_6): δ 0.97–1.06 (m, 2H), 1.11–1.19 (m, 2H), 1.24–1.25 (m, 1H), 1.37–1.42 (m, 2H), 1.76–1.79 (m, 2H), 1.87–1.90 (m, 2H), 2.39 (t, 2H, *J* = 7.2 Hz), 2.59–2.62 (m, 4H), 3.38–3.40 (m, 1H), 3.44–3.47 (m, 4H), 6.16 (d, 1H, *J* = 8.0 Hz), 7.23–7.26 (m, 1H), 7.44 (t, 1H, *J* = 7.6 Hz), 7.56 (t, 1H, *J* = 7.6 Hz), 7.86–7.88 (m, 1H), 8.04–8.11 (m, 3H), 8.50–8.51 (m, 1H). MS (ESI) *m*/*z*: 465.3 ([M+H] $^+$).

*6.1.8.8. 3-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1-methyl-1-phenylurea (**8h**).* Yield 91.7%; mp: 106–109 °C. HPLC t_R = 10.31 min, 98.9% purity. ^{1}H NMR (DMSO-d_6): δ 0.91–1.00 (m, 2H), 1.12–1.23 (m, 3H), 1.33–1.39 (m, 2H),1.71–1.77 (m, 4H), 2.36 (t, 2H, *J* = 7.2 Hz), 2.56–2.59 (m, 4H), 3.34 (s, 3H), 3.39–3.41 (m, 1H), 3.43–3.46 (m, 4H), 5.60 (d, 1H, *J* = 8.0 Hz), 7.16–7.20 (m, 1H), 7.23–7.25 (m, 2H), 7.34–7.38 (m, 2H), 7.43 (t, 1H, *J* = 7.6 Hz), 7.56 (t, 1H, *J* = 7.6 Hz), 8.03–8.06 (m, 2H). MS (ESI) *m*/*z*: 478.2 ([M+H] $^+$).

*6.1.9. Procedure G. General procedure for the preparation of compounds **9a-q***

Acyl chloride (1.7 mmol) in dichloromethane (2 mL) was added dropwise to a solution of **7** (0.5 g, 1.5 mmol) and triethylamine (0.4 mL, 3.0 mmol) in dichloromethane (5 mL) at 0–5 °C. The resulting mixture was stirred at room temperature for 7 h. Water (10 mL) was added to the reaction mixture and extracted with dichloromethane (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by recrystallization from ethanol to yield **9a-q** as free base. The free base of **9j**, **9n** and **9p** were then dissolved in methanol (5 mL) and a 5% hydrogenchloride solution (0.5 mL) was added. The resulting mixture was stirred for 1 h at room temperature. The resulting mixture was evaporated to until dry under reduced pressure and

purified by recrystallization from ethanol/water to yield **9j**, **9n** and **9p** hydrochloride.

*6.1.9.1. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)propionamide (**9a**).* Yield 93.5%; mp: 259–261 °C. HPLC t_R = 8.74 min, 98.7% purity. ^{1}H NMR (DMSO-d_6): δ 0.81–1.06 (m, 7H), 1.11–1.19 (m, 2H), 1.45–1.46 (m, 1H),1.74–1.80 (m, 4H), 2.03 (q, 2H, *J* = 8.0 Hz), 2.38 (t, 2H, *J* = 7.2 Hz), 3.19–3.24 (m, 4H), 3.43–3.46 (m, 4H), 3.70–3.72 (m, 1H), 5.56 (d, 1H, *J* = 7.6 Hz), 7.48 (t, 1H, *J* = 8.0 Hz), 7.63 (t, 1H, *J* = 8.0 Hz), 8.11–8.16 (m, 2H). MS (ESI) *m*/*z*: 401.4 ([M+H] $^+$).

*6.1.9.2. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)butyramide (**9b**).* Yield 92.8%; mp: 172–174 °C. HPLC t_R = 9.16 min, 99.1% purity. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, 3H, *J* = 8.0 Hz), 1.03–1.10 (m, 2H), 1.13–1.19 (m, 2H), 1.36–1.38 (m, 2H), 1.45–1.54 (m, 3H), 1.62–1.64 (m, 2H), 1.74–1.79 (m, 4H), 2.00 (t, 2H, *J* = 8.0 Hz), 3.19–3.24 (m, 4H), 3.43–3.46 (m, 4H), 3.60–3.62 (m, 1H), 5.58 (d, 1H, *J* = 7.6 Hz), 7.48 (t, 1H, *J* = 8.0 Hz), 7.60 (t, 1H, *J* = 8.0 Hz), 8.10–8.15 (m, 2H). MS (ESI) *m*/*z*: 415.4 ([M+H] $^+$).

*6.1.9.3. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-2,2,2-trifluoroacetamide (**9c**).* Yield 80.2%; mp: 170–172 °C. HPLC t_R = 9.92 min, 98.4% purity. ^{1}H NMR (DMSO-d_6): δ 0.97–1.08 (m, 2H), 1.29–1.40 (m, 5H), 1.77–1.80 (m, 4H), 2.39 (t, 2H, *J* = 8.0 Hz), 2.59–2.62 (m, 4H), 3.44–3.47 (m, 4H), 3.58–3.60 (m, 1H), 7.44 (t, 1H, *J* = 8.0 Hz), 7.57 (t, 1H, *J* = 8.0 Hz), 8.04–8.07 (m, 2H), 9.26 (d, 1H, *J* = 8.0 Hz). MS (ESI) *m*/*z*: 440.8 ([M+H] $^+$).

*6.1.9.4. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)methanesulfonamide (**9d**).* Yield 81.9%; mp: 152–154 °C. HPLC t_R = 8.88 min, 98.0% purity. ^{1}H NMR ($CDCl_3$): δ 1.02–1.19 (m, 2H), 1.20–1.29 (m, 3H), 1.42–1.58 (m, 2H), 1.81–1.84 (m, 2H), 2.05–2.08 (m, 2H), 2.64–2.66 (m, 4H), 2.68 (t, 2H, *J* = 7.8 Hz), 2.96 (s, 3H), 3.23–3.27 (m, 1H), 3.55–3.57 (m, 4H), 6.92 (d, 1H, *J* = 7.6 Hz), 7.33 (t, 1H, *J* = 8.0 Hz), 7.43 (t, 1H, *J* = 8.0 Hz), 7.81–7.86 (m, 2H). MS (ESI) *m*/*z*: 423.2 ([M+H] $^+$).

*6.1.9.5. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)propane-1-sulfonamide (**9e**).* Yield 87.0%; mp: 169–171 °C. HPLC t_R = 9.71 min, 98.8% purity. ^{1}H NMR (DMSO-d_6): δ 0.95–0.99 (m, 4H), 1.16–1.26 (m, 4H), 1.33–1.38 (m, 2H), 1.63–1.67 (m, 2H), 1.72–1.75 (m, 2H), 1.84–1.87 (m, 2H), 2.36 (t, 2H, *J* = 7.6 Hz), 2.55–2.58 (m, 4H), 2.92–3.02 (m, 3H), 3.41–3.44 (m, 4H), 7.00 (d, 1H, *J* = 7.6 Hz), 7.43 (t, 1H, *J* = 7.6 Hz), 7.56 (t, 1H, *J* = 7.6 Hz), 8.03–8.07 (m, 2H). MS (ESI) *m*/*z*: 451.1 ([M+H] $^+$).

*6.1.9.6. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)cyclohexanecarboxamide (**9f**).* Yield 87.0%; mp: 221–223 °C. HPLC t_R = 10.33 min, 97.5% purity. ^{1}H NMR ($CDCl_3$): δ 1.06–1.15 (m, 4H), 1.19–1.31 (m, 4H), 1.37–1.48 (m, 4H), 1.65–1.67 (m, 1H), 1.78–1.85 (m, 6H), 1.97–2.04 (m, 3H), 2.42–2.56 (m, 2H), 2.64–2.67 (m, 4H), 3.55–3.57 (m, 4H), 3.70–3.72 (m, 1H), 7.34 (t, 1H, *J* = 8.0 Hz), 7.45 (t, 1H, *J* = 8.0 Hz), 7.83–7.88 (m, 2H), 7.78 (d, 1H, *J* = 7.2 Hz). MS (ESI) *m*/*z*: 455.2 ([M+H] $^+$).

*6.1.9.7. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)cyclopropanecarboxamide (**9g**).* Yield 94.6%; mp: 213–215 °C. HPLC t_R = 9.06 min, 99.2% purity. ^{1}H NMR (DMSO-d_6): δ 0.59–0.61 (m, 4H), 0.95–1.00 (m, 2H), 1.13–1.23 (m, 4H), 1.37–1.49 (m, 2H), 1.74–1.78 (m, 4H), 2.37 (t, 2H, *J* = 7.6 Hz), 2.57–2.59 (m, 4H),3.42–3.44 (m, 4H), 3.45–3.47 (m, 1H), 7.43–7.45 (m, 1H), 7.54–7.56 (m, 1H), 7.90 (d, 1H, *J* = 6.8 Hz), 8.05–8.09 (m, 2H). MS (ESI) *m*/*z*: 413.1 ([M+H] $^+$).

*6.1.9.8. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)benzamide (**9h**).* Yield 91.1%; mp: 189–191 °C. HPLC t_R = 9.95 min, 97.4% purity. ^{1}H NMR (DMSO-d_6): δ 1.08–1.12 (m, 3H), 1.21–1.29 (m, 2H), 1.46–1.53 (m, 2H), 1.81–1.85 (m, 2H), 2.02–2.08 (m, 2H), 2.49 (t, 2H, *J* = 7.6 Hz), 2.64–2.67 (m, 4H), 3.50–3.53 (m, 4H), 4.17–4.19 (m, 1H), 7.30–7.42 (m, 4H), 7.48–7.53 (m, 2H), 7.79 (d, 1H, *J* = 8.4 Hz), 7.90–7.96 (m, 2H), 8.02 (d, 1H, *J* = 5.6 Hz). MS (ESI) *m*/*z*: 449.2 ([M+H] $^+$).

*6.1.9.9. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-4-methylbenzenesulfonamide (**9i**).* Yield 89.4%; mp: 143–146 °C. HPLC t_R = 10.83 min, 98.1% purity. ^{1}H NMR (DMSO-d_6): δ 0.76–0.85 (m, 2H), 1.06–1.15 (m, 3H), 1.24–1.31 (m, 2H), 1.56–1.63 (m, 4H), 2.29 (t, 2H, *J* = 7.6 Hz), 2.51–2.53 (m, 4H), 2.79–2.85 (m, 1H), 3.40–3.42 (m, 4H), 7.36–7.44 (m, 3H), 7.54–7.58 (m, 2H), 7.67–7.69 (m, 2H), 8.00–8.03 (m, 2H). MS (ESI) *m*/*z*: 499.0 ([M+H] $^+$).

*6.1.9.10. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)furan-2-carboxamide (**9j**) hydrochloride.* Yield 81.3%; mp: >270 °C. HPLC t_R = 10.97 min, 99.0% purity. ^{1}H NMR (DMSO-d_6): δ 1.03–1.09 (m, 2H), 1.30–1.40 (m, 3H), 1.65–1.68 (m, 2H),1.77–1.82 (m, 4H), 3.18–3.25 (m, 4H), 3.48 (t, 2H, *J* = 7.6 Hz), 3.59–3.61 (m, 2H), 3.68–3.73 (m, 1H), 4.07–4.08 (m, 4H), 6.60–6.61 (m, 1H), 7.09–7.10 (m, 1H), 7.47–7.49 (m, 1H), 7.59–7.62 (m, 1H), 7.80–7.81 (m, 1H), 8.11 (d, 1H, *J* = 5.2 Hz), 8.14–8.16 (m, 2H). MS (ESI) *m*/*z*: 439.3 ([M+H] $^+$).

*6.1.9.11. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)thiophene-2-carboxamide (**9k**).* Yield 88.5%; mp: 178–181 °C. HPLC t_R = 9.88 min, 98.2% purity. ^{1}H NMR (DMSO-d_6): δ 0.99–1.07 (m, 2H), 1.23–1.43 (m, 5H), 1.76–1.86 (m, 4H), 2.39 (t, 2H, *J* = 7.6 Hz), 2.59–2.61 (m, 4H), 3.44–3.46 (m, 4H), 3.67–3.70 (m, 1H), 7.11–7.14 (m, 1H), 7.42–7.45 (m, 1H), 7.71–7.72 (m, 1H), 7.77–7.78 (m, 1H), 8.04–8.07 (m, 2H), 8.21 (d, 1H, *J* = 8.0 Hz). MS (ESI) *m*/*z*: 455.0 ([M+H] $^+$).

*6.1.9.12. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1H-pyrrole-2-carboxamide (**9l**).* Yield 80.0%; mp: 200–202 °C. HPLC t_R = 9.54 min, 96.3% purity. ^{1}H NMR (DMSO-d_6): δ 1.00–1.06 (m, 2H), 1.19–1.32 (m, 3H), 1.43–1.47 (m, 2H), 1.48–1.81 (m, 4H), 2.66 (t, 2H, *J* = 7.6 Hz), 2.88–2.91 (m, 4H), 3.52–3.53 (m, 4H), 3.63–3.66 (m, 1H), 4.81 (s, 1H), 6.04–6.06 (m, 1H), 6.75–6.77 (m, 1H), 6.81–6.82 (m, 1H), 7.44–7.46 (m, 1H), 7.55–7.57 (m, 1H), 8.05–8.08 (m, 2H), 8.15 (d, 1H, *J* = 7.6 Hz). MS (ESI) *m*/*z*: 438.0 ([M+H] $^+$).

*6.1.9.13. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)nicotinamide (**9m**).* Yield 78.7%; mp: 176–178 °C. HPLC t_R = 8.05 min, 98.1% purity. ^{1}H NMR ($CDCl_3$): δ 1.08–1.14 (m, 3H), 1.20–1.28 (m, 2H), 1.44–1.50 (m, 2H), 1.79–1.81 (m, 2H), 2.01–2.05 (m, 2H), 2.45 (t, 2H, *J* = 7.6 Hz), 2.66–2.68 (m, 4H), 3.56–3.59 (m, 4H), 4.12–4.14 (m, 1H), 7.41–7.43 (m, 1H), 7.50–7.52 (m, 1H), 7.65–7.66 (m,1H), 7.79–7.83 (m, 2H), 8.31 (d, 1H, *J* = 7.6 Hz), 8.47–8.48 (m, 1H), 8.90–8.91 (m, 1H), 9.24 (d, 1H, *J* = 2.0 Hz). MS (ESI) *m*/*z*: 450.0 ([M+H] $^+$).

*6.1.9.14. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)thiophene-2-sulfonamide (**9n**) hydrochloride.* Yield 72.5%; mp: > 270 °C. HPLC t_R = 12.15 min, 99.1% purity. ^{1}H NMR (DMSO-d_6): δ 1.08–1.14 (m, 3H), 1.20–1.28 (m, 2H), 1.44–1.50 (m, 2H), 1.79–1.81 (m, 2H), 2.01–2.05 (m, 2H), 2.45 (t, 2H, *J* = 7.6 Hz), 2.66–2.68 (m, 4H), 3.56–3.59 (m, 4H), 4.12–4.14 (m, 1H), 7.15–7.17 (m, 1H), 7.43–7.47 (m, 1H), 7.56–7.60 (m, 2H), 7.87–7.91 (m, 2H), 8.08–8.13 (m, 2H). MS (ESI) *m*/*z*: 490.5 ([M+H]

$^{+}$).

*6.1.9.15. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)pyridine-3-sulfonamide (**9o**).* Yield 81.7%; mp: 137–140 °C. HPLC t_R = 9.36 min, 98.8% purity. ^{1}H NMR (DMSO-d_6): δ 0.87–0.93 (m, 2H), 1.03–1.05 (m, 1H), 1.10–1.23 (m, 3H), 1.34–1.36 (m, 1H), 1.59–1.66 (m, 4H), 2.33 (t, 2H, *J* = 7.6 Hz), 2.96–2.98 (m, 4H), 3.41–3.43 (m, 4H), 3.74–3.79 (m, 1H), 7.35–7.36 (m, 1H), 7.42–7.46 (m,1H), 7.63–7.66 (m,1H), 7.95 (d, 1H, *J* = 8.0 Hz), 8.06–8.08 (m, 2H), 8.19–8.21 (m, 1H), 8.81–8.82 (m, 1H), 8.97–8.98 (m, 1H). MS (ESI) *m*/*z*: 485.5 ([M+H] $^{+}$).

*6.1.9.16. Ethyl (trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)carbamate (**9p**) hydrochloride.* Yield 89.3%; mp: 218–219 °C. HPLC t_R = 11.44 min, 98.5% purity. ^{1}H NMR (DMSO-d_6): δ 0.97–1.04 (m, 2H), 1.13–1.18 (m, 5H), 1.24–1.25 (m, 1H), 1.60–1.64 (m, 2H), 1.73–1.75 (m, 2H), 1.79–1.81 (m, 2H), 3.17–3.23 (m, 5H), 3.41 (t, 2H, *J* = 7.6 Hz), 3.58–3.60 (m, 2H), 3.95 (q, 2H, *J* = 4.8 Hz), 4.06–4.09 (m, 2H), 7.00 (d, 1H, *J* = 5.2 Hz), 7.46–7.49 (m, 1H), 7.59–7.61 (m, 1H), 8.11–8.15 (m, 2H). MS (ESI) *m*/*z*: 416.9 ([M+H] $^{+}$).

*6.1.9.17. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-2-methoxyacetamide (**9q**).* Yield 86.3%; mp: 132–133 °C. HPLC t_R = 8.81 min, 99.7% purity. ^{1}H NMR (DMSO-d_6): δ0.93–1.02 (m, 2H), 1.22–1.25 (m, 3H), 1.34–1.40 (m, 2H), 1.70–1.76 (m, 4H), 2.37 (t, 2H, *J* = 7.6 Hz), 2.57–2.59 (m, 4H), 3.29 (s, 3H), 3.43–3.45 (m, 4H), 3.54–3.58 (m, 1H), 3.76 (s, 2H), 7.43 (t, 1H, *J* = 7.6 Hz), 7.52–7.58 (m, 2H), 8.03–8.07 (m, 2H). MS (ESI) *m*/*z*: 417.4 ([M+H] $^{+}$).

*6.1.10. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)-1H-indole-2-carboxamide (**9r**) hydrochloride*

A mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.3 g, 1.8 mmol) and 4-dimethylaminopyridine (0.1 g, 1.2 mmol) in dichloromethane (5 mL) was added at room temperature with stirring to a solution of **7** (0.5 g, 1.5 mmol) and indole-2-carboxylic acid (0.3 g, 1.7 mmol) in dichloromethane (5 mL). The resulting mixture was stirred for 12 h. Water (10 mL) was added to the reaction mixture and extracted with dichloromethane (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in methanol (5 mL) and 5% hydrogenchloride solution (0.5 mL) was added. The resulting mixture was stirred for 1 h at room temperature. After stirring, the mixture was evaporated until dry under reduced pressure and purified by recrystallization from ethanol/water to yield **9r** (0.7 g, 90.1%) as a white solid. Mp: 269–270 °C. HPLC t_R = 12.65 min, 99.7% purity. ^{1}H NMR (DMSO-d_6): δ 1.02–1.11 (m, 2H), 1.32–1.45 (m, 5H), 1.81–1.90 (m, 4H), 2.41 (t, 2H, *J* = 7.6 Hz), 2.59–2.61 (m, 4H), 3.44–3.46 (m, 4H), 3.76–3.78 (m, 1H), 7.01–7.04 (m, 1H), 7.15–7.19 (m, 2H), 7.41–7.46 (m, 2H), 7.55–7.61 (m, 2H), 8.05–8.08 (m, 2H), 8.30 (d, 1H, *J* = 5.2 Hz), 11.58 (d, 1H, *J* = 0.8 Hz). MS (ESI) *m*/*z*: 488.0 ([M+H] $^{+}$).

*6.1.11. tert-Butyl(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)carbamate (**10**)*

A mixture of **7** (0.5 g, 1.5 mmol), triethylamine (0.4 mL, 3.0 mmol) and di(tert-butyl) carbonate (0.4 g, 1.7 mmol), and dichloromethane (10 mL) was stirred at room temperature for 5 h. Water (10 mL) was then added to the resulting mixture was added water and extracted with dichloromethane (1 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by recrystallization from ethanol to yield **10** (0.6 g, 96.7%) as a white solid. Mp: 175–178 °C. ^{1}H NMR (DMSO-d_6): δ 0.96–1.03 (m, 2H), 1.08 (s, 9H), 1.12–1.17 (m, 2H), 1.22–1.23 (m, 1H), 1.58–1.61 (m, 2H), 1.71–1.73 (m, 2H), 1.78–1.80 (m, 2H), 3.15–3.17 (m, 4H), 3.19–3.22 (m, 1H), 3.40 (t, 2H, *J* = 7.6 Hz), 3.56–3.58 (m, 2H), 4.05–4.07 (m, 2H), 6.52 (d, 1H, *J* = 7.6 Hz), 7.45–7.48 (m, 1H), 7.57–7.59 (m, 1H), 8.12–8.15 (m, 2H).

*6.1.12. Ethyl trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)-N-methylcyclohexanamine (**11**)*

10 (0.5 g, 1.1 mmol) in THF (3 mL) was added dropwise with stirring to a solution of lithium aluminum hydride (0.1 g, 3.4 mmol) in THF (5 mL) at 0–5 °C. The resulting mixture was stirred at room temperature for 10 h. The reaction was quenched with water (0.5 mL). 20% sodium hydroxide solution (0.5 mL) and water (1.5 mL) were then added and stirred for 0.5 h. The resulting solution was filtered. The filtrate was evaporated *in vacuo* to give a yellow oil (0.8 g) which was purified using Aluminium oxide, which was eluted with 1–10% methanol/dichloromethane to **11** (0.2 g, 61.4%) as a colorless solid. Mp: 135–137 °C. ^{1}H NMR ($CDCl_3$): δ 0.96–1.11 (m, 4H), 1.22–1.24 (m, 1H), 1.42–1.45 (m, 2H), 1.75–1.86 (m, 5H), 2.43 (t, 2H, *J* = 4.0 Hz), 2.59–2.63 (m, 1H), 2.65–2.67 (m, 4H), 2.96 (d, 3H, *J* = 3.6 Hz), 3.55–3.57 (m, 4H), 7.35 (t, 1H, *J* = 8.0 Hz), 7.45 (t, 1H, *J* = 8.0 Hz), 7.88–7.93 (m, 2H).

*6.1.13. N-(trans-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexy)-N-methylthiophene-2-sulfonamide (**12**)*

Compound **11** (0.2 g, 0.6 mmol) in dichloromethane (5 mL) was treated with thiophene-2-sulfonyl chloride (0.1 g, 0.7 mmol) in dichloromethane (2 mL) using procedure G. The crude product was purified by recrystallization from ethanol to yield **12** (0.2 g, 66.4%) as a colorless solid. Mp: 159–161 °C. HPLC t_R = 10.93 min, 97.1% purity. ^{1}H NMR (DMSO-d_6): δ 0.93–1.02 (m, 2H),1.19–1.21 (m, 1H),1.28–1.42 (m, 6H), 1.71–1.74 (m, 2H), 2.33 (t, 2H, *J* = 7.2 Hz), 2.54–2.56 (m, 4H), 2.70 (s, 3H), 3.40–3.42 (m, 4H), 3.60–3.66 (m, 1H), 7.22–7.24 (m, 1H), 7.43 (t, 1H, *J* = 7.6 Hz), 7.55 (t, 1H, *J* = 7.6 Hz), 7.65–7.66 (m, 1H), 7.97–7.98 (m, 1H), 8.02–8.06 (m, 2H). MS (ESI) *m*/*z*: 505.1 ([M+H] $^{+}$).

*6.1.14. N-(cis-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)acetamide (**16**)*

The *trans*/*cis* mixture 15 (9.0 g, 34.2 mmol) was obtained via esterification (procedure B), reduction (procedure C), and activation (procedure D) using *tans*/*cis* mixture **13** (11.8 g, 59.2 mmol) as starting material. Then a mixture of **14** (9.0 g, 34.2 mmol), 3-(piperazin-1-yl)benzo[*d*]isothiazole (8.7 g, 37.6 mmol), sodium carbonate (10.9 g, 102.6 mmol), potassium iodide (0.01 g, 0.05 mmol), and acetonitrile (135 mL) was stirred at reflux for 36 h. The resulting solution was cooled to ambient temperature and filtered. The filtrate was evaporated *in vacuo* to give a brown oil (5.9 g), which was purified using silica gel, eluting with 1–5% methanol/dichloromethane to give a colorless solid (2.6 g). This material was further recrystallized from ethanol (26 mL) to afford **16** (1.9 g, 14.4%) as a white solid. Mp: 147–148 °C. ^{1}H NMR ($CDCl_3$): δ 1.23–1.25 (m, 2H), 1.50–1.54 (m, 3H), 1.62–1.67 (m, 4H), 1.84–1.88 (m, 2H), 1.99 (s, 3H), 2.45 (t, 2H, *J* = 4.0 Hz), 2.67–2.69 (m, 4H), 3.57–3.59 (m, 4H), 4.01–4.03 (m, 1H), 5.50 (d, 1H, *J* = 2.0 Hz), 7.35 (t, 1H, *J* = 8.0 Hz), 7.47 (t, 1H, *J* = 8.0 Hz), 7.81 (d, 1H, *J* = 4.0 Hz), 7.90 (d, 1H, *J* = 4.0 Hz).

*6.1.15. cis-4-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl) cyclohexanamine (**17**)*

Compound **16** (1.5 g, 3.9 mmol) was treated with 5%HCl (20 mL) using procedure F to yield **17** (1.2 g, 89.2%) as a colorless semisolid. ^{1}H NMR ($CDCl_3$): δ 1.34–1.39 (m, 4H), 1.44–1.47 (m, 4H), 1.50–1.55 (m, 2H), 1.72–1.74 (m, 3H), 2.37 (t, 2H, *J* = 4.0 Hz), 2.60–2.61 (m, 4H), 2.87–2.89 (m, 1H), 3.49–3.50 (m, 4H), 7.27 (t, 1H, *J* = 8.0 Hz), 7.38 (t, 1H, *J* = 8.0 Hz), 7.72 (d, 1H, *J* = 4.0 Hz), 7.82 (d, 1H, *J* = 4.0 Hz).

*6.1.16. General procedure for the preparation of compounds **18a-b***

Compound **17** (0.5 g, 1.5 mmol) was treated with acyl chloride (0.2 g, 1.8 mmol) in dichloromethane (5 mL) using procedure G to give **18a-b**.

*6.1.16.1. N-(cis-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)furan-2-carboxamide (**18a**) hydrochloride.* Yield 79.3%; mp: 163–165 °C. HPLC t_R = 9.40 min, 99.6% purity. ^{1}H NMR (DMSO-d_6): δ 1.54–1.58 (m, 7H), 1.66–1.69 (m, 2H), 1.79–1.81 (m, 2H), 3.17 (t, 2H, J = 4.0 Hz), 3.23–3.31 (m, 2H), 3.52–3.55 (m, 2H), 3.59–3.62 (m, 2H), 3.86–3.88 (m, 1H), 4.00–4.09 (m, 2H), 6.61–6.62 (m, 1H), 7.14–7.15 (m, 1H), 7.48 (t, 1H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.6 Hz), 7.82 (s, 1H), 7.97–7.99 (m, 1H), 8.11–8.15 (m, 2H). MS (ESI) m/z: 439.1 ([M+H]$^+$).

*6.1.16.2. Ethyl (cis-4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) ethyl)cyclohexyl)carbamate (**18b**) hydrochloride.* Yield 81.5%; mp: 202–204 °C. HPLC t_R = 9.74 min, 98.9% purity. ^{1}H NMR (DMSO-d_6): δ 1.04–1.07 (m, 1H), 1.15–1.19 (m, 4H), 1.68–1.70 (m, 2H), 1.98–1.99 (m, 1H), 2.50 (t, 3H, J = 1.6 Hz), 3.17 (t, 2H, J = 5.6 Hz), 3.24–3.27 (m, 3H), 3.43–3.90 (m, 6H), 3.95–4.09 (m, 5H), 7.08–7.10 (m, 1H), 7.48 (t, 1H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.6 Hz), 8.10–8.15 (m, 2H). MS (ESI) m/z: 417.3 ([M+H]$^+$).

*6.1.17. 3-Hydroxy-6-chloro-1,2-benzisothiazole (**25**)*

Sulfuryl dichloride (13.6 g, 100.9 mmol) in chloroform (20 mL) was added dropwise to a stirred solution of 2-(benzylthio)-4-chlorobenzoic acid chloride **23** [44] (20.0 g, 67.3 mmol) in chloroform (200 mL). After stirring for 2 h, the resulting solution was added to ammonia hydrate (200 mL) at room temperature and stirred for 2 h. The reaction was quenched with 30% hydrochloric acid (700 mL) in an ice bath and stirred for 20 min. The resulting mixture was filtered and washed with water (100 mL) to give **25** (6.5 g, 52.0%) as a yellow solid. Mp: 198–200 °C. ^{1}H NMR ($CDCl_3$): δ 7.40 (d, 1H, J = 7.6 Hz), 7.96–7.99 (m, 2H), 8.17 (s, 1H).

*6.1.18. 2-(Benzylthio)-4-nitrobenzoic acid (**22**)*

A mixture of 2-fluoro-4-nitrobenzoic acid (25 g, 135.1 mmol), phenylmethanethiol (18.5 g, 148.6 mmol), and *N*-ethyl-*N*-isopropylpropan-2-amine (118 mL) in *N*,*N*-dimethylformamide (300 mL) was stirred at 65 °C for 35 h. The resulting solution was evaporated *in vacuo* to less than a certain amount volume to give a brown oil, which was poured into water (600 mL). 30% hydrochloric acid (90 mL) was added to the resulting mixture and stirred for another 1 h. The solution was filtered and washed with water (100 mL). The crude product was purified by recrystallization from ethanol to yield **22** (19.8 g, 55.5%) as a yellow solid. Mp: 130–131 °C. ^{1}H NMR (DMSO-d_6): δ 4.37 (s, 2H), 7.27–7.31 (m, 1H), 7.34–7.38 (m, 2H), 7.47–7.48 (m, 2H), 8.00 (dd, 1H, J = 8.4 Hz, J = 2.0 Hz), 8.09 (d, 1H, J = 8.4 Hz), 8.20 (d, 1H, J = 2.0 Hz), 13.81 (br, 1H).

*6.1.19. 3-Hydroxy-6-nitro-1,2-benzisothiazole (**26**)*

Oxalyl dichloride (13.2 g, 103.7 mmol) and *N*,*N*-dimethylformamide (4 mL) were added dropwise to a stirred solution of **22** (15.0 g, 51.9 mmol) in chloroform (150 mL) at 0–5 °C. The resulting mixture was stirred at reflux for 3 h. The resulting solution was cooled to room temperature. Sulfuryl dichloride (7.7 g, 57.0 mmol) in chloroform (10 mL) was added dropwise. After stirring for 2 h, the resulting solution was added to ammonia hydrate (187 mL) at room temperature and stirred for 2 h. The reaction was quenched with 30% hydrochloric acid (600 mL) in an ice bath, and stirred for 20 min. The resulting mixture was filtered and washed with water (50 mL) to give **26** (3.2 g, 31.0%) as a yellow solid. Mp: 266–268 °C. ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 1H, J = 8.0 Hz), 8.25–8.27 (m, 2H), 8.59 (s, 1H).

*6.1.20. 3-Chloro-6-nitro-1,2-benzisothiazole (**28**)*

A mixture of **26** (3.6 g, 18.4 mmol), PCl_5 (4.6 g, 22.0 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (6.5 mL) in $POCl_3$ (10 mL) was heated to 120 °C with stirring. After 3 h the mixture was cooled to room temperature, poured into ice water, and extracted with ethyl acetate. The organic phase was concentrated *in vacuo*. The residue was purified using silica gel, eluting with 1–10% ethyl acetate/hexane to give **28** (2.0 g, 49.5%) as a colorless solid. Mp: 146–147 °C. ^{1}H NMR ($CDCl_3$): δ 8.17 (t, 1H, J = 4.0 Hz), 8.61–8.65 (m, 2H).

*6.1.21. 1-(6- nitro-1,2-benzisothiazol-3-yl)-3-piperazine (**30**)*

A mixture of **28** (0.44 g, 2.1 mmol), and piperazine (1.8 g, 21.0 mmol) in chlorobenzene (20 mL) was heated to 80 °C with stirring for 16 h. Cooled to room temperature, the resulting solution was partitioned between water (100 mL) and ethyl acetate (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified using silica gel, eluting with 0.1–1% methanol/dichloromethane to give **30** (2.0 g, 49.5%) as a colorless solid. Mp: 164–166 °C. ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 1H), 2.80–2.81 (m, 4H), 3.00–3.01 (m, 4H), 8.11–8.15 (m, 2H), 8.32 (s, 1H).

*6.1.22. General procedure for the preparation of compounds **31**–**32***

Compound **29** [44] (5.0 g, 19.7 mmol) and **30** (1.5 g, 5.7 mmol) were separately treated with sodium carbonate (2.5 equivalent) and **5** (1.1 equivalent) in acetonitrile using procedure E. The crude product was purified by recrystallization from ethanol to yield **31**–**32**.

*6.1.22.1. N-(trans-4-(2-(4-(6-Chlorobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)acetamide (**31**).* Yield 69.3%; Mp: 251–252 °C. ^{1}H NMR ($CDCl_3$): δ 1.08–1.14 (m, 3H), 1.23–1.31 (m, 2H), 1.47–1.54 (m, 2H), 1.83–1.87 (m, 2H), 2.09–2.13 (m, 2H), 2.31 (s, 3H), 2.50 (t, 2H, J = 7.6 Hz), 2.68–2.70 (m, 4H), 3.54–3.57 (m, 4H), 4.10–4.13 (m, 1H), 7.23 (d, 1H, J = 3.6 Hz), 7.50 (d, 1H), J = 8.0 Hz, 7.88–8.04 (m, 2H).

*6.1.22.2. N-(trans-4-(2-(4-(6-nitrobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)acetamide (**32**).* Yield 71.1%; Mp: > 270 °C. ^{1}H NMR ($CDCl_3$): δ 1.02–1.07 (m, 2H), 1.16–1.25 (m, 3H), 1.37–1.40 (m, 1H), 1.53–1.56 (m, 2H), 1.82–1.87 (m, 3H), 2.30 (s, 3H), 2.42 (t, 2H, J = 7.6 Hz), 2.56–2.59 (m, 4H), 3.48–3.50 (m, 4H), 3.58–3.63 (m, 1H), 8.08–8.10 (d, 1H, J = 8.4 Hz), 8.16–8.18 (m, 1H), 8.29 (s, 1H), 9.10 (d, 1H, J = 2.0 Hz).

*6.1.23. General procedure for the preparation of compounds **33**–**34***

Compound **31** (2.0 g, 4.8 mmol) or **32** (1.0 g, 2.3 mmol) was treated with 5% HCl (100 mL) using procedure F to yield **33**–**34**.

*6.1.23.1. trans-4-(2-(4-(6-Chlorobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexanamine (**33**).* Yield 82.3%; Mp: 98–100 °C. ^{1}H NMR (DMSO-d_6): δ 0.94–1.05 (m, 4H),1.13–1.20 (m, 3H), 1.34–1.39 (m, 2H), 1.68–1.76 (m, 2H), 1.74–1.87 (m, 2H), 2.34 (t, 2H, J = 7.6 Hz), 2.48–2.52 (m, 1H), 2.58–2.60 (m, 4H), 3.46–3.48 (m, 4H), 6.98 (d, 1H, J = 8.0 Hz), 7.45–7.48 (m, 1H), 8.03 (d, 1H, J = 8.4 Hz), 8.22 (d, 1H, J = 2.0 Hz).

*6.1.23.2. trans-4-(2-(4-(6-Nitrobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexanamine (**34**).* Yield 85.6%; Mp: 131–133 °C. ^{1}H NMR ($CDCl_3$): δ 0.95–1.07 (m, 4H), 1.15–1.22 (m, 3H), 1.37–1.41 (m, 2H), 1.69–1.72 (m, 2H), 1.77–1.80 (m, 2H), 2.39 (t, 2H, J = 8.0 Hz), 2.50–2.55 (m, 1H), 2.60–2.62 (m, 4H),3.49–3.52 (m, 4H), 7.94 (d, 1H, J = 8.0 Hz), 8.10–8.13 (m, 1H), 8.63 (d, 1H, J = 4.0 Hz).

*6.1.24. General procedure for the preparation of compounds **35**–**36***

Compound **33** (0.5 g, 1.3 mmol) and **34** (0.5 g, 1.3 mmol) were separately treated with acyl chloride (0.1 g, 1.5 mmol) in dichloromethane (2 mL) using procedure G. The crude product was purified by recrystallization from ethanol to yield **35**–**36**.

*6.1.24.1. N-(trans-4-(2-(4-(6-Chlorobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)furan-2-carboxamide (**35**).* Yield 89.2%; mp: 204–206 °C. HPLC t_R = 10.26 min, 99.8% purity. ^{1}H NMR (DMSO-d_6): δ 0.97–1.05 (m, 2H), 1.23–1.25 (m, 2H), 1.30–1.41 (m, 3H), 1.77–1.79 (m, 4H), 2.38 (t, 2H, J = 7.6 Hz), 2.51–2.57 (m, 4H), 3.42–3.47 (m, 4H), 3.66–3.70 (m, 1H), 6.59–6.61 (m, 1H), 7.08 (d, 1H, J = 3.2 Hz), 7.45 (dd, 1H, J = 8.4 Hz, J = 2.0 Hz), 7.80 (d, 1H, J = 0.8 Hz), 8.04–8.25 (m, 2H),8.25 (d, 1H, J = 2.0 Hz). MS (ESI) m/z: 473.1 ([M+H] $^+$).

*6.1.24.2. N-(trans-4-(2-(4-(6-nitrobenzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)cyclohexyl)furan-2-carboxamide (**36**).* Yield 90.5%; mp: 230–232 °C. HPLC t_R = 9.70 min, 99.0% purity. ^{1}H NMR (DMSO-d_6): δ 1.00–1.03 (m, 2H),1.34–1.39 (m, 5H),1.77–1.80 (m, 4H), 1.71–1.74 (m, 2H), 2.40 (t, 2H, J = 7.6 Hz), 2.59–2.61 (m, 4H), 3.45–3.47 (m, 4H), 3.64–3.69 (m, 1H), 6.59–6.61 (m, 1H), 7.07 (d, 1H, J = 3.6 Hz), 7.80 (s, 1H), 8.10 (d, 1H, J = 8.4 Hz), 8.17 (dd, 1H, J = 8.4 Hz, J = 4.0 Hz), 9.14 (d, 1H, J = 4.0 Hz). MS (ESI) m/z: 484.2 ([M+H] $^+$).

*6.1.25. N-(trans-4-(2-(4-(6-Aminobenzo[d]isothiazol-3-yl) piperazin-1-yl)ethyl)cyclohexyl)furan-2-carboxamide (**37**)*

Hydrazine hydrate (0.5 mL) was added with stirring to a solution of **36** (0.1g, 0.3 mmol) and Raney Nickel (0.01 g) in methanol (10 mL). The resulting solution was stirred at room temperature for 7 h and filtered. The filtrate was evaporated *in vacuo*. The residue was purified using Aluminum oxide, eluting with 1–2% methanol/dichloromethane to afford **37** (0.08 g, 85.3%) as a yellow solid. Mp: 182–184 °C. HPLC t_R = 8.16 min, 97.2% purity. ^{1}H NMR (DMSO-d_6): δ 0.99–1.02 (m, 2H), 1.23–1.24 (m, 1H), 1.33–1.39 (m, 4H), 1.77–1.79 (m, 4H), 2.36 (t, 2H, J = 7.6 Hz), 2.50–2.53 (m, 4H), 3.34–3.37 (m, 4H), 3.63–3.68 (m, 1H), 5.64 (s, 2H), 6.59–6.60 (m, 1H), 6.66–6.69 (m, 1H), 6.88 (d, 1H, J = 2.0 Hz), 7.07–7.08 (m, 1H), 7.63 (d, 1H, J = 8.4 Hz), 7.79–7.80 (m, 1H), 8.10 (d, 1H, J = 8.0 Hz). MS (ESI) m/z: 454.3 ([M+H] $^+$).

*6.1.26. tert-Butyl 4-(2-ethoxy-2-oxoethyl)piperidine-1-carboxylate (**38**)*

tert-Butyl 4-oxopiperidine-1-carboxylate (20.0 g, 100.4 mmol) was treated with triethyl phosphonoacetate (24.8 g, 110.4 mmol) in THF (250 mL), and subsequent 10% Pd/C (1.0 g) using procedure A to obtain **38**. Yield 96.5%,^{1}H NMR ($CDCl_3$): δ 1.14–1.17 (m, 2H), 1.26 (t, 3H, J = 4.0 Hz), 1.49 (s, 9H), 1.68–1.72 (m, 2H), 1.93–1.97 (m, 1H), 2.23 (d, 2H, J = 8.0 Hz), 2.72–2.74 (m, 2H), 4.09–4.11 (m, 2H), 4.12 (q, 2H, J = 4.0 Hz).

*6.1.27. tert-Butyl 4-(2-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate (**39**)*

Compound **38** (20.0 g, 73.7 mmol) was treated with sodium borohydride (13.9 g, 368.5 mmol) in THF (200 mL) and subsequently mesyl chloride (73.7 mmol) using procedure C to achieve **39**. Yield 86.9%, ^{1}H NMR ($CDCl_3$): δ 1.13–1.15 (m, 2H), 1.45 (s, 9H), 1.66–1.68 (m, 2H), 1.69–1.72 (m, 3H), 2.70–2.72 (m, 2H), 3.01 (s, 3H), 4.08–4.10 (m, 2H), 4.29 (t, 2H, J = 4.0 Hz).

*6.1.28. 3-(4-(2-(Piperidin-4-yl)ethyl)piperazin-1-yl)benzo[d] isothiazole (**40**)*

A mixture of **39** (4.0 g, 13.0 mmol), 3-(piperazin-1-yl)benzo[*d*] isothiazole (2.6 g, 11.8 mmol), sodium carbonate (3.8 g, 35.5 mmol), potassium iodide (0.01 g, 0.05 mmol), and acetonitrile (100 mL) was stirred at reflux for 32 h. The resulting solution was cooled to ambient temperature and filtered. The filtrate was evaporated *in vacuo* to give a brown oil (6.3 g). A mixture of this material and TFA (7.5 mL) in dichloromethane (60 mL) was stirred at room temperature for 20 h. The resulting solution was evaporated *in vacuo* to remove most of the TFA and partitioned between aqueous water (40 mL) and dichloromethane (100 mL). Aqueous 50% NaOH (20 mL) was added to the solution in an ice bath and stirred for 20 min. The solution was extracted with dichloromethane (50 mL) and the combined extracts were washed with brine (100 mL), dried with Na_2SO_4, and evaporated *in vacuo* to give **40** (2.6 g, 66.8%) as a light yellow solid. Mp: 61–63 °C. ^{1}H NMR ($CDCl_3$): δ 1.23–1.25 (m, 3H), 1.44–1.52 (m, 3H), 1.71–1.73 (m, 2H), 2.46 (t, 2H, J = 4.0 Hz), 2.59–2.64 (m, 2H), 2.66–2.68 (m, 4H), 3.09–3.11 (m, 2H), 3.56–3.58 (m, 4H), 7.35 (t, 1H, J = 4.0 Hz), 7.46 (t, 1H, J = 4.0 Hz), 7.80 (d, 1H, J = 4.0 Hz), 7.90 (d, 1H, J = 4.0 Hz).

*6.1.29. General procedure for the preparation of compound **41***

Compound **40** (2.5 g, 7.6 mmol) in dichloromethane (25 mL) was treated with acyl chloride (9.1 mmol) in dichloromethane (10 mL) using procedure G to obtained the free base of **41**. Then, a mixture of oxalic acid dihydrate and the corresponding free base in ethyl acetate (10 mL) were added and stirred at room temperature for 2 h. The reaction was filtered; the filter cake was washed with acetone (5 mL) to give **41** as a white solid.

*6.1.29.1. Ethyl 4-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl) piperidine-1-carboxylate oxalate (**41a**).* Yield 73.1%; mp: 190–193 °C. HPLC t_R = 9.52 min, 99.3% purity. ^{1}H NMR (DMSO-d_6): δ 1.14–1.16 (m, 2H), 1.62–1.65 (m, 3H), 1.75–1.78 (m, 2H), 2.82 (t, 2H, J = 4.0 Hz), 3.05–3.08 (m, 2H), 3.26–3.29 (m, 4H), 3.68–3.71 (m, 4H), 4.30–4.34 (m, 2H), 6.62 (s, 1H), 6.94–6.95 (m, 1H), 7.47 (t, 1H, J = 7.6 Hz), 7.59 (t, 1H, J = 7.6 Hz), 7.83 (s, 1H), 8.09–8.14 (m, 2H). MS (ESI) m/z: 425.0 ([M+H] $^+$).

*6.1.29.2. (4-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl) piperidin-1-yl)(furan-2-yl)methanone oxalate (**41b**).* Yield 76.8%; mp: 196–198 °C. HPLC t_R = 9.16 min, 99.5% purity. ^{1}H NMR (DMSO-d_6): δ 1.00–1.08 (m, 2H),1.17 (t, 3H, J = 7.2 Hz),1.60–1.69 (m, 5H), 2.75 (t, 2H, J = 4.0 Hz), 3.06–3.08 (m, 2H), 3.28–3.31 (m, 4H), 3.67–3.70 (m, 4H), 3.94–3.97 (m, 2H), 4.02 (q, 2H, J = 7.2 Hz), 7.47 (t, 1H, J = 7.6 Hz), 7.59 (t, 1H, J = 7.6 Hz), 8.09–8.14 (m, 2H). MS (ESI) m/z: 403.3 ([M+H] $^+$).

*6.1.30. trans-cyclohexane-1,4-dimethanol (**42**)*

Thionyl chloride (16.6 g, 139.5 mmol) was added dropwise to a stirred solution of *trans*-cyclohexane-1,4-dicarboxylic acid (10.0 g, 58.1 mmol) in methanol (160 mL) at 0–20 °C. The resulting mixture was stirred at reflux for 4 h. The resulting solution was evaporated *in vacuo* and the residue was partitioned between aqueous water (50 mL) and dichloromethane (100 mL). The organic layer was washed with aqueous $NaHCO_3$ (40 mL), brine (50 mL) and dried (Na_2SO_4), and evaporated *in vacuo* to give a colorless solid (9.8 g, 84.0%). Next, a mixture of this solid (9.8 g) and $NaBH_4$ (18.5 g, 488.2 mmol) in THF (196 mL) was stirred at reflux for 0.5 h. Then methanol (69 mL) was added to the resulting solution at room temperature.

The resulting solution was stirred at reflux for 12 h and quenched with 30% hydrochloric acid (20 mL) in and ice bath. The resulting mixture was stirred for 1 h and alkalized with 50% sodium hydroxide solution (30 mL). The resultant mixture was extracted with dichloromethane (3 × 60 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give **42** (2.4 g, 28.6%) as a colorless solid. Mp: 63–65 °C. ^{1}H NMR ($CDCl_3$): δ 0.93–1.03 (m, 4H), 1.45–1.47 (m, 2H), 1.84–1.88 (m, 4H),

3.40–3.46 (m, 4H).

*6.1.31. (trans-4-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) methyl)cyclohexyl)methyl methanesulfonate (**43**)*

Methylsulfonyl chloride (4.2 g, 36.6 mmol) in ethyl acetate (10 mL) was added dropwise with stirring to a solution of **42** (2.4 g, 16.6 mmol) and triethylamine (9.0 mL, 66.6 mmol) in ethyl acetate (40 mL) at 0 °C. The resulting mixture was stirred for 3 h at ambient temperature. The resulting mixture was filtered and washed with water (2 × 50 mL) to give a colorless solid (3.93 g, 78.7 mmol). A mixture of this solid (2.6 g, 11.9 mmol), 3-(piperazin-1-yl)benzo[*d*] isothiazole (3.9 g, 13.1 mmol), sodium carbonate (2.5 g, 23.8 mmol), potassium iodide (0.01 g, 0.05 mmol), and acetonitrile (100 mL) was stirred at reflux for 22 h. The resulting solution was cooled to ambient temperature and filtered and washed with acetonitrile (30 mL) to yield a colorless solid which was further purified using silica gel, eluting with 2.5% methanol/dichloromethane to give **43** (2.2 g, 44.1%) as a white solid. Mp: 138–140 °C. ^{1}H NMR ($CDCl_3$): δ 0.94–0.98 (m, 2H), 1.02–1.10 (m, 2H), 1.52–1.53 (m, 1H), 1.72–1.74 (m, 2H), 1.85–1.94 (m, 4H), 2.23 (d, 2H, J = 4.0 Hz), 2.61–2.63 (m, 4H), 3.00 (s, 3H), 3.54–3.55 (m, 4H), 4.04 (d, 2H, J = 4.0 Hz), 7.34–7.36 (m, 1H), 7.45–7.47 (m, 1H), 7.34–7.36 (m, 1H), 7.80–7.81 (m, 1H), 7.90–7.92 (m, 1H).

*6.1.32. (trans-4-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) methyl)cyclohexyl)methanamine (**44**)*

A mixture of **43** (1.0 g, 2.4 mmol), potassium phthalimide (0.5 g, 2.6), and *N*,*N*-dimethylformamide (15 mL) was stirred at 100 °C for 1.5 h. The resulting solution was poured into ice water (150.0 g) and stirred for 1 h. The resultant mixture was extracted with dichloromethane (2 × 75 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to give a semi-solid (1.1 g). A mixture of this material (1.1 g), hydrazine hydrate (0.2 g, 2.6 mmol), and ethanol (40 mL) was stirred at reflux for 12 h. The resulting mixture was cooled to ambient temperature, filtered and washed with ethanol (20 mL) to give **44** (0.5 g, 66.3%) as a white solid. Mp: 198–200 °C. ^{1}H NMR ($CDCl_3$): δ 0.91–0.94 (m, 4H), 1.26–1.28 (m, 2H), 1.54 (s, 2H), 1.82–1.83 (m, 2H), 1.89–1.90 (m, 2H), 2.23 (d, 2H, J = 4.0 Hz), 2.54 (d, 2H, J = 4.0 Hz), 2.61–2.63 (m, 4H), 3.54–3.56 (m, 4H), 7.34–7.36 (m, 1H), 7.45–7.47 (m, 1H), 7.34–7.36 (m, 1H), 7.80–7.81 (m, 1H), 7.90–7.92 (m, 1H).

*6.1.33. N-((trans-4-((4-(benzo[d]isothiazol-3-yl)piperazin-1-yl) methyl)cyclohexyl)methyl)furan-2-carboxamide (**45**)*

Compound **44** (0.2 g, 0.6 mmol) in dichloromethane (5 mL) was treated with pyromrcyl chloride (0.1 g, 0.7 mmol) in dichloromethane (1 mL) using procedure G. The crude product was purified by recrystallization from ethanol to yield **45** (0.2 g, 75.8%). Mp: 159–160 °C. HPLC t_R = 9.24 min, 97.1% purity. ^{1}H NMR (DMSO-d_6): δ 0.81–0.95 (m, 4H), 1.47–1.50 (m, 2H), 1.72–1.74 (m, 2H), 1.81–1.82 (m, 2H), 2.16 (d, 2H, J = 4.8 Hz), 2.54–2.56 (m, 4H), 3.06 (t, 2H, J = 4.4 Hz), 3.43–3.45 (m, 4H), 6.60–6.61 (m, 1H), 7.07 (d, 1H, J = 2.0 Hz), 7.43 (t, 1H, J = 5.2 Hz), 7.55 (t, 1H, J = 5.2 Hz), 7.81 (s, 1H), 8.03–8.06 (m, 2H), 8.31–8.32 (m, 1H). MS (ESI) *m*/*z*: 439.2 ([M+H]$^+$).

6.2. Single crystal X-ray crystallographic study

The X-ray crystallographic analysis of **6** was carried out on a colorless crystal, with dimensions 0.10 mm × 0.23 mm × 0.25 mm. The crystal was grown from the slow evaporation of an acetone solution of **6** at room temperature. A suitable crystal was selected and mounted on a Bruker SMART APEX-II CCD diffractometer using CuK_α radiation (λ = 1.54178 Å). Data collection was carried out at 298 (2) K. The crystal structure solution was worked out by the full matrix least-squares method using a SHELXL97. All the non-hydrogen atoms were located in different Fourier maps. The hydrogen atoms were fixed geometrically and refined isotropically. Graphical representations were generated using ORTEP. Crystallographic data for **6** has been deposited with the Cambridge Crystallographic Data Centre (CCDC 1414993).

6.3. Biology evaluation

6.3.1. Animals

Male Sprague-Dawley (SD) rats (150–290 g) were used as experimental animals in this study. Rats were housed under standardized conditions and had free access to standard rat chow and water. Rats were randomly assigned to different experimental groups, each separately kept in a cage. All research involving animals in this study follow the guidelines of the byelaw of experiments on animals, and have been approved by the Ethics and Experimental Animal Committee of Shanghai Institute of Pharmaceutical Industry.

6.3.2. In vitro binding assays

6.3.2.1. 5-HT$_{1A}$ receptor [53]. Cell membrane homogenates (36 μg protein) were incubated for 60 min at 22 °C with 0.3 nM [^{3}H]8-OH-DPAT in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgSO_4$, 0.5 mM EDTA and 2 μg/ml aprotinine. Nonspecific binding was determined in the presence of 10 μM 8-OH-DPAT. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI. The filtered material was rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The inhibition results were expressed as a percentage of sample binding compared to the control radioligand specific binding. The standard reference compound was 8-OH-DPAT and was tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

6.3.2.2. 5-HT$_{2A}$ receptor [54]. Cell membrane homogenates (40 μg protein) were incubated for 60 min at 22 °C with 0.5 nM [^{3}H] ketanserin in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4). Nonspecific binding was determined in the presence of 1 μM ketanserin. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI. The filtrate was rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The inhibition results were expressed as a percentage of sample binding compared to the control radioligand specific binding. The ketanserin was used as a standard reference compound and was tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

6.3.2.3. Dopaminergic D$_{2L}$ receptor [55]. Cell membrane homogenates (16 μg protein) were incubated for 60 min at 22 °C with 0.3 nM [^{3}H]methyl-spiperone in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 10 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding was determined in the presence of 10 μM butaclamol. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI. The filtrate was rinsed several times with ice-cold 50 mM Tris-HCl using

a 96-sample cell harvester (Unifilter, Packard). The filters were dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The inhibition results were expressed as a percentage of sample binding compared to the control radioligand specific binding. The butaclamol was used as a standard reference compound and was tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

6.3.2.4. Dopaminergic D_3 receptor [56]. Cell membrane homogenates (8 μg protein) were incubated for 60 min at 22 °C with 0.3 nM [^{3}H] methyl-spiperone in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$ and 5 mM EDTA. Nonspecific binding was determined in the presence of 10 μM (+)butaclamol. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI. The filtrate was rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results were expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound was (+)butaclamol, which was tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

6.3.2.5. Muscarinic M_1 receptor [57]. Cell membrane homogenates (45 μg protein) are incubated for 60 min at 22 °C with 2 nM [^{3}H] pirenzepine in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding is determined in the presence of 1 μM atropine. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is pirenzepine, which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

6.3.2.6. hERG affinity evaluation. CHO-K1 (Chinese Hamster Ovary) cells stably transfected with human hERG cDNA were used. The cells were harvested by trypsinization and maintained in Serum Free Medium at room temperature before recording. The test solutions were prepared in the Extracellular Solution on the day of patch clamp assay. The assay can tolerate up to 1% DMSO. After whole cell configuration was achieved, the cell was held at −80 mV. A −50 mV pulse was delivered for 50 ms to measure the leaking current, which was subtracted from the tail current on-line. Then the cell was depolarized to +20 mV for 5 s, followed by a 5 s pulse to −50 mV to reveal the hERG tail current. This paradigm was delivered once every 15 s to monitor the current amplitude. The Extracellular Solution (control) was applied first and the cell was stabilized in the solution for 2 min. Then the test compound was applied from low to high concentrations sequentially on the same cell. The cells were incubated with each test concentration for 2 min. Cisapride was tested concurrently at multiple concentrations to obtain an IC_{50} value. The percent inhibition of the hERG channel is calculated by comparing the tail current amplitude before and after application of the compound (the current difference is normalized to control).

6.3.3. In vitro pharmacology for intrinsic activity assessment

6.3.3.1. Dopamine D_2, serotonin 5-HT_{1A} and serotonin 5-HT_{2A} receptors [58,59]. CHO-K1 cells expressing three receptors (CHOK1/D_2/Gα15, CHO-K1/5-HT_{1A}/Gα15, and CHO-K1/5-HT_{2A}/Gα15) were seeded in a 384-well Poly-D-Lysine protein coating 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.

20 μL of dye-loading solution (20 mM 2X (8 uM) Fluo-4 Direct™ Loading Buffer with 2.5 mM Probenecid) was added to the 384-well cell culture plate using pipette. Then, the plate was placed into a 37 °C/5% CO_2 incubator for 50 min, followed by 10 min at room temperature. Cell plate was removed from incubator and placed into FLIPR. The compound plate and tip box were then placed into FLIPR.

For the DRC plate, 10 μL of assay buffer was transferred from 384-well plate to the cell plate, followed by transfer of 10 μL of the compounds from the DRC plate to the cell plate. Then, the "Max-Min" starting from Read 90 to Maximum allowed was calculated. The EC_{80} values for each cell line were calculated using FLIPR. Finally, 6 × EC_{80} concentrations of agonist reference compounds were prepared.

For the compound plate (agonist test and antagonist test), 10 μL of references and compounds was transferred from the compound plate to the cell plate, followed by transfer of 10 μL of 6 × EC_{80} concentrations of agonist reference compounds to the cell plate. For agonist test, the "Max-Min" starting from Read 1 to 90 was calculated. For antagonist test, the "Max-Min" starting from Read 90 to Maximum allowed was calculated. Data were analyzed using Prism.

6.3.3.2. Dopamine D_3 receptors. For detection of the agonism action of the compounds at D_3 receptor, the [^{35}S]GTPγS binding assay was performed at 30 °C for 40 min in reaction buffer containing 50 mM Tris, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, and 1 mM DL-dithiothreitol (DTT). The assay mixture (200 μL) contained 20 μg of membrane protein, 0.1 nM [^{35}S]GTPγS, and 40 μM guanosine triphosphate (GDP) with various concentration of the compound. The antagonism effects of the compounds were tested in the existence of 100 μM quinpirole. Nonspecific binding was measured in the presence of 100 μM 50-guanylimidodiphosphate (Gpp(NH)p). The reaction was terminated by addition of 3 mL of ice-cold washing buffer (50 mM Tris, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, and 100 mM NaCl) and was rapidly filtered with GF/C glass fiber filters (Whatman) and rinsed three times. Filters were dried and radioactivity was determined by liquid scintillation counting.

6.3.4. Behavioral tests

6.3.4.1. PCP-induced hyperlocomotion. Male SD rats (200–250 g, 8–10 rats in each group) were used. Animals were individually placed into a Plexiglas open field arena (40 × 40 × 45 cm, Jiliang Co. Ltd., Shanghai, China) for 2 min 10 min after intraperitoneal injection of the test compounds (0.1, 0.2 and 0.4 mg/kg) or cariprazine (0.1, 0.2 and 0.4 mg/kg), animals were treated with PCP (5 mg/kg, i.p.), and placed back into the experimental apparatus. Animals were habituated for 10 min before the 1.5-h measurement period. The results of the locomotor activity tests were expressed as the mean ± SEM. The percent inhibition of activity was calculated for each dose. Statistical evaluation was performed by two-way ANOVA followed by the Tukey Test. +++, $P < 0.001$ versus vehicle treated control group (Veh). *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$ versus phencyclidine treated group (PCP).

6.3.4.2. Conditioned avoidance response (CAR) [60]. Male SD rats (150–180 g, 6 rats in each group) were used. CAR behavior was assessed using auto-mated shuttle-boxes placed in a sound-attenuated chamber (73 × 43 × 37 cm) (LM100,

Hamilton–Kinder Scientific, USA). Daily sessions consisted of 30 trials with a 10-s continuous sound and light as conditioned stimulus, paired with a 10-s electric foot shock (shock intensity: 0.8 mA) as an unconditioned stimulus. Rats who achieved greater than 80% avoidance responses were chosen for drug treatments. Test compounds (i.p. 1.0 and 2.0 mg/kg) and Cariprazine (i.p. 1.0 and 2.0 mg/kg) were administered 1 h before testing via single intraperitoneal injection. The test session consisted of 20 trials. Results were expressed as percentage of avoidance. Data was expressed as Mean ± SEM (n = 6). ***, P < 0.001 versus vehicle treated control group (Basal).

6.3.4.3. Novel object recognition paradigm. Male SD rats (160–180 g, N = 50, 40 PCP- and 10 saline-treated) were used. Rats were randomly assigned to receive either saline (once a day, i.p.) or PCP (7.5 mg/kg once a day, i.p.) for 14 days. Subsequently, rats were given a 6-day drug-free period prior to NOR testing. Rats were habituated in groups to an empty test box for 5–10 min on day 1. The test consisted of two 3-min trials separated by an inter-trial interval (ITI) of 1 h. In the first (training session) trial, animals were placed in the test box and allowed to explore two identical objects. In the second (retention session) trial, one of the objects was replaced by a novel one. **9j** 0.1, or 0.2 mg/kg (PO), cariprazine 0.1 mg/kg (PO), or saline was administered 60 min prior to testing. The time spent in exploring any one of the two objects (training session) or the novel one (retention session) which including the animals licking, sniffing, or touching the object but not including time spent leaning against, standing or sitting on the object was recorded for used to score recognition memory. Data were analyzed by post-hoc Student's t-test; *P < 0.05 for time spent exploring familiar versus novel object.

6.3.4.4. Catalepsy test. Male SD rats (180–250 g) were tested 30 min, 60 min and 120 min after oral administration with test compound (1.0, 3.0 and 10.0 mg/kg), cariprazine (1.0, 3.0 and10.0 mg/kg) and risperidone (1.0, 3.0 and10.0 mg/kg). The rats were placed on a bar (0.9 cm in diameter and 11 cm in height above the tabletop) with its forepaws on the bar. Rats were considered cataleptic if they did not correct their body posture within 60 s.

6.3.4.5. Pharmacokinetic study. Pharmacokinetic studies were performed in male SD rats (24 in each group) weighing 250–290 g. Pharmacokinetic parameters of blood, brain, and oral bioavailability were obtained by single intravenous (i.v.) and p.o. administration of the test compound 0.5 mg/kg and 2.0 mg/kg, respectively. The test compound was dissolved in mixed solution of DMSO (5%), Solutol HS 15 (10%) and PBS (85%). Heparinized samples of blood were collected at 5 min, 15min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h after oral or intravenous administration. Plasma was obtained after centrifugation and stored frozenat −20 °C for analysis.

Plasma and brain samples were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and the chromatographic column was Thermo C18 column (2.1 × 50 mm, 5 μm) using a gradient elution. The mobile phase was 0.1% formic acid aqueous solution and 0.1% formic acid acetonitrile solution at a flow rate of 0.4 mL/min. The original data was calculated and collected using AB Sciex mass spectrum software Analyst 1.6.1.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.07.038.

References

[1] A. Fernández, C. Gómez, R. Hornero, J.J. Lopéz-Ibor, Complexity and schizophrenia, Prog. Neuropsychopharmacol. Biol. Psychiatry 45 (2013) 267–276.
[2] S. Leucht, T. Burkard, J. Henderson, M. Maj, N. Sartorius, Physical illness and schizophrenia: a review of the literature, Acta Psychiatr. Scand 116 (2007) 317–333.
[3] A. Schmitt, P. Falkai, Schizophrenia spectrum and related neuropathology, Eur. Arch. Psychiatry Clin. Neurosci. 265 (2015) 85–86.
[4] W.T. Carpenter Jr., J.M. Davis, Another view of the history of antipsychotic drug discovery and development, Mol. Psychiatry 17 (2012) 1168–1173.
[5] S. Miyamoto, N. Miyake, L.F. Jarskog, W.W. Fleischhacker, J.A. Lieberman, Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents, Mol. Psychiatry 17 (2012) 1206–1227.
[6] J.W. Newcomer, Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review, CNS Drugs 19 (2005) 1–93.
[7] P.M. Haddad, I.M.Anderson, Antipsychotic-related QTc prolongation, torsade de pointes and sudden death, Drugs 62 (2002) 1649–1671.
[8] A.B. Minns, R.F. Clark, Toxicology and overdose of atypical antipsychotics, J. Emerg. Med. 43 (2012) 906–913.
[9] R. Morphy, C. Kay, Z. Rankovic, From magic bullets to designed multiple ligands, Drug Discov. Today 9 (2004) 641–651.
[10] R. Morphy, Z. Rankovic, Designed multiple ligands. An emerging drug discovery paradigm, J. Med. Chem. 48 (2005) 6523–6543.
[11] R. Morphy, Z. Rankovic, Fragments, network biology and designing multiple ligands, Drug Discov. Today 12 (2007) 156–160.
[12] E.H. Wong, F.I. Tarazi, M. Shahid, The effectiveness of multi-target agents in schizophrenia and mood disorders: relevance of receptor signature to clinical action, Pharmacol. Ther. 126 (2010) 173–185.
[13] A. Newman-Tancredi, The importance of 5-HT_{1A} receptor agonism in antipsychotic drug action: rationale and perspectives, Curr. Opin. Investig. Drugs 11 (2010) 802–812.
[14] T.A. Mestre, M. Zurowski, S.H. Fox, 5-Hydroxytryptamine 2A receptor antagonists as potential treatment for psychiatric disorders, Expert Opin. Invest. Drugs 22 (2013) 411–421.
[15] C. Missale, S.R. Nash, S.W. Robinson, M. Jaber, M.G. Caron, Dopamine receptors: from structure to function, Physiol. Rev. 78 (1998) 189–225.
[16] N. Griffon, P. Sokoloff, J. Diaz, D. Levesque, F. Sautel, J.C. Schwartz, P. Simon, J. Costentin, F. Garrido, A. Mann, The dopamine D_3 receptor and schizophrenia: pharmacological, anatomical and genetic approaches, Eur. Neuropsychopharmacol. 5 (1995) 3–9.
[17] J.N. Joyce, M.J. Millan, Dopamine D_3 receptor antagonists as therapeutic agents, Drug Discov. Today 10 (2005) 917–925.
[18] M.J. Millan, B. Di Cara, A. Dekeyne, F. Panayi, L. De Groote, D. Sicard, L. Cistarelli, R. Billiras, A. Gobert, Selective blockade of dopamine D(3) versus D(2) receptors enhances frontocortical cholinergic transmission and social memory in rats: a parallel neurochemical and behavioural analysis, J. Neurochem. 100 (2007) 1047–1061.
[19] M.J. Millan, A. Dekeyne, J.M. Rivet, T. Dubuffet, G. Lavielle, M. Brocco, S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: II. Functional and behavioral profile compared with GR218,231 and L741,626, J. Pharmacol. Exp. Ther. 293 (2000) 1063–1073.
[20] S. Butini, G. Campiani, S. Franceschini, F. Trotta, V. Kumar, E. Guarino, G. Borrelli, I. Fiorini, E. Novellino, C. Fattorusso, M. Persico, N. Orteca, K. Sandager-Nielsen, T.A. Jacobsen, K. Madsen, J. Scheel-Kruger, S. Gemma, Discovery of bishomo(hetero)arylpiperazines as novel multifunctional ligands targeting dopamine D(3) and serotonin 5-HT(1A) and 5-HT(2A) receptors, J. Med. Chem. 53 (2010) 4803–4807.
[21] E. Agai-Csongor, G. Domany, K. Nogradi, J. Galambos, I. Vago, G.M. Keseru, I. Greiner, I. Laszlovszky, A. Gere, E. Schmidt, B. Kiss, M. Vastag, K. Tihanyi, K. Saghy, J. Laszy, I. Gyertyan, M. Zajer-Balazs, L. Gemesi, M. Kapas, Z. Szombathelyi, Discovery of cariprazine (RGH-188): a novel antipsychotic acting on dopamine D3/D2 receptors, Bioorg. Med. Chem. Lett. 22 (2012) 3437–3440.

[22] B. Kiss, I. Laszlovszky, A. Horvath, Z. Nemethy, E. Schmidt, G. Bugovics, K. Fazekas, I. Gyertyan, E. Agai-Csongor, G. Domany, Z. Szombathelyi, Sub-nanomolar dopamine D_3 receptor antagonism coupled to moderate D_2 affinity results in favourable antipsychotic-like activity in rodent models: I. neurochemical characterisation of RG-15, Schmiedeb. Arch. Pharmacol. 378 (2008) 515–528.

[23] D.I. Cho, M. Zheng, K.M. Kim, Current perspectives on the selective regulation of dopamine D(2) and D(3) receptors, Arch. Pharmacal Res. 33 (2010) 1521–1538.

[24] I. Gyertyan, B. Kiss, K. Saghy, J. Laszy, G. Szabo, T. Szabados, L.I. Gemesi, G. Pasztor, M. Zajer-Balazs, M. Kapas, E.A. Csongor, G. Domany, K. Tihanyi, Z. Szombathelyi, Cariprazine (RGH-188), a potent D_3/D_2 dopamine receptor partial agonist, binds to dopamine D_3 receptors *in vivo* and shows antipsychotic-like and procognitive effects in rodents, Neurochem. Int. 59 (2011) 925–935.

[25] I. Gyertyán, B. Kiss, K. Sághy, I. Laszlovszky, A. Horváth, J. Laszy, É. Schmidt, G. Szabó, Gy Domány, É. Ágai-Csongor, K. Tihanyi, Z. Szombathelyi, P.3.d.006 Pharmacological profile of RGH-188, a novel dopamine D3/D2 receptor antagonist/partial agonist atypical antipsychotic, Eur. Neuropsychopharmacol. 16 (2006) S432.

[26] T. Veselinovic, M. Paulzen, G. Grunder, Cariprazine, a new, orally active dopamine D2/3 receptor partial agonist for the treatment of schizophrenia, bipolar mania and depression, Expert Rev. Neurother. 13 (2013) 1141–1159.

[27] B. Kiss, A. Horvath, Z. Nemethy, E. Schmidt, I. Laszlovszky, G. Bugovics, K. Fazekas, K. Hornok, S. Orosz, I. Gyertyan, E. Agai-Csongor, G. Domany, K. Tihanyi, N. Adham, Z. Szombathelyi, Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: *in vitro* and neurochemical profile, J. Pharmacol. Exp. Ther. 333 (2010) 328–340.

[28] H.Y. Meltzer, S. Matsubara, J.C. Lee, Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values, J. Pharmacol. Exp. Ther. 251 (1989) 238–246.

[29] P.A. Janssen, C.J. Niemegeers, F. Awouters, K.H. Schellekens, A.A. Megens, T.F. Meert, Pharmacology of risperidone (R64766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties, J. Pharmacol. Exp. Ther. 244 (1988) 685–693.

[30] F. Boeckler, H. Lanig, P. Gmeiner, Modeling the similarity and divergence of dopamine D2-like receptors and identification of validated ligand-receptor complexes, J. Med. Chem. 48 (2005) 694–709.

[31] G.J. Macdonald, C.L. Branch, M.S. Hadley, C.N. Johnson, D.J. Nash, A.B. Smith, G. Stemp, K.M. Thewlis, A.K. Vong, N.E. Austin, P. Jeffrey, K.Y. Winborn, I. Boyfield, J.J. Hagan, D.N. Middlemiss, C. Reavill, G.J. Riley, J.M. Watson, M. Wood, S.G. Parker, C.R. Ashby Jr., Design and synthesis of *trans*-3-(2-(4-((3-(3-(5-methyl-1,2,4-oxadiazolyl))- phenyl)carboxamido)cyclohexyl)ethyl)-7-methylsulfonyl-2,3,4,5-tetrahydro-1H-3-ben zazepine (SB-414796): a potent and selective dopamine D_3 receptor antagonist, J. Med. Chem. 46 (2003) 4952–4964.

[32] M. Leopoldo, F. Berardi, N.A. Colabufo, P. De Giorgio, E. Lacivita, R. Perrone, V. Tortorella, Structure-affinity relationship study on N-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides as potent and selective dopamine D(3) receptor ligands, J. Med. Chem. 45 (2002) 5727–5735.

[33] G. Campiani, S. Butini, F. Trotta, C. Fattorusso, B. Catalanotti, F. Aiello, S. Gemma, V. Nacci, E. Novellino, J.A. Stark, A. Cagnotto, E. Fumagalli, F. Carnovali, L. Cervo, T. Mennini, Synthesis and pharmacological evaluation of potent and highly selective D_3 receptor ligands: inhibition of cocaine-seeking behavior and the role of dopamine D_3/D_2 receptors, J. Med. Chem. 46 (2003) 3822–3839.

[34] A. Hackling, R. Ghosh, S. Perachon, A. Mann, H.D. Holtje, C.G. Wermuth, J.C. Schwartz, W. Sippl, P. Sokoloff, H. Stark, N-(omega-(4-(2-methoxyphenyl) piperazin-1-yl)alkyl)carboxamides as dopamine D_2 and D_3 receptor ligands, J. Med. Chem. 46 (2003) 3883–3899.

[35] A.H. Newman, J. Cao, C.J. Bennett, M.J. Robarge, R.A. Freeman, R.R. Luedtke, N-(4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl, butenyl and butynyl)arylcarboxamides as novel dopamine D(3) receptor antagonists, Bioorg. Med. Chem. Lett. 13 (2003) 2179–2183.

[36] T.R. Belliotti, S.R. Kesten, J.R. Rubin, D.J. Wustrow, L.M. Georgic, K.T. Zoski, H.C. Akunne, L.D. Wise, Novel cyclohexyl amides as potent and selective D_3 dopamine receptor ligands, Bioorg. Med. Chem. Lett. 7 (1997) 2403–2408.

[37] S.Y. Ablordeppey, R. Altundas, B. Bricker, X.Y. Zhu, E.V. Kumar, T. Jackson, A. Khan, B.L. Roth, Identification of a butyrophenone analog as a potential atypical antipsychotic agent: 4-[4-(4-chlorophenyl)-1,4-diazepan-1-yl]-1-(4-fluorophenyl)butan-1-one, Bioorg. Med. Chem. 16 (2008) 7291–7301.

[38] Y. Chen, Y. Lan, S. Wang, H. Zhang, X. Xu, X. Liu, M. Yu, B.F. Liu, G. Zhang, Synthesis and evaluation of new coumarin derivatives as potential atypical antipsychotics, Eur. J. Med. Chem. 74 (2014) 427–439.

[39] G. Neves, R. Menegatti, C.B. Antonio, L.R. Grazziottin, R.O. Vieira, S.M. Rates, F. Noel, E.J. Barreiro, C.A. Fraga, Searching for multi-target antipsychotics: discovery of orally active heterocyclic N-phenylpiperazine ligands of D2-like and 5-HT_{1A} receptors, Bioorg. Med. Chem. 18 (2010) 1925–1935.

[40] H.R. Howard, J.A. Lowe 3rd, T.F. Seeger, P.A. Seymour, S.H. Zorn, P.R. Maloney, F.E. Ewing, M.E. Newman, A.W. Schmidt, J.S. Furman, G.L. Robinson, E. Jackson, C. Johnson, J. Morrone, 3-Benzisothiazolylpiperazine derivatives as potential atypical antipsychotic agents, J. Med. Chem. 39 (1996) 143–148.

[41] M.H. Norman, G.C. Rigdon, F. Navas 3rd, B.R. Cooper, Cyclic benzamides as mixed dopamine D2/serotonin 5-HT2 receptor antagonists: potential atypical antipsychotic agents, J. Med. Chem. 37 (1994) 2552–2563.

[42] M.H. Norman, G.C. Rigdon, W.R. Hall, F. Navas 3rd, Structure-activity relationships of a series of substituted benzamides: potent D2/5-HT2 antagonists and 5-HT1a agonists as neuroleptic agents, J. Med. Chem. 39 (1996) 1172–1188.

[43] A. Orjales, R. Mosquera, A. Toledo, C. Pumar, L. Labeaga, A. Innerarity, New 3-benzisothiazolyl and 3-benzisoxazolylpiperazine derivatives with atypical antipsychotic binding profile, Eur. J. Med. Chem. 37 (2002) 721–730.

[44] N.J. Hrib, J.G. Jurcak, D.E. Bregna, K.L. Burgher, H.B. Hartman, S. Kafka, L.L. Kerman, S. Kongsamut, J.E. Roehr, M.R. Szewczak, A.T. Woods-Kettelberger, R. Corbett, Structure-activity relationships of a series of novel (piperazinylbutyl)thiazolidinone antipsychotic agents related to 3-[4-[4-(6-fluorobenzo[b]thien-3-yl)-1-piperazinyl]butyl]-2,5,5- trimethyl-4-thiazolidinone maleate, J. Med. Chem. 39 (1996) 4044–4057.

[45] N.R. Swerdlow, M.A. Geyer, Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia, Schizophr. Bull. 24 (1998) 285–301.

[46] J.M. Uslaner, S.M. Smith, S.L. Huszar, R. Pachmerhiwala, R.M. Hinchliffe, J.D. Vardigan, S.J. Nguyen, N.O. Surles, L. Yao, J.C. Barrow, V.N. Uebele, J.J. Renger, J. Clark, P.H. Hutson, T-type calcium channel antagonism produces antipsychotic-like effects and reduces stimulant-induced glutamate release in the nucleus accumbens of rats, Neuropharmacology 62 (2012) 1413–1421.

[47] G. Martel, R. Jaffard, J.L. Guillou, Identification of hippocampus-dependent and hippocampus independent memory components in step-down inhibitory avoidance tasks, Behav. Brain Res. 207 (2010) 138–143.

[48] H.Y. Meltzer, L. Rajagopal, M. Huang, Y. Oyamada, S. Kwon, M. Horiguchi, Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia, Int. J. Neuropsychopharmacol. 16 (2013) 2181–2194.

[49] J.C. Neill, S. Barnes, S. Cook, B. Grayson, N.F. Idris, S.L. McLean, S. Snigdha, L. Rajagopal, M.K. Harte, Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism, Pharmacol. Ther. 128 (2010) 419–432.

[50] H.A. Nasrallah, Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles, Mol. Psychiatry 13 (2008) 27–35.

[51] A. Newman-Tancredi, M.S. Kleven, Comparative pharmacology of antipsychotics possessing combined dopamine D2 and serotonin 5-HT1A receptor properties, Psychopharmacol. (Berl) 216 (2011) 451–473.

[52] X. Xiberas, J.L. Martinot, L. Mallet, E. Artiges, H.C. Loc, B. Maziere, M.L. Paillere-Martinot, Extrastriatal and striatal D(2) dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia, Br. J. Psychiatry 179 (2001) 503–508.

[53] J.G. Mulheron, S.J. Casanas, J.M. Arthur, M.N. Garnovskaya, T.W. Gettys, J.R. Raymond, Human 5-HT_{1A} receptor expressed in insect cells activates endogenous G(o)-like G protein(s), J. Biol. Chem. 269 (1994) 12954–12962.

[54] D.W. Bonhaus, C. Bach, A. DeSouza, F.H. Salazar, B.D. Matsuoka, P. Zuppan, H.W. Chan, R.M. Eglen, The pharmacology and distribution of human 5-hydroxytryptamine 2B (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors, Br. J. Pharmacol. 115 (1995) 622–628.

[55] D.A. Hall, P.G. Strange, Evidence that antipsychotic drugs are inverse agonists at D_2 dopamine receptors, Br. J. Pharmacol. 121 (1997) 731–736.

[56] R.G. MacKenzie, D. VanLeeuwen, T.A. Pugsley, Y.H. Shih, S. Demattos, L. Tang, R.D. Todd, K.L. O'Malley, Characterization of the human dopamine D_3 receptor expressed in transfected cell lines, Eur. J. Pharmacol. 266 (1994) 79–85.

[57] F. Dorje, J. Wess, G. Lambrecht, R. Tacke, E. Mutschler, M.R. Brann, Antagonist binding profiles of five cloned human muscarinic receptor subtypes, J. Pharmacol. Exp. Ther. 256 (1991) 727–733.

[58] R.H. Porter, K.R. Benwell, H. Lamb, C.S. Malcolm, N.H. Allen, D.F. Revell, D.R. Adams, M.J. Sheardown, Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells, Br. J. Pharmacol. 128 (1999) 13–20.

[59] S. Offermanns, M.I. Simon, Alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C, J. Biol. Chem. 270 (1995) 15175–15180.

[60] Y. Guo, H. Zhang, X. Chen, W. Cai, J. Cheng, Y. Yang, G. Jin, X. Zhen, Evaluation of the antipsychotic effect of bi-acetylated l-stepholidine (l-SPD-A), a novel dopamine and serotonin receptor dual ligand, Schizophr. Res. 115 (2009) 41–49.