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Novel multi-target azinesulfonamides of cyclic amine derivatives as potential antipsychotics with pro-social and pro-cognitive effects

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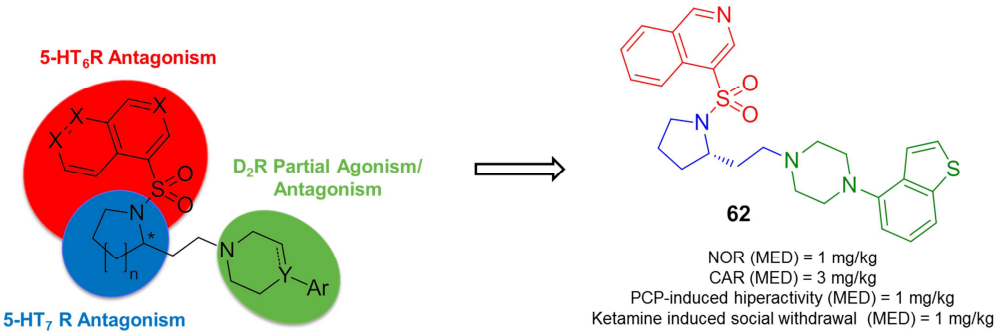

Abstract

Currently used antipsychotics are characterized by multireceptor mode of action. While antagonism of dopamine D_2 receptors is responsible for the alleviation of "positive" symptoms of schizophrenia and the effects at other, particularly serotonergic receptors are necessary for their additional therapeutic effects, there is no consensus regarding an "ideal" target engagement. Here, a detailed SAR analysis in a series of 45 novel azinesulfonamides of cyclic amine derivatives, involving the aryl-piperazine/piperidine pharmacophore, central alicyclic amine and azinesulfonamide groups has led to the selection of (*S*)-4-((2-(2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)pyrrolidin-1-yl)sulfonyl)isoquinoline (**62**). The polypharmacology profile of **62**, characterized by partial 5-HT_{1A}R agonism, 5-HT_{2A}/5-HT_7/D_2/D_3R antagonism, and blockade of SERT, reduced the "positive"-like, and "negative"-like symptoms of psychoses. Compound **62** produced no catalepsy, demonstrated a low hyperprolactinemia liability and displayed pro-cognitive effects in the novel object recognition task and attentional set-shifting test. While association of *in vitro* features with the promising *in vivo* profile of **62** is still not fully established, its clinical efficacy should be verified in further stages of development.

Keywords: multitarget directed ligands, polypharmacology, designed multiple ligands, 5-HT_{1A} partial agonist, 5-HT_7 antagonist, dopamine D_2 receptors, schizophrenia, cognitive flexibility

1. Introduction

The key action of typical antipsychotic medications is attributed to the blockade of dopamine D_2 receptors (D_2Rs) in the mesolimbic and nigrostriatal brain areas [1]. However, apart from the reduction of the most disturbing "positive" symptoms (hallucinations and delusions) [2], this mechanism is also responsible for the undesired side-effects, including the induction of extrapyramidal symptoms (EPS) [3] and for hyperprolactinemia [4].

Second generation antipsychotics exhibit significant affinity for various serotonin receptors. Their prominent feature is an additional antagonism of 5-HT_{2A}Rs (or, more precisely, a high 5-HT_{2A}/D_2 receptor affinity ratio) [5], which contributes to the side-effects reduction. For instance, an inverse agonist of 5-HT_{2A}Rs, pimavanserin alleviates symptoms of psychosis without causing EPS [6]. The stimulation of 5-HT_{1A}Rs also contributes to the alleviation of the negative and extrapyramidal symptoms and improves cognitive impairment [7]. Clinical studies have demonstrated that the 5-HT_{1A}R partial agonist tandospirone ameliorates cognitive decline in schizophrenic patients. In addition, 5-HT_{1A}R activation contributes also to the antidepressant and anti-anxiety properties of antipsychotics [8,9]. Next, the blockade of 5-HT_6Rs has emerged as a novel mechanism to treat cognitive impairment in schizophrenia [10]. In this regard, antagonists and inverse agonists of 5-HT_6R display pro-cognitive actions in preclinical and clinical settings [11]. Finally, recent preclinical data demonstrate that the 5-HT_7R antagonist SB-269970 exerts pro-social effects [12], most likely contributing to an alleviation of the "negative" symptoms of schizophrenia (including, emotional flattening and social withdrawal) as well as to the pro-social and pro-cognitive effects of the modern antipsychotics [13].

Although the introduction and development of second generation antipsychotics have revolutionized the pharmacotherapy of schizophrenia, there is no clear consensus regarding the precise mechanism of action of an "ideal" antipsychotic medication being effective

against all three core symptoms and devoid of the undesired side-effects [14]. Because the etiology and pathophysiology of psychoses remain elusive, a multi-target approach remains the conventional strategy for the development of schizophrenia pharmacotherapies [15].

We designed compounds with a multi-receptor profile by combining privileged structures (i.e., molecular frameworks that confer biological activity within a class of targets) present in selected antipsychotics and compounds evaluated in preclinical and clinical trials (Figure 1).

Figure 1.

Specifically, we used an alicyclic amine fragment modified with an arylsulfonamide moiety as a privileged structure for 5-HT_7R, the azinesulfonyl fragment present in potent 5-HT_6R and 5-HT_7R antagonists [16,17], different privileged amine cores stimulating 5-HT_{1A}Rs (like eltoprazine and ziprasidone) and acting as antagonists/partial agonists at dopamine D_2Rs (like ziprasidone, risperidone, bifeprunox and brexpiprazole), integrating these pharmacophores into one molecule (Figure 1).

We report the chemical synthesis and structure-activity relationship results, followed by neurochemical, functional, pharmacokinetic and toxicological evaluation. Present study revealed compound **62** as a putative novel atypical antipsychotic addressing the “positive”-like symptoms, reversing the “negative”-like symptoms, and cognitive deficits associated with schizophrenia without causing catalepsy with a minimal side-effects profile.

2. Results and Discussion

2.1. Chemistry

The synthesis of final compounds **38**–**82** was performed according to the multistep procedure shown in Scheme 1. Boc-protected β-homo-amino acids **1**–**5** were reduced to the corresponding primary alcohols **6**–**10**, which were subsequently oxidized using 2-iodoxybenzoic acid (IBX) [18] to yield aldehyde derivatives **11**–**15**. These were further used for reductive amination of selected arylpiperazines/arylpiperidines/tetrahydropyridines in the presence of sodium triacetoxyborohydride, giving intermediates **16**–**37**. After the removal of the protecting group, the final quinoline- and isoquinoline-sulfonamides of cyclic amine derivatives **38**–**82** were obtained while treating the secondary amines with the selected azinesulfonyl chloride in the basic condition. The azinylsulfonyl chlorides were synthesized from respective haloquinolines or isoquinoline *via* their S-methyl analogs according to the previously reported method [19].

Scheme 1.

(*S*)-(Boc-azepan-2-yl)acetic acid (**5**) used for the preparation of compounds **75**, **76** and **81**, **82** was synthesized following the routes presented in Scheme 2. A starting 2-acetamido-5-hexenoic acid was submitted to enzymatic treatment giving (*S*)-2-amino-5-hexenoic acid (**83**). Upon the introduction of the Boc protection, this was further alkylated with allyl bromide to give **84**. The latter compound was submitted to ring closing metathesis, using Grubbs second-generation catalyst [20], in refluxing dichloromethane for 3 days, to yield cyclized Boc-tetrahydro-1*H*-azepine-2-carboxylic acid (**85**).

Scheme 2.

After the double bond reduction, using palladium on activated charcoal, in a mixture of EtOH/THF/H_2O at 36°C under a hydrogen atmosphere, the resulting intermediate Boc-hexahydro-1*H*-azepine-2-carboxylic acid (**86**) was then submitted to Arndt-Eistert β-homologation reaction. The reaction involved the creation of a diazoketone intermediate (**87**) and its further conversion to acetic acid analog **5** in the presence of CF_3COOAg as a catalyst [21].

2.2. Structure-affinity relationships studies

Our approach focused on the development of a putative novel antipsychotic, which, by extending the idea of multitarget ligands fit the classical pharmacophore for monoaminergic receptor (mainly serotonin and dopamine) ligands consisting of the protonated amine anchored by Asp3.32 (amino acid numbering according to [22]) and specific hydrophobic recognition involving aromatic interaction (CH–π or π–π) with the Phe6.51/Phe6.52/Trp6.48 aromatic cluster [23]. Moreover, in the case of compounds optionally containing the terminal fragment connected with the basic center *via* alkylene linker, the additional aromatic and H-bond interactions stabilize ligand binding in the second transmembrane pocket created between TMHs 7–3 (involving specific amino acids characteristic for serotonin and dopamine receptors) [23].

2.2.1. The effect of the type of amine

We first focused on the amine pharmacophore moieties present in bifeprunox, which behaves as 5-HT_{1A}R agonist and D_2R partial agonist, and eltoprazine (classified as 5-HT_{1A}R

partial agonist). Our results indicate that compounds bearing 1,3-benzoxazol-2-on-4-yl-piperazine (**I**) were classified as dual 5-HT_{1A}/D_2R ligands (Table 1), while 1,4-benzodioxin-5-yl-piperazine derivatives (**II**, eltoprazine) displayed a high affinity for 5-HT_{1A}/D_2Rs and high-to-moderate affinity for the 5-HT_7 sites. It was further found that a 5-chloroindol-3-yl-tetrahydropyridine (**III**) core did not result in a high affinity for 5-HT_{2A}Rs, which are crucial for atypical antipsychotic activity.

Table 1.

Our amine scaffold replacement strategy involving introduction of 6-fluorobenzo[*d*]isoxazol-3-yl-piperidine (**IV**, present in risperidone) did not improve affinity for 5-HT_{1A} receptors. Compounds **49**–**51** displayed a mixed 5-HT_{2A}/5-HT_7/D_2R profile, with a moderate affinity for 5-HT_{1A} and 5-HT_6Rs. These compounds showed a higher affinity for 5-HT_6R in comparison to risperidone. It was further found, that replacement of 6-fluorobenzo[*d*]isoxazol-3-yl-piperidine with benzo[*d*]isothiazol-3-yl-piperazine (**V**), present in ziprasidone, resulted in compounds **52**–**57** with a high affinity for the four receptors, except for a moderate-to-high affinity for 5-HT_6R.

The replacement of benzo[*d*]isothiazol-3-yl-piperazine (**V**) with benzo[*d*]isoxazol-3-yl-piperazine (**VI**) generally had no influence on the affinity for 5-HT_{2A}, 5-HT_7 or D_2Rs, while it decreased the affinity for 5-HT_{1A}R. This receptor pattern is in line with the one observed for 6-fluorobenzo[*d*]isoxazol-3-yl-piperidine. This result indicates that the benzo[*d*]isoxazole moiety determines the affinity for 5-HT_{2A}, 5-HT_7 and D_2Rs regardless of the type of connected alicyclic amine (i.e. piperidine or piperazine). It thus seems, that piperidine and piperazine are equipotent regarding their interaction with the studied receptors.

Indeed, the introduction of benzothiophen-4-yl-piperazine (**VII**), present in brexpiprazole and responsible for its partial agonism of D_2 receptors, conferred multimodality of all the compounds for the five studied receptors. The representative compounds **61**–**66** with benzothiophen-4-yl-piperazine were classified as high affinity ligands for 5-HT_{1A}/5-HT_{2A}/ 5-HT_6/5-HT_7/D_2Rs. Interestingly, the replacement of the benzothiophen-4-yl-piperazine fragment with indol-4-yl-piperazine (**VIII**) decreased the affinity of compounds **67**–**70** for 5-HT_6R.

2.2.2. The effect of the azine moiety

Our previous reports on azinesulfonamides of long-chain arylpiperazine derivatives with multimodal mechanism [17,24] and selective 5-HT_6R antagonists [16] disclosed the influence of the type of azinesulfonamide moiety (the position of the sulfonamide group in the α- or β-position of the azine moiety and the localization of the sulfonamide group in a pyridine or benzene ring) on receptor affinity. Thus, for the present study only beneficial α-position azinesulfonamides were selected. Unfortunately, the quantitative influence of 5-quinolyl, and 4- and 5-isoquinolyl moieties toward the tested receptor panel was difficult to unequivocally establish.

2.2.3. The effects of stereochemistry and size of alicyclic amine

As stereochemistry plays a role in ligand recognition by different serotonin and dopamine receptors [25–28], we focused on the relevance of chirality to receptor activity profile. It was found that for some amine scaffolds an interaction with 5-HT_6 and to less extent 5-HT_7Rs was dependent on stereochemistry. This observation was pronounced in the case of 5-HT_6Rs,

where enantiomers (*R*) displayed 5–to–19-fold higher affinity than their (*S*) counterparts (e.g., **45**, **49**, **53**, **61** *vs* **47**, **51**, **56**, **64**, Table 1).

Encouraged by these data we then applied a scaffold hopping approach to investigate any influence of the size of the alicyclic amine on the receptor profile (Table 2).

Table 2.

For this purpose, we synthesized analogs with (*S*) azetidine, six-member ring (*R*) piperidine and its even higher seven-member analog – (*S*) azepane. The replacement of pyrrolidine with azetidine generally decreased the binding, even though affinity for 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2Rs stayed at the same level. Increasing the ring size from a pyrrolidine to piperidine and azepane also decreased the affinity for all receptors with the most pronounced decrease or even loss of affinity of the evaluated compounds for 5-HT_6R (Table 2).

2.3. Preliminary pharmacokinetic study

Next, based on the binding data (Table 2), a series of 6 selected compounds, **53**, **54**, **56**, **57**, **62**, **65** exhibiting a high affinity for 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2Rs, and a high-to-moderate affinity for 5-HT_6R, were submitted to a preliminary pharmacokinetic study in male Wistar rats. The results indicate that the three 4-isoquinolyl derivatives better penetrate the blood−brain barrier and are taken up more by the brain than the two 5-isoquinolyl analogs (Table 1. SI).

2.4. In vitro intrinsic activity evaluation

Subsequently, the selected 4-isoquinolyl derivatives (**53**, **62** and **65**) were submitted for a detailed evaluation of their functional profile using *in vitro* methods (Table 3). Among them, compound **53** with benzo[*d*]isothiazol-3-yl-piperazine behaved as an antagonist at 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, D_2, and D_3Rs. In contrast, benzothiophen-4-yl-piperazine analogs **62** and **65** showed stimulatory activity in the agonist assays at 5-HT_{1A}Rs. The latter two compounds behaved as antagonists of 5-HT_{2A}, 5-HT_7, D_2 and D_3Rs (Table 3).

Table 3.

Interestingly, only compound **65** behaved as a potent 5-HT_6R antagonist. The functional activity of **62** and **65** at 5-HT_{1A}R was similar to that observed for clozapine, ziprasidone or brexpiprazole (behaving as 5-HT_{1A}R partial agonists).

2.5. Preliminary in vivo behavioral evaluation

Uncompetitive NMDA receptor antagonists (e.g., ketamine or PCP,) induce schizophrenia-like symptoms in healthy volunteers, thus administration of these psychotomimetics to rodents serves as a model of psychoses [29]. Consequently, compounds normalizing hyperactivity (distance traveled) display antipsychotic-like properties [30]. The preliminary experiments (Table 4) allowed the selection of compounds **53** and **62**, which produced an antipsychotic effect on PCP-induced locomotor hyperactivity in rats at doses lower than those evoking sedation (see Figure 1-SI and Figure 2-SI for details, respectively). In contrast compound **56** and **65** (both *R* enantiomer) did not reverse the PCP-induced hyperactivity and induced sedation. Importantly, clozapine (Figure 3-SI), and ziprasidone (Figure 4-SI) displayed a favorable profile by reducing PCP-induced hyperactivity at doses

lower than those inducing sedation, while haloperidol (Figure 5-SI) and olanzapine (Figure 6-SI) inhibited the PCP-induced hyperactivity at doses higher than those producing sedation.

Impaired cognitive processes constitute an integral part of the clinical picture of schizophrenia, and the improvement of cognitive deficits has become one of the priorities in the development of antipsychotics. Thus, the novel object recognition task (NORT) was used to study the influence of the investigated compounds on visual episodic memory [31]. This test is based on the spontaneous exploration of novel and familiar objects. The administration of ketamine or PCP reduces the ability to discriminate a novel object from a familiar one [13], and any compounds that have a protective effect against this deficit are regarded as potentially useful for treating cognitive deficits that are characteristic of psychoses [32].

Table 4.

Table 4 shows that compounds **53** (Figure 7-SI) and **62** (Figure 8-SI) (minimum effective dose; MED = 1 mg/kg, *i.p.*) ameliorated PCP-induced learning deficits in the NORT. In the same experimental settings, clozapine (Figure 9-SI), ziprasidone (Figure 10-SI) and olanzapine (Figure 11-SI) but not haloperidol (Figure 12-SI) displayed similar pro-cognitive effects.

2.6. Cardiac safety, interaction with cytochrome P450, and metabolic stability of compounds **53** *and* **62**

Since a cardiac toxicity is a common reason for increased cardiac death, ability of compound **53** (Figure 13-SI) and **62** (Figure 14-SI) to block human hERG potassium channels was investigated in a whole cell patch clamp assay in CHO-K1 cells with expression

of hERG channels. Compound **53** tested at 10 μM and 50 μM concentrations showed a propensity to block hERG, while **62** tested at the same concentrations displayed no statistically significant changes in ion currents. Additionally, **62** displayed no agonist properties for 5-HT_{2B}R (agonist effect @ 10^{-6}M counted 3 and 14 % for **53** and **62**, respectively) which are involved in valvulopathy and cardiac impairment [33].

As the QT interval prolongation is one of the safety issue often noted for atypical antipsychotics, cardiac safety of compounds **53** and **62** was further tested *in vivo* in a guinea pig model showing no QT prolongation or any cardiac liability at a dose of 5 mg/kg, and 10 mg/kg (*i.p.*), respectively. Compounds **53** (Table 4-SI) and **62** (Table 5-SI) at doses 5- and 10-fold higher than those used in the behavioral tests did not significantly change the normal ECG or frequency of heart rate in guinea pigs. The lack of changes in the ECG may suggest the safety of **53** and **62** regarding pro-arrhythmic activity while using behaviorally effective doses.

We also investigated whether compound **53** (2 and 5 mg/kg; Figure 15-SI) and compound **62** (3 and 10 mg/kg, Figure 16-SI) affected blood pressure in rats. The results demonstrate compounds **53** and **62** did not affect diastolic or systolic blood pressure in rats at any of the doses tested.

In vitro biotransformation studies using rat liver microsomes indicate that compounds **53** and **62** showed metabolic stability with intrinsic clearance values of 1.73 and 2.81 μL/min/mg, respectively. Additionally, to extend the potential drug-drug interaction at the level of the liver cytochrome P450, the influence of compounds **53** and **62** on five different cytochrome P450 isozymes – CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, was determined [34]. Compounds **53** and **62** only moderately inhibited CYP3A4 activity with IC_{50} counting 23 and 10 μM, respectively (Table 6-SI). The obtained values indicate that compounds **53** and **62** should not inhibit the CYP3A4 isoform *in vivo* since their

pharmacological concentrations in the rat brain and plasma are below 1 µM. Hence, the new compounds studied here are safe with respect to drug interaction at the level of cytochrome P450 as other atypical neuroleptics (e.g., clozapine or risperidone) which is not true for typical neuroleptics (e.g., phenothiazines or haloperidol), which are strong CYP2D6 inhibitors [35].

2.7. Advanced in vivo pharmacological evaluation of **53** *and* **62**

To further extend the evaluation of the antipsychotic properties of compounds **53** and **62**, the conditioned avoidance response (CAR) test was employed [36]. The CAR test is recognized as a reliable tool with high predictive validity for "positive" symptoms in antipsychotic development. Compounds **53** and **62**, at a dose of 3 mg/kg, suppressed the *avoidance* behavior without affecting *failures*, suggesting a "specific" antipsychotic-like action in the CAR test (Table 5).

Table 5.

Similar specific effects were apparent for risperidone and clozapine; however, haloperidol, olanzapine and ziprasidone significantly increased the *failures*, suggesting a sedative effect under these experimental conditions (Table 7-SI).

In the ketamine-disrupted social interaction test summarized in Table 5, treatment with **53** (Figure 17-SI) and **62** (Figure 18-SI) ameliorated social deficits at doses of 3 and 1 mg/kg, respectively. Similar effects were observed for 2 mg/kg aripiprazole (Figure 19-SI) and 0.1 mg/kg risperidone (Figure 20-SI). In contrast, clozapine (1 mg/kg; Figure 21-SI),

haloperidol (0.1 mg/kg, Figure 22-SI), and ziprasidone (1 mg/kg, Figure 23-SI) were inactive in the same experimental setting.

One of the major obstacles to the treatment with antipsychotics is their propensity to produce extrapyramidal side-effects [3]. To examine the tendency of the novel compounds to produce EPS-like side effects rats were scored for catalepsy for 240 min following *i.p.* administration. Haloperidol produced dose-dependent cataleptogenic effects with an MED ~ 0.2 mg/kg, (Figure 24-SI). Compound **53** at 30 mg/kg and olanzapine at 40 mg/kg shows the cataleptogenic effects (Figure 25-SI) In contrast, ziprasidone (30 mg/kg) produced insignificant cataleptogenic effects, and compound **62** (30 mg/kg) was devoid of this action (Table 5, Figure 25-SI).

The potential side effect profile of **62** was also assessed in terms of its ability to induce hyperprolactinemia. In contrast to olanzapine and ziprasidone (which significantly increased prolactin levels at a dose of 3 mg/kg), compound **62** did not affect plasma prolactin level in rats up to a dose of 3 mg/kg (Figure 26-SI).

Apart from the aforementioned functional profile (Table 3), extended pharmacological *in vitro* profiling was conducted for **62** to investigate its potential off-target activity (Table 6). The results show that **62** at 10^{-6} M inhibited the binding of the control to adrenergic α_1, α_{2C}, D_1, D_3, D_4, and 5-HT_{2C}, but not M_1 and 5-HT_3 by at least 50%. It also inhibited binding to SERT (K_i = 76 nM). It was found that **62** behaved as an antagonist of adrenergic α_{1A}, H_1, 5-HT_{1D}, 5-HT_{2C}Rs and showed modest antagonistic properties at α_{2C}, D_3 and 5-HT_{1B}Rs. Consequently, like other atypical antipsychotics, **62** displays a complex pharmacology with the desired profile, and appears to produce some potentially unwanted off-target effects. These effects, which result from the antagonistic action of **62** on α_{1A} and H_1 receptors, could suggest factors related to hypotension and sedation; however, these states were not found in this study.

Table 6.

The pharmacokinetic properties of **62** were then explored after administration of a single dose of 9 mg/kg *per os* indicating that compound **62** is quickly absorbed from the gastrointestinal tract (T_{max} = 10 min) and reaches a maximum concentration of C_{max} = 54 nM. A similar C_{max} value is reached in the brain after 60 min (T_{max}).

Schizophrenic patients develop cognitive dysfunctions, including inflexibility in modifying behavior in response to altering the relevance of stimuli. This aspect of executive function, which is controlled by the prefrontal cortex, is commonly assessed in humans using the WCST [37]. In fact, a poor WCST performance is a key behavioral symptom of schizophrenia [38]. We thus examined the cognitive effects of compound **62** in the attentional set-shifting test (ASST) [13,39]. While ketamine disturbed cognitive flexibility by significantly and specifically impairing rats' performance at the extradimensional (ED) stage, compound **62** (1 mg/kg) reversed this deficit (Figure 27-SI).

3. Conclusions

Currently used medications for schizophrenia can effectively control "positive" symptoms but with some exceptions they display limited impact on cognitive deficits and social withdrawal. These unmet medical needs were addressed in present study aimed at developing novel antipsychotic. By combining the concept of multimodal ligands with privileged structural elements favorable for interactions with selected monoaminergic receptors, we designed, synthesized, and characterized a new series of azinesulfonamides of alicyclic amine derivatives with arylpiperazine/piperidine moieties. SAR exploration of this library revealed that the (isoquinolin-4-ylsulfonyl)-(*S*)-pyrrolidinyl fragment and the 1,2-benzothiazol-3-yl-

and benzothiophen-4-yl-piperazine fragments were favorable for binding the 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2, and D_3 receptors. Additionally, the interaction of the designed series with 5-HT_6R was highly dependent on the stereochemistry of the alicyclic amine. From a diverse library, (*S*)-4-((2-(2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl) pyrrolidin-1-yl)sulfonyl)isoquinoline (**62**) was identified as a potential novel antipsychotic. Taking advantage of the concept of D_2R blockade, which is the only clinically validated approach, **62** combines a multi-receptor mechanism with prominent 5-HT_{1A}R partial agonism, 5-HT_{2A}/5-HT_7/D_3R antagonism, and blockade to SERT. Because it reverses PCP-induced hyperactivity and avoidance behavior in the CAR test, **62** appears to be useful for addressing the positive symptoms of schizophrenia. Furthermore, its ability to reverse the social interaction deficit in a ketamine model and memory decline in PCP- and ketamine-disrupted conditions reveals that **62** can alleviate the negative symptoms and displays pro-cognitive properties. Additionally, **62** displayed no cardiac toxicity, and no tendency of inducing catalepsy. Further mechanistic studies are necessary to better understand the distinct behavioral attribute profiles observed. Additionally, the receptor multimodality of **62** could potentially provide opportunities for the treatment of various other CNS disorders, such as anxiety, depression, psychoses associated with dementia, and stress and impulse control disorders. Finally, since social withdrawal accompanies autism spectrum disorders and because only two atypical antipsychotics – risperidone and aripiprazole – are registered for treatment of autism, further studies should reveal the activity of novel antipsychotics with a 5-HT_7R component against the core symptoms and communication impairments of autism.

4. Experimental Section

4.1. Chemistry

Organic synthesis were carried out at ambient temperature, unless indicated otherwise. Organic solvents used in this study (Sigma-Aldrich, Chempur) were of reagent grade and were used without purification. All other commercially available reagents were of the highest purity (from Sigma-Aldrich, Fluorochem, TCI). All workup and purification procedures were carried out with reagent-grade solvents under ambient atmosphere.

Mass spectra were recorded on a UPLC-MS/MS system consisted of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column; 2.1 × 100 mm, and 1.7 µm particle size, equipped with Acquity UPLC BEH C18 VanGuard pre-column; 2.1 × 5 mm, and 1.7 µm particle size. The column was maintained at 40°C, and eluted under gradient conditions from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL min^{-1}. Eluent A: water/formic acid (0.1%, v/v); eluent B: acetonitrile/formic acid (0.1%, v/v). Chromatograms were made using Waters eλ PDA detector. Spectra were analyzed in 200–700 nm range with 1.2 nm resolution and sampling rate 20 points/s.

MS detection settings of Waters TQD mass spectrometer were as follows: source temperature 150°C, desolvation temperature 350°C, desolvation gas flow rate 600 L h^{-1}, cone gas flow 100 L h^{-1}, capillary potential 3.00 kV, cone potential 40 V. Nitrogen was used for both nebulizing and drying gas. The data were obtained in a scan mode ranging from 50 to 2000 m/z in time 1.0 s intervals. Data acquisition software was MassLynx V 4.1 (Waters). The UPLC/MS purity of all the final compounds was confirmed to be 95% or higher.

^{1}H NMR and ^{13}C NMR spectra were obtained in Varian BB 200 or AV-III-500-HD spectrometer using TMS (0.00 ppm) as an internal standard in $CDCl_3$ and were recorded at 300 or 500 and 75 MHz, respectively. The *J* values are reported in Hertz (Hz), and the splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets), qd (quartet of doublet), ddd (doublet of doublet of doublets), m (multiplet), br.s (broad singlet). Elemental analyses for C, H, N and S were carried out using the elemental Vario EI III Elemental Analyser (Hanau, Germany). All values are given as percentages, and were within ±0.4% of the calculated values.

Melting points (Mp) were determined with a Büchi apparatus and are uncorrected.

Characterization data for representative compounds are summarized below, while all characterization data for all of intermediate and remaining final compounds are reported in the Supporting Information.

4.1.1 General procedure for the synthesis of intermediates **6**–**10**

To a mixture of 2-((2*S*)-1-tert-butoxycarbonylpyrrolidin-2-yl)acetic acid (2.0 g, 8.7 mmol) in anhydrous CH_2Cl_2 (20 mL) lithium aluminum hydride 2.0 M in THF (7.23 mL, 13.05 mmol) was added dropwise and the reaction mixture was heated to 55^{o}C in inert atmosphere (under argon). After stirring for 4 hours, the reaction mixture was cooled to room temperature, diluted with AcOEt (20 mL) and treated with saturated $NaHCO_3$ (3 mL). Then the solution was concentrated under reduce pressure. To the residue were added CH_2Cl_2 (20 mL) and the organic layer was washed with water and brine, and then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give intermediate **6** (1.72 g, 8.0 mmol) as light oil (yield 91.9%). The same procedure was used for the synthesis of intermediates **7**–**10**.

4.1.2. General procedure for the synthesis of intermediates **11**–**15**

2–Iodoxybenzoic acid (IBX, 6.72 g, 24 mmol) was dissolved in DMSO (50 mL), stirred for 10 min. at room temperature and treated with a solution of intermediate **6** (1.72 g, 8.0 mmol) in DMSO (50 mL). After stirring overnight at room temperature the reaction mixture was diluted with Et_2O (100 mL), cooled to 0°C and quenched by addition of water (100 mL). The inorganic layer was diluted with brine (50 mL) and extracted with AcOEt (3 × 20 mL). The combined organic layers were washed with saturated $NaHCO_3$, water and brine, and then dried over anhydrous Na_2SO_4. After concentration under reduced pressure the crude product was purified by column chromatography on silica gel, using a mixture of AcOEt/Hex (30/70 *v*/*v*) as eluting system yielding intermediate **11** (1.12 g, 5.24 mmol) as light oil (yield 65.5%). The same procedure was used for the synthesis of intermediates **12**–**15**.

4.1.3. General procedure for the synthesis of intermediates **16**–**37**

To a solution of intermediate **11** (1 g, 4.7 mmol), 1-(benzothiophen-4-yl)piperazine (1.5 g, 7 mmol) in THF (30 mL) was added sodium triaceoxyborohydride (1.98 g, 9.4 mmol) and anhydrous AcOH (1 mL). After stirring for 4 hours at room temperature, saturated $NaHCO_3$ was added to the mixture, and the aqueous layer was extracted with AcOEt (3 × 20 mL). The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel, using a mixture of CH_2Cl_2/MeOH (9/0.5 *v*/*v*), to give the Boc-protected derivative **24** (1.70 g, 4.14 mmol) as light oil (yield 88%). The same procedure was used for the synthesis of intermediates **16**–**37**.

4.1.4. General procedure for the synthesis of final compounds **38**–**82**

Removal of Boc function of the intermediates **16**–**37** was accomplished by treatment with a mixture of TFA/CH_2Cl_2 (80/20 *v*/*v*). Upon removal of organic solvents under reduced

pressure, TFA salts of deprotected intermediates **16**–**37** were left under vacuum overnight. Then, a mixture of the proper secondary amine (0.8 mmol) in CH_2Cl_2 (5 mL), and Et_3N (2.4 mmol) was cooled down (ice bath), and the selected azinesulfonyl chloride (0.96 mmol) was added in one portion. The reaction mixture was stirred for 2-6 hours on ice bath. Then, the solvent was evaporated and the final sulfonamides **38**–**82** were purified by column chromatography using CH_2Cl_2/MeOH as eluting system.

4.2. Characterization data for representative final compounds

4.2.1. (S)-7-(4-(2-(1-(Isoquinolin-4-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)benzo[d] oxazol-2(3H)-one (**39**)

Yellow oil (150 mg, 78% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 100%, R_t = 3.94; $C_{26}H_{29}N_5O_4S$, MW 507.6, Monoisotopic Mass 507.19, $[M+H]^+$ 508.5. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.60–1.72 (m, 4H), 1.73–1.81 (m, 1H), 1.85–1.94 (m, 1H), 2.34–2.46 (m, 2H), 2.51–2.59 (m, 2H), 2.60–2.69 (m, 2H), 3.32 (t, *J* = 4.6 Hz, 4H) 3.44–3.52 (m, 2H), 4.07 (tt, *J* = 8.0, 3.9 Hz, 1H), 6.59–6.71 (m, 2H), 7.02–7.10 (m, 1H), 7.77 (ddd, *J* = 8.1, 7.0, 1.0 Hz, 1H), 7.92 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 8.05 (br.s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.86 (dd, *J* = 8.7, 0.8 Hz, 1H), 9.13 (s, 1H), 9.43 (d, *J* = 0.5 Hz, 1H).

4.2.2. (S)-4-((2-(2-(4-(2,3-Dihydrobenzo[b][1,4]dioxin-5-yl)piperazin-1-yl)ethyl)pyrrolidin-1-yl)sulfonyl)isoquinoline (**42**)

White solid (140 mg, 69% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 97%, R_t = 4.42; $C_{27}H_{32}N_4O_4S$, MW 508.63, Monoisotopic Mass 508.21, $[M+H]^+$ 509.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.63–1.82 (m, 4H), 1.89–2.00 (m, 1H), 2.05–2.18 (m, 1H), 2.24–2.36 (m, 1H), 3.34 (td, *J* = 3.1, 1.3 Hz, 4H), 3.62–3.81 (m, 4H), 3.90–4.01 (m, 2H), 4.29 (t, *J* = 3.9 Hz, 2H), 4.42 (t, *J* = 2.9 Hz, 2H),

4.49–4.57 (m, 2H), 6.79–6.94 (m, 2H), 7.32 (dd, *J* = 6.9, 1.6 Hz, 1H), 7.95–8.08 (m, 1H), 8.30 (t, *J* = 7.6 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 9.00 (d, *J* = 8.6 Hz, 1H), 9.26 (s, 1H), 9.96 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.2, 29.7, 30.5, 30.7, 51.7, 55.1, 58.4, 64.7, 104.8, 108.9, 122.6, 126.5, 128.5, 128.6, 128.9, 131.7, 132.7, 147.4, 155.7.

4.2.3. (R)-5-((2-(2-(4-(5-Chloro-1H-indol-3-yl)-5,6-dihydropyridin-1(2H)-yl)ethyl) pyrrolidin-1-yl)sulfonyl)quinoline (**47**)

Brown oil (140 mg, 71% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 99%, R_t = 5.03; $C_{28}H_{29}ClN_4O_2S$, MW 521.07, Monoisotopic Mass 520.17, $[M+H]^+$ 521.5. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.58–1.75 (m, 4H), 1.76–1.87 (m, 1H), 2.03–2.16 (m, 1H), 2.44–2.55 (m, 4H), 2.58–2.78 (m, 2H), 3.18 (dd, *J* = 10.8, 2.1 Hz, 2H), 3.31–3.39 (m, 2H), 3.95–4.05 (m, 1H), 6.06–6.08 (m, 1H), 7.07–7.14 (m, 2H), 7.29 (s, 1H), 7.53 (dd, *J* = 8.8, 4.7 Hz, 1H), 7.73–7.80 (m, 2H), 8.26 (dd, *J* = 7.0, 1.2 Hz, 1H), 8.32 (d, *J* = 8.2 Hz, 1H), 8.96 (dd, *J* = 4.1, 1.2 Hz, 1H), 9.07 (br. s., 1H), 9.27 (dd, *J* = 8.8, 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.2, 28.5, 31.1, 33.0, 48.4, 50.1, 52.9, 54.8, 58.4, 112.5, 117.1, 118.3, 120.0, 122.3, 122.7, 123.0, 125.0, 125.6, 126.1, 127.7, 129.5, 129.9, 133.8, 134.4, 135.2, 135.5, 148.5, 151.1.

4.2.4. (R)-6-Fluoro-3-(1-(2-(1-(quinolin-5-ylsulfonyl-pyrrolidin-2-yl)ethyl)piperidin-4-yl)-1,2-benzoxazole (**51**)

Brown oil (120 mg, 65% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 100%, R_t = 4.5; $C_{27}H_{29}FN_4O_3S$, MW 508.61, Monoisotopic Mass 508.2, $[M+H]^+$ 509.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.61–1.69 (m, 6H), 1.72–1.89 (m, 3H), 1.99–2.11 (m, 8H), 3.37 (t, *J* = 6.3 Hz, 2H), 3.95–4.00 (m, 1H), 7.06 (td, *J* = 8.89, 2.1 Hz, 1H), 7.23 (d, *J* = 2.1 Hz, 1H), 7.58 (dd, *J* = 8.8, 4.2 Hz, 1H), 7.69 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 8.26–8.37 (m, 2H), 9.01 (dd, *J* = 4.1, 1.5

Hz, 1H), 9.26 (d, *J* = 8.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.2, 29.7, 30.9, 30.9, 31.1, 48.5, 55.1, 58.4, 97.6, 117.4, 122.6, 124.5, 128.5, 128.6, 128.9, 131.7, 132.7, 147.4, 149.9, 157.7, 164.2, 166.3.

4.2.5. (S)-3-(4-(2-(1-(Isoquinolin-4-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)-1,2-benzothiazole (**53**)

Yellow oil (250 mg, 78% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 4.85; $C_{26}H_{29}N_5O_2S_2$, MW 507.67, Monoisotopic Mass 507.18, $[M+H]^+$ 508.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.56–1.66 (m, 2H), 1.69–1.78 (m, 2H), 1.87–1.99 (m, 2H), 2.11–2.20 (m, 2H), 2.26–2.37 (m, 2H), 3.37–3.48 (m, 4H), 3.52–3.64 (m, 2H), 3.69–3.79 (m, 2H), 4.10–4.22 (m, 1H), 7.40–7.48 (m, 1H), 7.51–7.58 (m, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.98–8.09 (m, 2H), 8.28 (t, *J* = 7.8 Hz, 1H), 8.51 (d, *J* = 8.1 Hz, 1H), 8.98 (d, *J* = 8.6 Hz, 1H), 9.17 (s, 1H), 9.81 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 23.7, 29.4, 30.7, 46.5, 48.6, 51.0, 51.6, 54.2, 58.0, 120.4, 123.1, 124.4, 124.9, 127.0, 127.9, 128.3, 131.2, 131.7, 132.8, 133.8, 135.6, 138.3, 152.5, 161.6. Anal. calcd for $C_{26}H_{29}N_5O_2S_2 \cdot 3HCl \cdot 2H_2O$: C: 47.82, H: 5.56, N: 10.72, S: 9.82; Found C: 47.91, H: 5.47, N: 10.75, S: 9.84. $C_{26}H_{29}N_5O_2S_2 \cdot 3HCl \cdot 2H_2O$: 201.1–202.3°C.

4.2.6. (S)-3-(4-(2-(1-(Isoquinolin-5-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)-1,2-benzothiazole (**54**)

Yellow oil (220 mg, 71% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 98%, R_t = 4.35; $C_{26}H_{29}N_5O_2S_2$, MW 507.67, Monoisotopic Mass 507.18, $[M+H]^+$ 508.3. UPLC ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.58–1.66 (m, 2H), 1.69–1.76 (m, 2H), 1.83–1.94 (m, 2H), 2.11–2.20 (m, 2H), 2.28–2.37 (m, 2H), 3.35–3.48 (m, 4H), 3.52–3.67 (m, 2H), 3.72–3.81 (m, 2H), 4.12–4.22 (m, 1H), 7.19–7.24 (m, 1H), 7.51–7.58 (m, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.98–8.09 (m, 2H), 8.20 (d,

J = 8.2 Hz, 1H), 8.42 (s, 1H), 8.62–8.72 (m, 2H), 9.33 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.2, 31.0, 32.8, 38.7, 48.3, 50.0, 53.0, 55.1, 58.4, 68.1, 120.5, 122.6, 123.9, 124.9, 127.6, 127.7, 128., 129.9, 133.7, 134.6, 135.5, 148.6, 151.1, 152.7, 163.9. Anal. calcd for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: C: 52.17, H: 5.56, N: 11.70, S: 10.71; Found C: 52.24, H: 5.45, N: 11.82, S: 11.01. Mp for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: 201.5–202.4°C.

4.2.7. (R)-3-(4-(2-(1-(Isoquinolin-4-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)-1,2-benzothiazole (**56**)

Brown oil (200 mg, 75% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 4.81; $C_{26}H_{29}N_5O_2S_2$, MW 507.67, Monoisotopic Mass 507.18, $[M+H]^+$ 508.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.59–1.75 (m, 4H), 1.81–1.91 (m, 2H), 2.03–2.16 (m, 2H), 2.38–2.47 (m, 2H), 3.40–3.46 (m, 4H), 3.55 (t, *J* = 4.7 Hz, 2H), 3.69–3.79 (m, 2H), 4.05 (dt, *J* = 8.1, 3.9 Hz, 1H), 7.31–7.38 (m, 1H), 7.45 (t, *J* = 7.52 Hz, 1H), 7.70–7.83 (m, 2H), 7.85–7.94 (m, 2H), 8.07 (d, *J* = 8.1 Hz, 1H), 8.84 (d, *J* = 8.7 Hz, 1H), 9.10 (s, 1H), 9.39 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.3, 31.0, 32.9, 48.5, 50.0, 53.0, 55.2, 58.5, 120.5, 123.9, 124.6, 127.5, 128.5, 128.6, 128.8, 129.0, 131.7, 132.6, 145.0, 152.7, 157.6, 163.9. Anal. calcd for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: C: 52.17, H: 5.56, N: 11.70, S: 10.71; Found C: 51.95, H: 5.95, N: 11.82, S: 10.11. Mp for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: 201.5–202.4°C.

4.2.8. (R)-3-(4-(2-(1-(Isoquinolin-5-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)-1,2-benzothiazole (**57**)

Yellow oil (240 mg, 76% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 4.31; $C_{26}H_{29}N_5O_2S_2$, MW 507.67, Monoisotopic Mass 507.18, $[M+H]^+$ 508.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.56–1.73 (m, 4H), 1.80–1.89 (m, 1H), 2.00–2.12 (m, 1H), 2.36–2.44 (m, 2H), 2.51–2.60 (m, 2H), 2.63–

2.70 (m, 2H), 3.37–3.43 (m, 2H), 3.54 (t, *J* = 4.9 Hz, 4H), 3.96–4.04 (m, 1H), 7.32–7.38 (m, 1H), 7.46 (td, *J* = 7.6, 1.0 Hz, 1H), 7.68–7.75 (m, 1H), 7.79–7.93 (m, 2H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.41 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.64–8.70 (m, 2H), 9.34 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.2, 31.0, 32.8, 48.4, 50.1, 53.00, 55.1, 58.5, 120.6, 123.9, 125.9, 127.5, 128.0, 129.1, 132.1, 133.5, 133.8, 145.1, 152.7, 153.2, 163.9. Anal. calcd for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: C: 52.17, H: 5.56, N: 11.70, S: 10.71; Found C: 52.45, H: 5.26, N: 11.42, S: 10.81. Mp for Anal. calcd for $C_{26}H_{29}N_5O_2S_2 \cdot 2HCl \cdot H_2O$: 201.5–202.4°C.

4.2.9. (S)-3-(4-(2-(1-(Quinolin-5-ylsulfonyl)pyrrolidin-2-yl)ethyl)piperazin-1-yl)-1,2-benzoxazole (**58**)

Yellow oil (150 mg, 70% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 4.25; $C_{26}H_{29}N_5O_3S$, MW 491.61, Monoisotopic Mass 491.2, $[M+H]^+$ 492.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.58–1.70 (m, 4H), 1.79–1.89 (m, 1H), 1.96–2.08 (m, 1H), 2.33–2.40 (m, 2H), 2.48–2.56 (m, 2H), 2.59–2.67 (m, 2H), 3.38 (d, *J* = 5.9 Hz, 2H), 3.56 (t, *J* = 5.0 Hz, 4H), 4.00 (tt, *J* = 7.9, 3.9 Hz, 1H), 7.22 (ddd, *J* = 7.9, 6.3, 1.4 Hz, 1H), 7.43–7.48 (m, 2H), 7.54–7.58 (m, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.79 (t, *J* = 7.9 Hz, 1H), 8.24–8.36 (m, 2H), 9.00 (dd, *J* = 4.2, 1.3 Hz, 1H), 9.26 (dt, *J* = 8.8, 0.7 Hz, 1H).

4.2.10. (S)-4-((2-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)pyrrolidin-1-yl)sulfonyl) isoquinoline (**62**)

Brown oil (230 mg, 75% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 5.06; $C_{27}H_{30}N_4O_2S_2$, MW 506.68, Monoisotopic Mass 506.18, $[M+H]^+$ 507.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.61–1.73 (m, 4H), 1.73–1.80 (m, 1H), 1.86–1.95 (m, 1H), 2.39–2.50 (m, 2H), 2.60–2.75 (m, 4H), 3.20 (t, *J* = 4.0 Hz, 4H), 3.47 (dd, *J* = 7.2, 5.9 Hz, 2H), 4.07–4.12 (m, 1H), 6.92 (dd, *J* = 7.7, 0.7

Hz, 1H), 7.27–7.32 (m, 1H), 7.39–7.44 (m, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.76 (ddd, *J* = 8.1, 7.0, 0.9 Hz, 1H), 7.91 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 8.88 (dd, *J* = 8.7, 0.8 Hz, 1H), 9.14 (s, 1H), 9.42 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.3, 31.1, 33.0, 48.5, 52.1, 53.5, 53.6, 55.2, 58.6, 112.2, 117.0, 121.9, 124.7, 124.9, 125.0, 128.4, 128.4, 128.6, 129.0, 129.1, 131.8, 132.5, 134.1, 141.1, 145.1, 148.4, 157.6. Anal. calcd for $C_{27}H_{30}N_4O_2S_2 \cdot 3HCl$: C: 52.64, H: 5.40, N: 9.09, S: 10.41; Found C: 52.61, H: 5.72, N: 8.76, S: 10.58. Mp for $C_{27}H_{30}N_4O_2S_2 \cdot 3HCl$: 202.5–203.8°C.

4.2.11. (R)-4-((2-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)pyrrolidin-1-yl)sulfonyl) isoquinoline (**65**)

Brown oil (260 mg, 74% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 100%, R_t = 5.27; $C_{27}H_{30}N_4O_2S_2$, MW 506.68, Monoisotopic Mass 506.18, $[M+H]^+$ 507.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.60–1.74 (m, 4H), 1.85–1.96 (m, 2H), 2.08–2.18 (m, 2H), 2.39–2.53 (m, 2H), 2.58–2.77 (m, 2H), 3.21 (t, *J* = 6.0 Hz, 4H). 3.47 (dd, *J* = 7.2, 5.9 Hz, 2H), 4.09 (tt, *J* = 8.12, 3.9 Hz, 1H), 6.92 (dd, *J* = 7.6, 0.6 Hz, 1H), 7.25–7.34 (m, 1H), 7.38–7.46 (m, 2H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.76 (ddd, *J* = 8.1, 7.1, 0.9 Hz, 1H), 7.92 (ddd, *J* = 8.1, 7.1, 0.9 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 8.88 (dd, *J* = 8.7, 0.7 Hz, 1H), 9.14 (s, 1H), 9.42 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.3, 31.0, 48.4, 52.1, 53.6, 55.2, 58.5, 112.2, 117.1, 117.9, 121.9, 125.0, 125.9, 133.5, 133.6, 145.1, 153.2. Anal. calcd for $C_{27}H_{30}N_4O_2S_2 \cdot 3HCl$: C: 52.64, H: 5.40, N: 9.09, S: 10.41; Found C: 52.68, H: 5.67, N: 8.89, S: 10.34. Mp for $C_{27}H_{30}N_4O_2S_2 \cdot 3HCl$: 202.2–203.5°C.

4.2.12. (S)-4-((2-(2-(4-(1H-Indol-4-yl)piperazin-1-yl)ethyl)pyrrolidin-1-yl)sulfonyl) isoquinoline (**68**)

Yellow oil (120 mg, 67% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 97%, R_t = 4.38; $C_{27}H_{31}N_5O_2S$, MW 489.63,

Monoisotopic Mass 489.22, $[M+H]^+$ 490.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.63–1.76 (m, 6H), 1.86–1.93 (m, 1H), 2.38–2.49 (m, 2H), 2.58–2.65 (m, 2H), 2.68–2.75 (m, 2H), 3.26–3.31 (m, 4H), 3.41–3.47 (m, 2H), 4.02–4.11 (m, 1H), 6.51–6.62 (m, 2H), 7.04–7.18 (m, 3H), 7.71–7.94 (m, 2H), 8.09 (s, 1H), 8.86 (d, *J* = 8.6 Hz, 1H), 9.11 (s, 1H), 9.40 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.3, 30.1, 31.0, 48.4, 51.2, 53.2, 55.2, 58.6, 101.2, 105.8, 106.6, 121.2, 122.6, 122.7, 124.6, 128.4, 128.6, 128.9, 131.8, 132.6, 137.0, 145.0, 157.6, 207.0.

4.2.13. (S)-5-((2-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)azetidin-1-yl)sulfonyl) quinoline (**77**)

Brown oil (150 mg, 66% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 99%, R_t = 5.06; $C_{25}H_{27}N_5O_2S_2$, MW 493.64, Monoisotopic Mass 493.16, $[M+H]^+$ 493.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.74–1.89 (m, 2H), 1.97–2.08 (m, 2H), 2.09–2.22 (m, 2H), 2.59–2.62 (m, 4H), 3.13–3.17 (m, 4H), 3.57–3.66 (m, 1H), 3.83–3.93 (m, 1H), 4.41 (qd, *J* = 8.2, 4.7 Hz, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 7.24–7.31 (m, 2H), 7.53–7.62 (m, 2H), 7.82 (dd, *J* = 8.5, 7.3 Hz, 1H), 8.29–8.41 (m, 2H), 9.03 (dd, *J* = 4.4, 1.5 Hz, 1H), 9.17 (d, *J* = 8.8 Hz, 1H).

4.2.14. (R)-5-((2-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)piperidin-1-yl)sulfonyl) quinoline (**79**)

Brown oil (160 mg, 69% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); UPLC purity = 99%, R_t = 5.14; $C_{28}H_{32}N_4O_2S_2$, MW 520.71, Monoisotopic Mass 520.2, $[M+H]^+$ 521.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.57–1.62 (m, 6H), 1.69–1.81 (m, 2H), 1.87–1.99 (m, 2H), 2.22–2.32 (m, 2H), 2.47–2.56 (m, 4H), 3.07–3.18 (m, 4H), 3.85–3.93 (m, 1H), 6.89 (dd, *J* = 7.8, 0.7 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H),

7.37–7.44 (m, 2H), 7.54–7.63 (m, 2H), 7.81 (dd, *J* = 8.5, 7.4 Hz, 1H), 8.31–8.40 (m, 2H), 8.96–9.02 (m, 1H), 9.04 (dd, *J* = 4.2, 1.6 Hz, 1H).

4.2.15. (S)-4-((2-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)ethyl)azepan-1-yl)sulfonyl) isoquinoline (**82**)

Brown oil (180 mg, 73% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); UPLC purity = 98%, R_t = 5.21; $C_{29}H_{34}N_4O_2S_2$, MW 534.74, Monoisotopic Mass 534.21, $[M+H]^+$ 535.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.28–1.40 (m, 2H), 1.43–1.56 (m, 4H), 1.61–1.75 (m, 3H), 2.02–2.23 (m, 4H), 2.27–2.38 (m, 4H), 3.00–3.11 (m, 4H), 3.94–4.13 (m, 2H), 6.86 (d, *J*= 7.03 Hz, 1H), 7.26 (d, *J* = 15.8 Hz, 1H), 7.33–7.40 (m, 2H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.71–7.77 (m, 1H), 7.88–7.94 (m, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 8.62 (d, *J* = 7.6 Hz, 1H), 9.19 (s, 1H), 9.39 (s, 1H).

4.3. In vitro pharmacology

4.3.1. Radioligand binding assays

Radioligand binding assays were employed for determining the affinity and the selectivity profile of the synthesized compounds for cloned serotonin: 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7 and dopamine D_{2L} receptors which were all stably expressed in HEK293 or CHO-K1 cells. According to the previously published procedures [24,26,40,41], the experiments were carried out using 2.5 nM [^{3}H]-8 OH DPAT (135.2 Ci/mmol) for 5-HT_{1A}R; 1 nM [^{3}H]-Ketanserin (53.4 Ci/mmol) for 5-HT_{2A}R; 2 nM [^{3}H]-LSD (83.6 Ci/mmol) for 5-HT_6R; 0.8 nM [^{3}H]-5-CT (80.1 Ci/mmol) for 5-HT_7R or 2.5 nM [^{3}H]-Raclopride (76.0 Ci/mmol) for D_{2L}R.

Non-specific binding is defined with 10 µM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas 10 µM of chlorpromazine, 10 µM of methiothepine or 10 µM of

haloperidol were used in 5-HT_{2A}R, 5-HT_6R and D_{2L} assays, respectively. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation [42].

HEK293 cells with stable expression of human serotonin 5-HT_{1A}R, 5-HT_6R, 5-HT_{7b}R or dopamine D_{2L}R (all prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's Modifier Eagle Medium containing 10% dialysed foetal bovine serum and 500 mg/ml G418 sulphate. For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37°C phosphate buffered saline (PBS) and were pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparations pellets were stored at –80°C. CHO-K1 cells with stable expression of human serotonin 5-HT_{2A}R were purchased from PerkinElmer BioSignal Inc and were maintained according to manufacturer's protocol.

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4°C, with incubation for 15 min at 37°C in between. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbate; for 5-HT_{2A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$ and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate.

All assays were incubated in a total volume of 200 ml in 96-well microtitre plates for 1 h at 37°C, except for 5-HT_{1A}R and 5-HT_{2A}R which were incubated at room temperature for 1 h and 1.5 h, respectively. The process of equilibration is terminated by rapid filtration through

Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader.

4.3.2. In vitro functional activity ago-/antagonism for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and D_2, D_3 receptors

The agonist and antagonist properties of compounds **53**, **62**, **65** against the human 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2, and D_3Rs were evaluated in functional cellular assays, performed at Eurofins Cerep, using HEK-293 and CHO cells, according to experimental conditions summarized in Table 2-SI, and described online at www.cerep.fr. Additionally, agonist and antagonist properties of compound **62** against the human 5-HT_{1A}R were performed at DiscoverX, according to the experimental conditions summarized in Table 2-SI.

4.3.3.Extended in vitro receptor binding and functional profile

The percentage of inhibition for selected compounds for adrenergic α_1, $\alpha_{2C,}$ β_1 and β_2Rs, dopaminergic D_1 and D_4Rs, histamine H_1, muscarinic M_1, serotoninergic 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C} and 5-HT_3Rs as well as for monoamine transporters SERT, NET and DaT were evaluated at Eurofins Cerep. Experimental conditions for these assays are described in Table 3-SI. Additionally, antagonist properties of compound **62** at α_{1A}, α_{2C}, H_1, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2C} and agonist 5-HT_{2B} were evaluated in functional cellular assays, performed at Eurofins Cerep, using HEK-293 and CHO cells, according to experimental conditions summarized in Table 2-SI, and described online at www.cerep.fr.

4.3.4. Interaction with cytochrome P450 (CYP) isoforms

Experiments were carried out on human cDNA-expressed cytochrome P450 isoforms (Supersomes) from Corning (Woburn, MA, USA). The enzymatic activity was assessed on the basis of the rate of CYP isoform-specific metabolic reactions (caffeine 3-N-demethylation, diclofenac 4'-hydroxylation, perazine N-demethylation, bufuralol 1'-hydroxylation and testosterone 6β-hydroxylation) as described previously [34]. The reactions proceeded in the absence and presence of the investigated compounds, added in vitro at the following concentrations: 0.01, 0.1, 1, 10, 50 and 100 μM. Briefly, the activity of CYP1A2 was studied by measuring the rate of caffeine 3-N-demethylation at a substrate concentration of 1 mM, and Supersomes CYP1A2 of 50 pmol/ml. The activity of CYP2C9 was studied by measuring the rate of diclofenac 4'-hydroxylation, at a substrate concentration of 10 μM, and Supersomes CYP2C9 of 100 pmol/ml. The activity of CYP2C19 was studied by measuring the rate of perazine N-demethylation at a substrate concentration of 250 μM, and Supersomes CYP2C19 of 50 pmol/ml. The activity of CYP2D6 was studied by measuring the rate of bufuralol 1'-hydroxylation, at a substrate concentration of 30 μM, and Supersomes CYP2D6 of 50 pmol/ml. The activity of CYP3A4 was studied by measuring the rate of testosterone 6β-hydroxylation, at a substrate concentration of 100 μM, and Supersomes CYP3A4 of 100 pmol/ml. An incubation time for all reactions was 30 min. Caffeine and its metabolite 3-N-desmethylcaffeine, diclofenac and its metabolite 4'-hydroxydiclofenac, perazine and its metabolite N-desmethylperazine, testosterone and its metabolite 6β-hydroxylation were analyzed by HPLC with UV detection. Bufuralol and its metabolite 1'-hydroxybufuralol were analyzed by HPLC with fluorimetric detection. The potency of a compound to inhibit enzyme activity was expressed as IC50.

4.3.5. In vitro binding to hERG assay

The propensity of tested compounds to block the human hERG potassium channels was investigated, in whole cell Patch Clamp assay in CHO-K1 cells with expression of hERG channels, by BLIRT, Gdańsk, Poland.

4.3.6. Metabolic stability

Test compounds incubation (20 µM) with RLM (0.4 mg/ml) was performed in 100 mM potassium phosphate buffer (PB), pH 7.4, at 37°C for 15 min in a final reaction mixture volume of 150 µl. The reaction was initiated by the addition of an NADPH-regenerating system (NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase in 100 mM PB, pH 7.4) to the incubation mixture. The blank probe without the NADPH – regenerating system was performed in parallel [43,44]. Subsequently, the samples (**53**, **62**) were incubated at 37°C for 5, 15, 30, 60, 90, and 120 min with gentle shaking. Next, an internal standard (pentoxiphylline, 20 µM) was added. The reaction was terminated at different time points with perchloric acid (69-72%, by volume). Thereafter, all samples were centrifuged and the supernatant was analyzed using UPLC/MS in order to determine the quantity of starting material left in solution. All samples were prepared in duplicate.

The in vitro half-time ($t_{1/2}$) for test compounds was determined from the slope of the linear regression of ln % parent compound remaining versus incubation time. The calculated $t_{1/2}$ was incorporated into the following equation to obtain intrinsic clearance (Cl_{int}) = (volume of incubation [µl]/ protein in the incubation [mg]) × $0.693/t_{1/2}$.

4.4. Safety cardiology

4.4.1. ECG in guinea pigs

Electrocardiographic measurement was carried out using the ASCARD (Mr Blue) apparatus, standard lead II, and paper speed of 50 mm/s. The tested compound were administered intraperitoneally (*i.p.*) into guinea pigs as suspension in TWEEN at dose 5 mg/kg. The observation ECG was made for 80 minutes from the injection. The ECG recording was carried out immediately in 10, 20, 30, 40, 50, 60, 70 and 80 min after administration of the tested compounds. Male guinea pigs were anaesthetised with urethan (1.4 g kg^{-1}, *i.p.*). The data are expressed as the mean ± SEM. The statistical significance was calculated using a one-way ANOVA and Dunnett test.

4.4.2. The effect on blood pressure in rats

The normotensive male Wistar rats (body weight 200–250 g) were anesthetized with thiopental (70 mg/kg) by *i.p.* injection. The left carotid artery was cannulated with polyethylene tubing filled with heparin solution in saline to facilitate pressure measurements using PowerLab Apparatus (ADInstruments). Blood pressure was measured: before administration of the compounds – time 0 min (control pressure) and 60 min thereafter. Experimental groups consisted of six animals each. Compounds were dissolved in water and administered intravenously.

4.5. The effect on prolactin level

Male Wistar rats were orally administered either vehicle (Tween 1%) or tested compounds. Sixty minutes after administration blood were collected from tail vein into a 1.5 ml Eppendorf tube containing 25 µl EDTA. Blood was immediately mixed with EDTA and centrifuged at 12 000 rpm for 15 minutes. The supernatant was collected in another tube as

plasma, and were stored in a deep-freezer until use. The prolactin level in the plasma samples were measured using an ELISA kit (Thermo Fisher Scientific). The statistical significance of differences between two groups was assessed by the Student's t test.

4.6. Behavioral test

The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology. Male Sprague–Dawley rats (Charles River, Germany) weighing ~150 g at the arrival were housed in the standard laboratory cages, under standard colony A/C controlled conditions: room temperature 21 ± 2°C, humidity (40–50 %), 12-hr light/dark cycle (lights on: 06:00) with ad libitum access to food and water. Rats were allowed to acclimatize for at least 7 days before the start of the experimental procedure. During this week animals were handled for at least 3 times. Behavioral testing was carried out during the light phase of the light/dark cycle. At least 1 h before the start of the experiment, rats were transferred to the experimental room for acclimation.

4.6.1. Drug treatment

Unless stated otherwise, all drugs were obtained from Sigma–Aldrich (Poznan, Poland). All drugs were dissolved in physiological saline and administered intraperitoneally (*i.p.*) in a volume of 1 ml/kg. Haloperidol was initially dissolved in 5 microliter of glacial acetic acid, supplemented with physiological saline and brought to a neutral pH with 1 N NaOH.

4.6.2. Preliminary behavioral evaluation: a comparison of doses affecting spontaneous activity with phencyclidine (PCP)-induced hyper-activity

Both spontaneous and PCP-induced locomotor activity were measured automatically in Opto-Varimex-4 Auto-Tracks (Columbus Instruments, Ohio, USA) located in sound-attenuated and ventilated boxes [45]. The Auto-Track System sensed the motion with a grid of infrared photocells (16 beams per axis) surrounding the arena.

The compounds were administered 30 min before placing the rats individually in auto-tracks for 30 min of spontaneous locomotor activity measurement. Thereafter the rats were removed from the boxes, injected with phencyclidine hydrochloride (PCP), at a dose of 5 mg/kg (*s.c.*) and then the PCP-induced locomotor activity was measured for 30 min, starting 15 min after rat reintroduction to the auto-tracks. The data (number of counts) collected every 5 min are presented as the total distance traveled.

4.6.3. Preliminary behavioral evaluation: Novel object recognition (NOR) test as a tool assessing the pro-cognitive drug action in phencyclidine (PCP)-disturbed conditions

The protocol described earlier was adapted from the original work [31,45]. Rats were tested in a dimly lit (25 lx) "open field" apparatus made of a dull gray plastic (66 × 56 × 30 cm). After each measurement, the floor was cleaned and dried. The procedure lasting for 2 days consisted of the habituation to the test arena (without any objects) for 5 min. The test session comprising of two trials separated by an inter-trial interval (ITI) of 1 h was carried out on the next day. During the first trial (familiarization, T1) two identical objects (A1 and A2) were presented in the opposite corners of the open field, approximately 10 cm from the walls. During the second trial (recognition, T2) one of the A objects was replaced by a novel object B, so that the animals were presented with the A=familiar and B=novel objects. Both trials lasted for 3 min and the animals were returned to their home cages after T1. The objects used were the glass beakers filled with the gravel and the plastic bottles filled with the sand.

The heights of the objects were comparable (~12 cm) and the objects were heavy enough not to be displaced by the animals. The sequence of presentations and the location of the objects was randomly assigned to each rat.

The animals explored the objects by looking, licking, sniffing or touching the object while sniffing, but not when leaning against, standing or sitting on the object. Any rat exploring the two objects for less than 5 s within 3 min of T1 or T2 was eliminated from the study. Exploration time of the objects and the distance traveled were measured using the Any-maze® (Stoelting Co., IL, USA) video tracking system. Based on exploration time (E) of two objects during T2, discrimination index (DI) was calculated according to the formula: $DI = (E_B–E_A)/(E_A+A_B)$, where E_A is defined as the time spent exploring the familiar object and E_B is the time spent exploring the novel object, respectively.

Phencyclidine (PCP), used to attenuate learning, was administered at the dose of 5 mg/kg (IP) 45 min before familiarization phase (T1). The compounds were administrated *i.p.*, 1 hour and 15 min before T1.

4.6.4. Advanced behavioral evaluation: Conditioned avoidance response test

As described earlier [45], the training and the testing were performed in four shuttle boxes (Med Associates, Inc, USA). Each box (44 × 21 × 18 cm) housed in the ventilated, sound-isolated cubicles and was divided in two equal-sized compartments by the guillotine doors [36]. The rats were allowed to move freely from one compartment to another at any time. The position of animal was tracked by 8 photocells in each of the boxes. A cue light was situated on the wall opposing the compartment entry.

The training and testing sessions were started by presenting the conditioned stimulus (CS, the light) for 10 s, followed by unconditioned stimulus (UCS, continuous foot shock of 0.25 mA at the start of training, and 0.37 mA at the end of training and during the tests) for the

maximum of 10 s. The procedure was repeated with 20 trials daily with an intra-trial interval of 20-40s. If a rat moved from one compartment into the other within 10 s of CS presentation, it avoided the foot shock and this shuttle response was recorded as avoidance. If the rat remained in the same compartment for more than 10 s and made a crossing upon receiving the foot shock, this response was recorded as an escape. If the rats did not respond neither during the 10 s of CS nor by 10 s of UCS, the trial was terminated and the failure was recorded. About 12-15 training sessions (during 2-3 weeks) was needed to start the tests. Approximately 20% of the rats has dropped off during the training. The test was started if the rats reached the stable avoidance level of above 80% on two subsequent days. The rats were used several times with 7 days a drug-free period between tests. Drugs were administered 30-60 minutes before the test (*i.p).*

4.6.5. Advanced behavioral evaluation: social interaction in ketamine-disturbed conditions (a measure of "negative" symptoms of schizophrenia)

The experiments were conducted in the open field arena (57 × 67 × 30 cm) made of black Plexiglas, as described earlier [46]. The arena was dimly illuminated with an indirect light of 18 Lux. The behavior of the rats was recorded by two cameras placed above the arena and connected to the Noldus MPEG recorder 2.1. Videos were analyzed off-line by the Noldus Observer XT, version 10.5.

Rats were individually housed for 5 days prior to the start of the procedure. On the fifth day of social isolation, all rats were transferred to the experimental room and individually adapted to the open field arena for 7 min. Afterward, the rats were handled, weighed and half were dyed with a gentian violet (2% Methylrosanilinium chloride) on the rear part of the body. On the test day (the sixth day of social isolation), two unfamiliar rats of matched body

weight (+/-5 g), one white and one dyed, were placed in the open field arena and their behavior was recorded for 10 min. Both rats in a given pair received the same treatment. Social interaction time was measured for each rat separately and expressed as a summed score per each pair of rats. The following active social behaviors were scored: sniffing (rat sniffs the conspecific's parts of the body, including the anogenital region), social grooming (rat licks and chews the fur of the conspecific), following (rat moves towards and follows the other rat), mounting (rat stands on the conspecific's back) and climbing (rat climbs over the conspecific's back). The time of active social behaviors was summed to yield a total score. Because both animals in a pair yielded approximately equal scores (for either total time spent in social interactions or separate social behaviors), social interaction time was expressed as a summed score for each pair of animals.

Ketamine at a dose of 20 mg/kg (*i.p.*) was given 30 min before the test. The test compounds were given 30-60 min (*i.p.*) before the ketamine injections.

4.6.6. Advanced behavioral evaluation: Catalepsy bar test

One hour *i.p.* drug administration, the catalepsy, a prolonged maintenance of an externally imposed abnormal posture, was assessed by means of the bar test [47]. Testing was accomplished by placing each rat in an upright position with his forepaws resting on a horizontal bar (0.9 cm of diameter), suspended in a wooden frame 7.5 cm above the cage floor. The rat was allowed to keep his forepaws on the bar for a measurement lasting for 30 s. The latency to remove both rat's forepaws from the bar was measured with a hand-held stopwatch. The test was repeated for each animal every 30 min up to 240 min. Animals were returned to their home cages between the tests. Each rat was tested twice, in the interval of one week.

4.6.7. Advanced evaluation of pro-cognitive effects in the attentional set shifting test (ASST)

The ASST assesses cognitive flexibility, i.e., the ability to modify behavior in response to alterations in the relevance of stimuli [39]. In this paradigm, rats must select a bowl containing a food reward (Honey Nut Cheerio, Nestle®) based on their ability to discriminate the odors associated with the pot and the digging media that covered the Cheerio bait in the pot.

The ASST requires rats to initially learn a rule and form an attentional "set" within the same stimulus dimensions. At the extra-dimensional (ED) shift stage, the essential phase of the task, the animals must switch their attention to a previously irrelevant stimulus dimension and, for example, discriminate between the odors and not between the media covering the bait. The animal's performance at the ED stage is considered an index of cognitive flexibility. A detailed description of the apparatus and procedure has been provided previously [48].

The procedure for each rat entailed three days: habituation, training and testing. During a single test session, the rats performed a series of 7 discriminations. In the simple discrimination (SD) that involved only one stimulus dimension, the pots differed along one of two dimensions (e.g., digging medium). For the compound discrimination (CD), the second (irrelevant) dimension (i.e., odor) was introduced, but the correct and incorrect exemplars of the relevant dimension remained constant. For the reversal of this discrimination (Rev 1), the exemplars and relevant dimension were unchanged, but the previously correct exemplar was now incorrect and the previously incorrect exemplar was now correct. The intra-dimensional (ID) shift was then presented; this shift consisted of new exemplars of both the relevant and irrelevant dimensions, with the relevant dimension remaining the same as before.

The ID discrimination was then reversed (Rev 2) so that the formerly positive exemplar became the negative one. This series of discriminations serves to progressively form an

attentional set. For the ED shift, a new pair of exemplars was again introduced, but this time, a relevant dimension was also changed. At this essential phase of the task, animals must switch their attention to a previously irrelevant stimulus dimension and, for example, discriminate between the odors and not between the media covering the bait. Finally, the last phase was the reversal (Rev 3) of the ED discrimination.

On the test day, the rats received ketamine (0 or 10 mg/kg *s.c.*) 75 min prior to the task, whereas compound **62** (1.0 mg/kg) was administered intraperitoneally (*i.p.*) 30 min prior to the ketamine injection. The number of animals in each experimental group was N = 6. Each rat was tested only once.

4.6.8. Statistics

Behavioral data were analyzed with Student's paired *t*-tests (objects exploration in NOR), one-way ANOVA (spontaneous activity, PCP-induced hyperactivity, Discrimination Index in NOR, CAR avoidances, escapes, failures, IT-crosses), two-way repeated measures ANOVA (social interaction: treatment x ketamine; catalepsy: treatment x time; ASST: treatment x phase) or three-way mixed-design ANOVA (blood pressure: systolic/diastolic x time x treatment), followed by Newman-Keul's or Dunnett's post hoc test (IBM/SPSS 21 or Prism 5.0 for Windows). The alpha value was set at $P<0.05$.

4.7. Pharmacokinetic studies for compound **62**

Experiments were carried out on male Wistar rats, 220 – 250 g (Charles River Laboratory, Germany), having free access to food and tape water. Rats received the compound **62** *per os* (9 mg/kg) and were decapitated in different time intervals (2, 5, 10, 15, 20, 30, 45 min, 1, 2, 4, 6, 12, 24 h). After decapitation the blood samples were centrifuged (2000 g, 10 min). The obtained blood plasma was separated and stored at -20°C until used for analysis. Brains were

stored at -80°C until homogenized and analyzed for the concentration of compounds. Plasma and brain concentrations of **62** were analyzed using LC-MS/MS system. Kinetic parameters were calculated using Winnolin Noncompartmental Analysis Program.

Abbreviations used:

AcOH Acetic acid; AcOEt Ethyl Acetate; Boc, tert-butoxycarbonyl; CF_3COOAg Silver trifluoroacetate; DMSO dimethylsulfoxide; Et_3N triethyl amine; EtOH Ethanol, Et_2O diethyl ether; THF tetrahydrofuran; Hex n-Hexane; $LiAlH_4$ Lithium Aluminium Hydride; MED, minimum effective dose; MeOH Methanol; $NaBH(OAc)_3$ Sodium triacetoxyborohydride; NMDA N-methyl-D-aspartate; PCP Phenciclidine; TFA trifluoroacetic acid; WCST, Wisconsin Card Sorting test

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Supporting Material

Supporting Information Available: characterization data for all intermediates and final compounds (excluded from the manuscript); MS, ^{1}H, ^{13}C NMR spectra for representative final compounds; Methods and details describing experimental conditions for *in vitro* and *in*

vivo characterization of reference and tested compounds i.e., radioligand binding assays toward serotonin and dopamine receptors, functional assays, metabolic stability, CYP450 interaction, hERG interaction, blood pressure and ECG tests, pharmacokinetic tests, prolactin level assay, PCP-induced hyperactivity test, novel object recognition task, conditioned avoidance response test, ketamine-disturbed social interaction test, catalepsy test, attentional set-shifting test.

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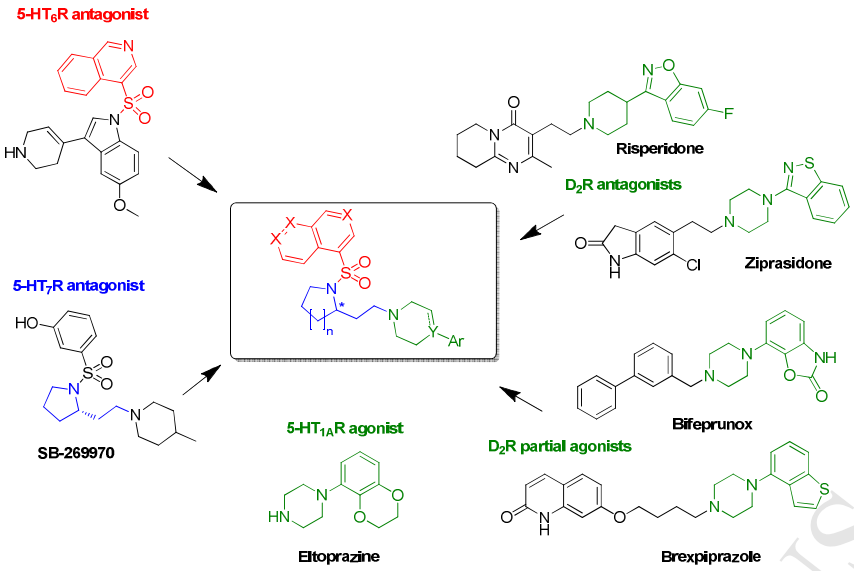
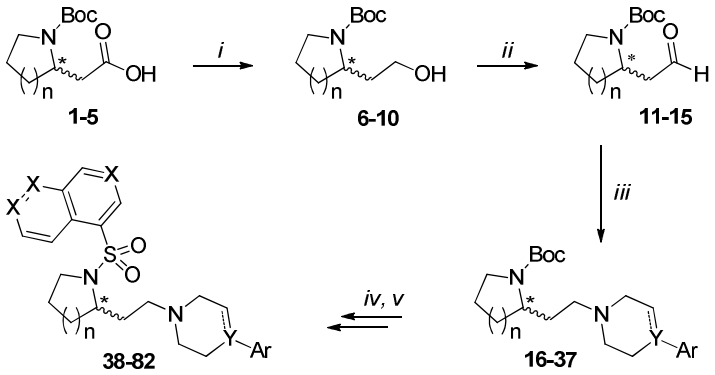
Figure 1.



Scheme 1.



n = 0, 1, 2, 3; Y = C, CH, N; X = CH, N;
Ar =(1,3-benzoxazol-2(3*H*)-one)-4-yl, 2,3-dihydro-1,4-benzodioxin-5-yl, 6-fluoro-1,2-benzoxazol-3-yl, 5-chloro-1*H*-indol-3-yl, 1,2-benzothiazol-3-yl, 1,2-benzoxazol-3-yl, benzothiophen-4-yl, 1*H*-indol-4-yl.

Scheme 2.

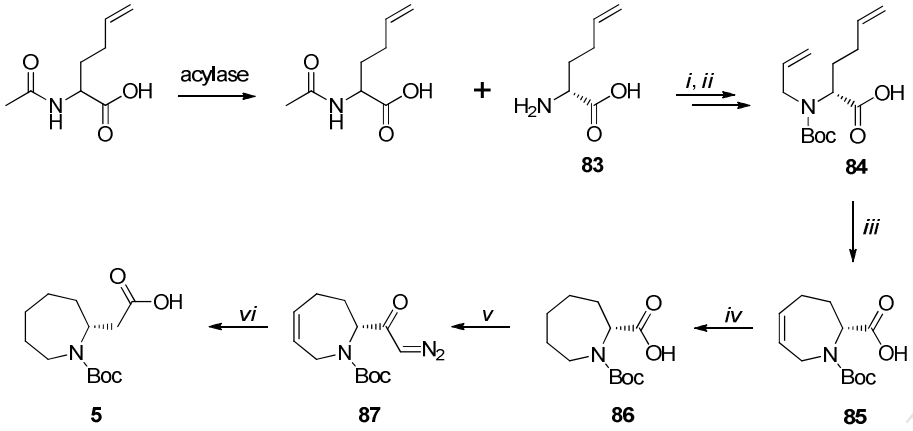
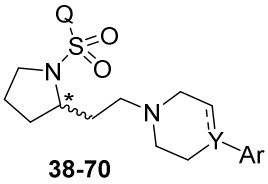


Table 1.



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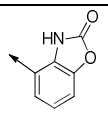
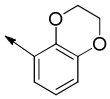
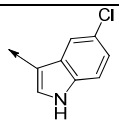
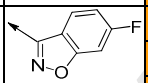
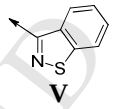
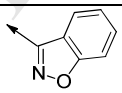
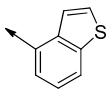
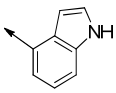
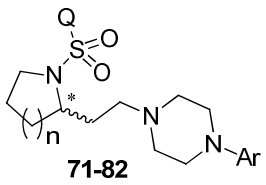
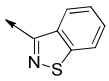
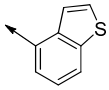
Compd	Q	Enant	Y	Ar	K_i [nM][a]					S[b]
					5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$	
38	5-Quinolyl	*S*	N	 **I**	23	2453	3912	1119	9	0.7
39	4-Isoquinolyl				27	2040	2990	672	5	0.69
40	4-Isoquinolyl	*R*			36	1970	1810	1340	12	0.72
41	5-Quinolyl	*S*	N	 **II**	6	110	2533	349	13	0.88
42	4-Isoquinolyl				6	91	1483	142	19	0.91
43	5-Quinolyl	*R*			5	296	996	66	12	0.82
44	4-Isoquinolyl				7	390	1263	52	22	0.84
45	5-Quinolyl	*S*	C	 **III**	146	1400	77	65	11	0.73
46	4-Isoquinolyl				66	238	60	97	13	0.84
47	5-Quinolyl	*R*			91	1150	18	12	15	0.76
48	4-Isoquinolyl				147	1904	23	8	12	0.72
49	5-Quinolyl	*S*	CH	 **IV**	147	12	169	12	1.7	0.9
50	4-Isoquinolyl				111	6	137	0.4	0.1	0.82
51	5-Quinolyl	*R*			120	5	45	12	2	0.95
52	5-Quinolyl	*S*	N	 **V**	25	16	311	7	1	0.87
53	4-Isoquinolyl				37	13	313	9	0.14	0.80
54	5-Isoquinolyl				37	18	360	12	0.5	0.83
55	5-Quinolyl	*R*			24	21	38	1	0.7	0.84
56	4-Isoquinolyl				14	10	40	3	0.9	0.88
57	5-Isoquinolyl				16	15	40	1	0.3	0.82
58	5-Quinolyl	*S*	N	 **VI**	179	49	291	7	4	0.6
59	4-Isoquinolyl				96	33	497	6	3	0.88
60	5-Isoquinolyl	*R*			86	41	92	10	3	0.87
61	5-Quinolyl	*S*	N	 **VII**	13	36	82	19	16	0.95
62	4-Isoquinolyl				18	9	116	19	11	1.01
63	5-Isoquinolyl				10	9	72	23	5	0.97
64	5-Quinolyl	*R*			15	56	13	1	4	0.86
65	4-Isoquinolyl				24	26	11	4	6	0.92
66	5-Isoquinolyl				13	19	9	3	5	0.93
67	5-Quinolyl	*S*	N	 **VIII**	14	244	2310	165	42	0.89
68	4-Isoquinolyl				8	82	2057	75	37	0.95
69	5-Quinolyl	*R*			5	451	641	14	8	0.78
70	4-Isoquinolyl				6	76	766	4	3	0.84
Haloperidol					1703	89	1083	408	2	0.81
Clozapine					143	13	4	>10000	71	1.1
Risperidone					1169	0.5	1107	0.4	0.6	1.01
Ziprasidone					2	1.7	20	5	0.8	0.96
Olanzapine					3442	4	7	206	4	1
Aripiprazole					30	63	89	44	0.8	0.79

[a] K_i values are the means of three independent binding experiments (SEM ≤ 21%)
[b] Atypically ratio = pK_i(5-HT_{2A})/ pK_i (D_2);
Compound multimodality color code: low affinity K_i > 500nM; moderate affinity 50 nM ≤ K_i ≤ 500 nM; high affinity K_i < 50 nM

Table 2.



71-82

Compd	Q	N	Enant	Ar	K_i [nM][a]					S[b]
					5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$	
71	5-Quinolyl	0	*S*	 **V**	54	30	344	57	11	0.95
72	4-Isoquinolyl				56	24	348	51	10	0.95
73	5-Quinolyl	2	*R*		28	29	949	52	7	0.93
74	4-Isoquinolyl				24	35	1259	69	5	0.90
75	5-Quinolyl	3	*S*		48	30	457	17	4	0.90
76	4-Isoquinolyl				50	26	623	19	3	0.89
77	5-Quinolyl	0	*S*	 **VII**	9	7	130	45	24	1.07
78	4-Isoquinolyl				16	16	104	31	29	1.03
79	5-Quinolyl	2	*R*		15	35	337	22	21	0.97
80	4-Isoquinolyl				12	64	221	32	23	0.94
81	5-Quinolyl	3	*S*		12	51	176	19	10	0.91
82	4-Isoquinolyl				8	29	190	24	6	0.92

[a] K_i values are the means of three independent binding experiments (SEM ≤ 21%)
[b] Atypically ratio = pK_i(5-HT$_{2A}$)/pK_i (D$_2$);
Compound multimodality color code: low affinity K_i > 500nM; moderate affinity 50 nM ≤ K_i ≤ 500 nM; high affinity K_i < 50 nM

Table 3.

Compd	EC_{50} [nM] (%ago@ $10e^{-6}$M)[a]	K_b [nM] (%inhib @ $10e^{-6}$M)[c]					
	5-HT_{1A}	**5-HT_{1A}**	**5-HT_{2A}**	**5-HT_6**	**5-HT_7**	**D_2**	**D_3**
53	(5.3)	11 (87) Antag	2.0 (100) Antag	974 (23)	2.5 (100) Antag	0.42 (100) Antag	8.6 (100) Antag
62[b]	28.3 (40.6) partial Ago	35.6 (32.5)	2.3 (100) Antag	920 (23) Antag	3.4 (94) Antag	3.0 (92) Antag	92 (73) Antag
65	11 (36) partial Ago	(80)	9.5 (100) Antag	53 (73) Antag	0.47 (100) Antag	0.74 (100) Antag	(78) Antag

[a] EC_{50} value or % of control agonist response at 10^{-6}M (8-OH-DPAT 10 µM) performed at Eurofins Cerep

[b] EC_{50} and K_b for **62** values performed in G_i cAMP assays at DiscoverX, USA

[c] K_b value or % inhibition of control agonist response at 10^{-6} M (5-HT_{1A} – serotonin 300 nM; 5-HT_{2A} – serotonin 100 nM; 5-HT_6 and 5-HT_7 – serotonin 300 nM; D_2 – dopamine 30 nM; D_3 – dopamine 10 nM), performed at Eurofins Cerep

Table 4.

Compd	MED [mg/kg][a]		Index	MED [mg/kg][a]
	Sedation	PCP-induced hyperactivity	MED Sedation / MED PCP-induced hyperactivity	NOR
53	3	1 (↓)	3	1
56	2	Inactive (1–3 mpk)	–	NT [b]
62	3	1 (↓)	3	1
65	3	Inactive (1–3 mpk)	1	NT [b]
Clozapine	3	1 (↓)	3	1
Haloperidol	0.06	0.6 (↓)	0.1	Inactive (0.05)
Olanzapine	1.25	1.875 (↓)	0.66	1.25
Ziprasidone	3	1 (↓)	3	1

MED represents minimum effective dose. [a] Compounds were administered *i.p.* 60 min before the test; [b] not tested; ↓ decrease and ↑ increase of PCP-induced hyperactivity, respectively. For detailed data and statistics, see the Supplementary material

Table 5.

Compd	MED [mg/kg]				
	CAR[a]: Avoidances	CAR: Failures	Ketamine-induced social withdrawal[a]	Catalepsy	Ketamine-disturbed ASST
53	3	No effect (3)	3	30	NT
62	3	No effect (3)	1	Inactive (30)	1
Clozapine	1	No effect (6)	Inactive (1)	NT	NT
Haloperidol	0.1	0.2	Inactive (0.1)	0.2	NT
Olanzapine	1	No effect (2)	NT	40	NT
Risperidone	0.1	No effect (0.3)	0.1	NT	NT
Ziprasidone	1	2	Inactive (1)	Inactive (30)	NT

MED represents minimum effective dose.[a] Compounds were administered *i.p.* 60 min before the test. *Note*: 2 mg/kg aripiprazole (Figure 19-SI) inhibited ketamine-induced social deficit but was not investigated in other tests

Table 6.

Receptor	%inhib @ 10^{-6} M[a]	K_i [nM][b]	K_b [nM][c]
	62		
α_{1A}	94	–	13 (Antag)
α_{2C}	99	–	120 (Antag)
β_1	31	–	–
β_2	25	–	–
D_1	10	–	–
D_2	100	11	3.0 (Antag)
D_3	98	–	92 (Antag)
D_4	10	–	–
H_1	96	–	25 (Antag)
M_1	6	–	–
5-HT_{1A}	100	18	28.3 (Partial ago)
5-HT_{1B}	84	–	210 (Antag)
5-HT_{1D}	95	–	2.8 (Antag)
5-HT_{2A}	100	9	2.3 (Antag)
5-HT_{2B}	100	–	(14% agonist effect)
5-HT_{2C}	80	–	24 (Antag)
5-HT_3	15	–	–
5-HT_6	23	116	920 (Antag)
5-HT_7	100	19	3.4 (Antag)
SERT	84	76	–
NET	11	–	–
DAT	19	–	–

[a] % inhibition of control binding @ 10^{-6}M

[b] K_i values are the means of three independent binding experiments (SEM ≤ 21%)

[c] K_b value or % inhibition of control agonist response at 10^{-6}M (for details see Table 2-SI)

List of captions

Figure 1. Multireceptor design strategy used in the present study.

Scheme 1. Synthesis of azinesulfonamides of cyclic amine derivatives 38–82. Reaction and conditions: *i*) $LiAlH_4$ 2.0 M in THF, anh CH_2Cl_2, 55°C, 4 h, (yields 68–99%); *ii*) 2-iodoxybenzoic acid (IBX), DMSO, r.t., 12 h, (yields 64–73%); *iii*) arylpiperazines or arylpiperidines or tetrahydropirydines, $NaBH(OAc)_3$, AcOH, THF, r.t., 4 h, (yields 62–89%); *iv*) TFA/CH_2Cl_2 (80/20 v/v), r.t., 2 h, (yields 96–99%); *v*) azinesulfonyl chloride, Et_3N, CH_2Cl_2, 0°C, 2–6 h (yields 60–80%).

Scheme 2. Synthesis of (*S*)-(Boc-azepan-2-yl)acetic acid (**5**). Reaction and conditions: *i*) Boc_2O, K_2CO_3, $H_2O/(CH_3)_2CO$, r.t., (yield 70%); *ii*) allyl bromide, NaH, THF, 0°C then reflux, 12 h, (yield 61%); *iii*) Grubbs second-generation catalyst (3 mmol%), CH_2Cl_2, 36°C, 3 days (yield 67%); *iv*) H_2, Pd/C, EtOH/THF/H_2O (20/20/1; *v*/*v*/*v*), 40°C, 3 h, (yield 96%); *v*) Et_3N, THF, methyl chloroformate, 0°C, 40 min, then CH_2N_2 in Et_2O, -30°C, 1 h, and 12 h r.t. (yield 90%); *vi*) CF_3COOAg, Et_3N, THF, -5°C then r.t., 1.5 h (yield 83%).

Table 1. Structures and receptor binding profiles of compounds **38–70**.

Table 2. Structures and receptor binding profiles of compounds **71**–**82**.

Table 3. Functional profile of selected compounds **53**, **62** and **65** for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2, and D_3 receptors.

Table 4. Behavioral effects of selected compounds **53**, **56**, **62** and **65** and model antipsychotics in in vivo preliminary assays.

Table 5. Behavioral effects of **53** and **62** on conditioned avoidance response (a measure of "positive" symptoms of schizophrenia), social interactions in ketamine-disturbed conditions

(a measure of “negative” symptoms of schizophrenia) and catalepsy (extrapyramidal side-effects).

Table 6. Extended receptor binding profile and functional profile for **62**.

Highlights:

- Putative novel atypical antipsychotics with multi-receptor profile
- Compound **62** showed favorable ADME properties
- Compound **62** produced no catalepsy, demonstrated low cardiac and hyperprolactinemia liability
- Compound **62** addressed “positive”-like symptoms of schizophrenia and displayed pro-social and pro-cognitive profile