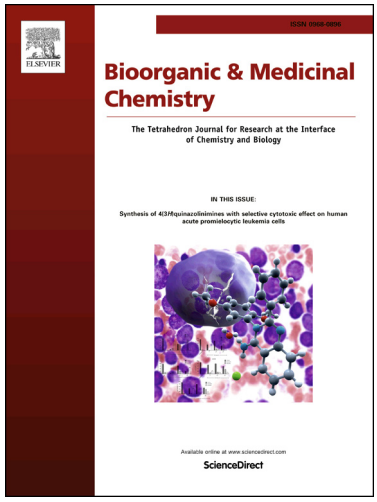
# Accepted Manuscript

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# The impact of the halogen bonding on $D_2$ and 5-$HT_{1A}$/5-$HT_7$ receptor activity of azinesulfonamides of 4-[(2-ethyl)piperidinyl-1-yl]phenylpiperazines with antipsychotic and antidepressant properties

Anna Partyka,[a] Rafał Kurczab,[b] Vittorio Canale,[c] Grzegorz Satała,[b] Krzysztof Marciniec,[d] Agnieszka Pasierb,[c] Magdalena Jastrzębska-Więsek,[a] Maciej Pawłowski,[c] Anna Wesołowska,[a] Andrzej J Bojarski,[b] Paweł Zajdel[c,*]

[a] *Department of Clinical Pharmacy,* [c] *Department of Medicinal Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Krakow, Poland*

[b] *Institute of Pharmacology, Polish Academy of Sciences, Department of Medicinal Chemistry, 12 Smętna Street, 31-343 Krakow, Poland*

[d] *Department of Organic Chemistry, Medical University of Silesia, 4 Jagiellońska Street, 41-200 Sosnowiec, Poland*

*Corresponding author:
Department of Medicinal Chemistry
Jagiellonian University Medical College
e-mail: pawel.zajdel@uj.edu.pl
Tel.: +48 12 620 54 50; fax: +48 12 620 54 05

**Abstract**

A series of azinesulfonamides of long-chain arylpiperazine derivatives with semi-rigid alkylene spacer was designed, synthesized, and biologically evaluated using *in vitro* methods for their affinity for dopaminergic $D_2$ and serotoninergic 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and 5-$HT_7$ receptors. Docking to homology models revealed a possible halogen bond formation in complexes of the most potent ligands and the target receptors. The study allowed for the identification of compound 5-({4-(2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (**21**), which behaved as $D_2$, 5-$HT_{1A}$ and 5-$HT_7$ receptor antagonist. In preliminary *in vivo* studies, compound **21** displayed distinct antipsychotic properties in the MK-801-evoked hyperactivity test in mice at a dose of 10 mg/kg, and exerted antidepressant-like effect in a forced swim test in mice (MED = 0.625 mg/kg, *i.p.*).

Keyword: azinesulfonamides, semi-rigid long-chain arylpiperazines, multimodal dopamine/serotonin ligands, multi-target ligands, halogen bonding, schizophrenia, depression.

## 1. Introduction

Despite recent advances in the pharmacology and neurophysiology which allowed for the development of new therapeutic strategies, the outcome of pharmacological treatments of schizophrenia as well as affective disorders e.g., depression, bipolar disorder still remains unsatisfactory. Indeed, in the last decades a large amount of efforts were aimed to improve the therapeutic efficiency of clinically used antipsychotics and antidepressants avoiding tolerance, long time of onset of action or remission of a disease and to reduce the unwanted side effects (sexual dysfunction, extrapyramidal symptoms, nausea/emesis, weight gain, endocrinopathies and cardiovascular effects).[1,2]

Since schizophrenia displays high comorbidity with other serious CNS disorders (depression, cognitive impairment and anxiety) a multi-target strategy might show a broad-based efficacy.[3–5] The essential characteristic of all currently used antipsychotics is their ability to block $D_2$ receptors to reverse positive symptoms of schizophrenia. It has been further found, that antagonism at 5-$HT_7$ receptors (5-$HT_7$Rs) may contribute to alleviation of negative symptoms (e.g., social withdrawal),[6] while both antagonism at 5-$HT_7$Rs and partial agonism at 5-$HT_{1A}$Rs may contribute to pro-cognitive effects. Furthermore, there is strong evidence supporting the notation that antagonism at 5-$HT_7$Rs as well as partial agonism at 5-$HT_{1A}$Rs may be involved in the antidepressant and/or anxiolytic activity of second generation antipsychotics such as amisulpride, lurasidone and cariprazine (Figure 1).[7–9]

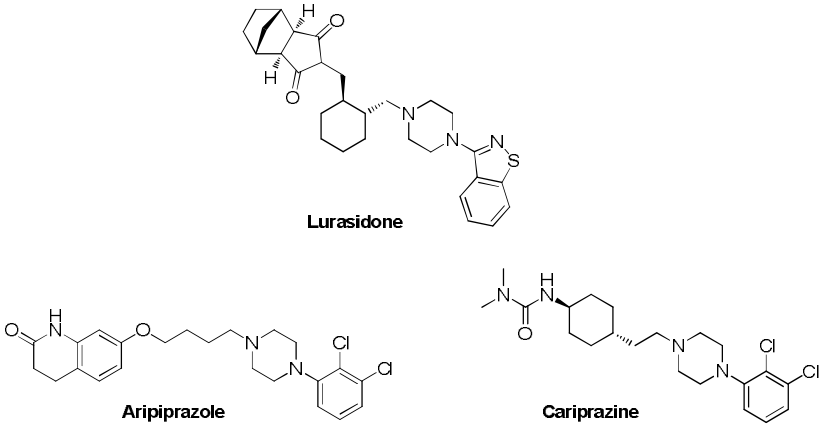



**Figure 1**. Chemical structures of atypical antipsychotics lurasidone, aripiprazole and cariprazine.

Our research group has previously reported on the series of flexible quinoline- and isoquinoline-sulfonamides of long-chain arylpiperazines (LCAPs) with halogen substituent at the phenylpiperazine moiety which showed high affinity for 5-$HT_7$ receptor and a mixed 5-$HT_{2A}$/5-$HT_7$/$D_2$R antagonist profile, and displayed antidepressant and anxiolytic properties (Figure 2).[10–12]

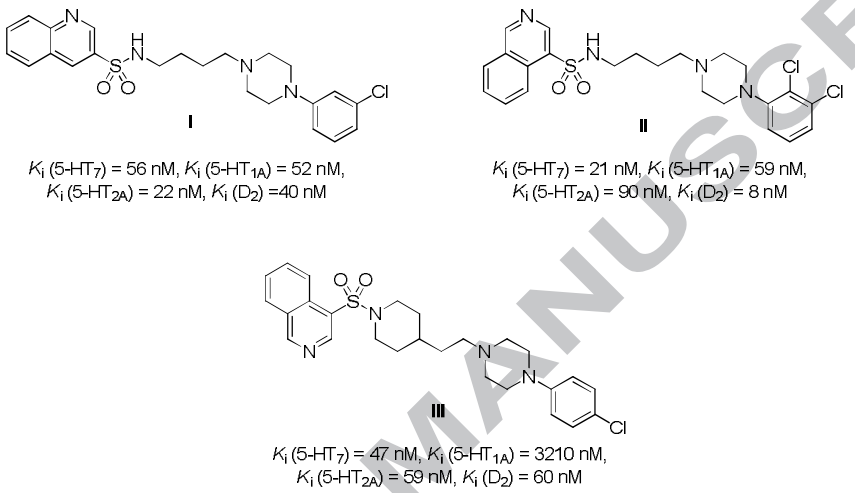



**Figure 2**. The chemical structure of compounds **I–III** as multimodal $D_2$/5-$HT_{1A}$/5-$HT_{2A}$/5-$HT_7$ receptor ligands.

Moreover, inspired by previous findings on a group of *N*-alkylated azinesulfonamides,[13] we identified compound **III** (4-(4-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-piperidine-1-sulfonyl)-isoquinoline) with partially rigidified polimethylene spacer,[10] which behaved as $D_2$/ 5-$HT_{2A}$/5-$HT_7$ receptor antagonist. Compound **III** showed antipsychotic-like properties in MK-801-induced hyperlocomotor activity in mice (MED = 5 mg/kg, *i.p*), significant antidepressant-like activity in the forced swim test (FST) in mice (MED = 30 mg/kg, *i.p.*), and anxiolytic-like effect in the plus-maze test in rats (MED = 10 mg/kg, *i.p.*).

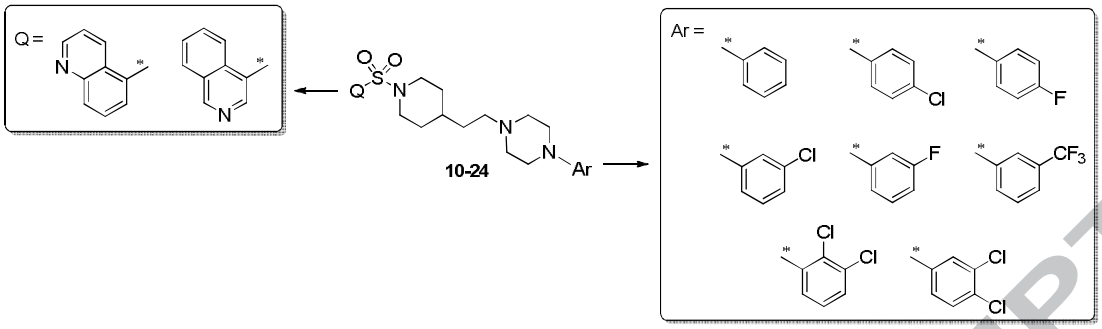
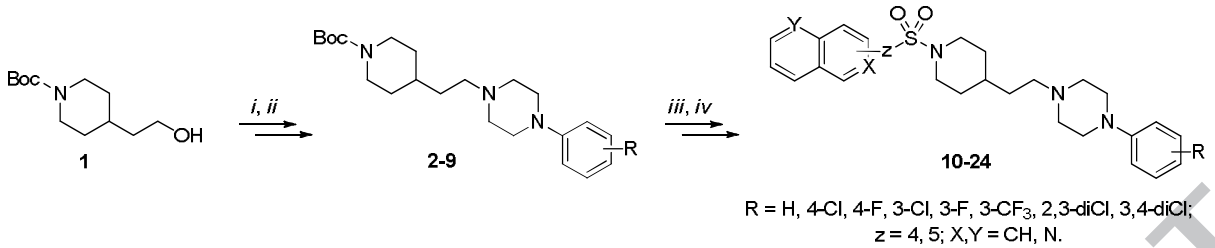



**Figure 3.** Structural modifications for the designed series of azinesulfonamide derivatives of 4-[(2-ethyl)piperidin-1yl]phenylpiperazines.

Continuing study in this group of LCAP derivatives, we designed a new series of close analogs of compound **III** with semi-rigid alkylene spacer (Figure 3). Structural modifications aimed to verify an influence of the type of azinesulfonamide fragment as well as an impact of the halogen substitution pattern at the phenylpiperazine moiety on the receptor affinity through the directional interaction called halogen bonds. Herein, we report on the synthesis, *in vitro* evaluation for $D_2$, 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and 5-$HT_7$ receptors, followed by determination of *in vitro* functional receptor profile for selected compounds. Finally, we present results of preliminary pharmacological evaluation of the most *in vitro* active agents in mouse models of schizophrenia and depression.

## 2. Chemistry

The synthesis of final compounds **10–24** was performed according to the Scheme 1. In the first step, commercially available 1-Boc-protected-4-(2-hydroxyethyl)piperidine (**1**) was treated with *para*-toluenesulfonyl chloride followed by nucleophilic substitution with the substituted phenylpiperazines to obtain intermediates **2–9**. After removal of Boc-protecting group, the resulted secondary amines reacted with the selected azinesulfonyl chloride in basic condition yielding final compounds **10–24**.




**Scheme 1**. Synthesis of azinesulfonamides of LCAP with semi-rigid alkylene spacer **10–24**. Reagents and conditions: *i*) *p*-toluenesulfonyl chloride, 4-(dimethylamino)aniline, triethylamine, $CH_2Cl_2$, 0°C, 12 h; *ii*) substituted phenylpiperazines, THF/Toluene (30/70, *v*/*v*), 70°C, 12 h; *iii*) TFA/$CH_2Cl_2$ (80/20, *v*/*v*), rt, 2 h; *iv*) azinesulfonyl chloride, TEA, $CH_2Cl_2$, 0°C, 2–6 h.

The azinylsulfonyl chlorides were obtained from their respective bromo derivatives according to the previously reported method.[14] All of the final compounds were converted into their water-soluble hydrochloride salts.

### 3. Molecular Modeling

Homology models built on $D_3$ receptor crystal structure template developed and tested in our previous studies,[15–17] were used to investigate the molecular mechanism of binding of the synthesized library of compounds for the interaction with $D_2$, 5-$HT_{1A}$, 5-$HT_7$Rs and to support results obtained from *in vitro* radioligand binding assays. The recently studied procedure involving Quantum Mechanics/Molecular Mechanics-driven molecular docking with binding free energy calculations was applied to assess the contribution of halogen bonding to the quality of binding mode.[18] To visualize (plotting interaction spheres) the possible contribution of halogen bonding for resulting ligand-receptor complexes, the Halogen Bonding Webserver was used (access 19.03.2017, http://www.halogenbonding.com/).[19]

## 4. *In vitro* pharmacology

### 4.1. *In vitro* radioligand binding evaluation

Radioligand binding assays were performed to determine the affinity and selectivity profiles of the synthesized compounds in competition binding experiments for human dopaminergic $D_2$ and serotonin 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and 5-$HT_7$ receptors, which were all stably expressed in HEK293 cells, according to the previously published procedures.[10,20,21] The inhibition constants ($K_i$) were calculated from the Cheng-Prusoff equation.[22] Results were expressed as means of at least three separate experiments.

### 4.2. *In vitro* functional evaluation

The functional properties of selected compounds **16**, **21** and **22** against the human $D_2$, 5-$HT_7$ and 5-$HT_{1A}$Rs were determined in functional cAMP cellular assays, performed at Eurofins Cerep using CHO and HEK-293 cells, according to procedures described online at www.cerep.fr.

## 5. *In vivo* pharmacology

The central activities of the most potent $D_2$/5-$HT_{1A}$/5-$HT_7$ receptor antagonist (i.e. **16**, **21** and **22**) were tested in *in vivo* behavioral test commonly used to predict antipsychotic- and antidepressant-like activity. MK-801-induced hyperactivity test in CD-1 mice was used to determine the antipsychotic-like activity of the tested compounds while the potential antidepressant properties were evaluated in the forced swim test (FST) in Swiss albino mice.[23,24] Moreover, the influence of the antipsychotic- and/or antidepressant-effective doses on the spontaneous locomotor activity in mice was investigated in order to exclude the possibility of competing behaviors such as general locomotor activity. Citalopram, aripiprazole and compound **III** were used as references.

## 6. Results and Discussion

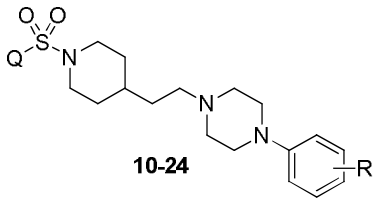
### 6.1. Structure-activity relationship studies

The newly synthesized azinesulfonamides **10–24** were evaluated in *in vitro* binding experiments for their affinity for $D_2$, 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and 5-$HT_7$Rs. In general, binding assays revealed that new compounds displayed high-to-low affinity for $D_2$Rs (30–1821 nM) and 5-$HT_{1A}$ ($K_i$ = 29–975 nM), high-to-moderate affinity for 5-$HT_7$R ($K_i$ = 14–209 nM), and finally low affinity for 5-$HT_6$Rs ($K_i$ = 309–8279 nM) (Table 1). It is worth noting, that all the newly synthesized compounds showed moderate affinity for 5-$HT_{2A}$R ($K_i$ = 139–691 nM) and displayed lower affinity for these sites in comparison to compound **III** ($K_i$ = 59 nM) (Table 1).

Following our previous studies,[10,25] suggesting a preferential position of sulfonamide group in α-position of the azinyl moiety, regardless localization of the sulfonamide group in pyridine or benzene rings, 4-isoquinolyl and 5-quinolyl moieties were selected for design of analogs of compound **III**. It was observed that a kind of azinyl moiety only slightly influenced affinity of synthesized compounds for tested receptors.

Then, regarding the preliminary studies on the engagement of halogens in interaction of LCAP derivatives with 5-$HT_{1A}$ and 5-$HT_7$ receptors,[26] a special interest was focused on impact of halogen atoms (an influence of the kind of halogen and/or its position at the phenylpiperazine moiety) in the binding of compounds **10–24** with $D_2$, 5-$HT_{1A}$, and 5-$HT_7$ receptors. Although halogen atoms have been routinely used in drug development processes to improve biological activity for a given target(s) and/or pharmacokinetic properties (e.g., membrane permeability, oral absorption), their role has been recently assigned to stabilize the ligand–receptor complexes *via* formation of specific interactions called halogen bond.[27,28]

**Table 1.** The binding data of compound **III** and the synthesized compounds **10–24** for $D_2$, 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and 5-$HT_7$Rs




**10-24**

| Cmpd | Q | R | $K_i$ [nM][a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | **$D_2$** | **5-$HT_{1A}$** | **5-$HT_{2A}$** | **5-$HT_6$** | **5-$HT_7$** |
| **III**[b] | 4-isoquinolyl | 4-Cl | 60 | 3210 | 59 | 16650 | 47 |
| **10** | 5-quinolyl | H | 1237 | 749 | 646 | 6712 | 209 |
| **11** | 4-isoquinolyl | H | 1218 | 543 | 410 | 2412 | 174 |
| **12** | 5-quinolyl | 4-Cl | 141 | 740 | 152 | 1020 | 37 |
| **13** | 5-quinolyl | 4-F | 223 | 759 | 139 | 1459 | 29 |
| **14** | 4-isoquinolyl | 4-F | 418 | 975 | 156 | 1863 | 14 |
| **15** | 5-quinolyl | 3-Cl | 76 | 54 | 197 | 445 | 37 |
| **16** | 4-isoquinolyl | 3-Cl | 37 | 32 | 157 | 1185 | 18 |
| **17** | 5-quinolyl | 3-F | 1821 | 609 | 665 | 8279 | 165 |
| **18** | 4-isoquinolyl | 3-F | 1011 | 370 | 691 | 3802 | 142 |
| **19** | 5-quinolyl | 3-$CF_3$ | 149 | 40 | 530 | 640 | 56 |
| **20** | 4-isoquinolyl | 3-$CF_3$ | 148 | 30 | 161 | 1016 | 52 |
| **21** | 5-quinolyl | 2,3-diCl | 36 | 45 | 286 | 401 | 55 |
| **22** | 4-isoquinolyl | 2,3-diCl | 30 | 29 | 371 | 309 | 39 |
| **23** | 5-quinolyl | 3,4-diCl | 121 | 257 | 336 | 791 | 52 |
| **24** | 4-isoquinolyl | 3,4-diCl | 145 | 324 | 179 | 1041 | 36 |

[a] $K_i$ values are means of three independent binding experiments (SEM ≤ 21%);
[b] Data taken from Ref. 10

Binding data revealed that unsubstituted phenylpiperazine compounds **10** and **11** displayed low affinity for all the tested receptors. On the other hand, the introduction of a chlorine atom in 4-position increased the affinity for $D_2$ and 5-$HT_7$Rs up to 8- and 5-fold, respectively (**10** *vs* **12**).

Then, the replacement of the 4-chloro atom with the 4-fluoro was not preferred for the interaction with $D_2R$ yet maintaining high affinity for 5-$HT_7$Rs (**13** *vs* **12**). The higher affinity of compounds **III** and **12** (with chlorine atom in 4-position) for $D_2$Rs than unsubstituted and 4-fluoro analogs might be explained by the ability of chlorine to stabilize the ligand-receptor (L-R) complex by the formation of a halogen bond (X-bond distance = 2.95 Å, σ-hole angle = 171.87°) with the backbone carbonyl group of Ser5.42 (Figure 4A). On the contrary, hydrophobic interactions (i.e., with Ala5.46 and Ile3.40, Figure 4B) of compounds **III** and **14**, seem to be more crucial for the interaction with 5-$HT_7$R ($K_i$ = 47 and 14 nM, respectively). In line with *in silico* experiments indicating a perturbation of L-R complex stability (lack of interaction with Asp3.32), both 4-chloro (**III**) and 4-fluoro (**14**) derivatives displayed low affinity for 5-$HT_{1A}$Rs (Figure 4C).

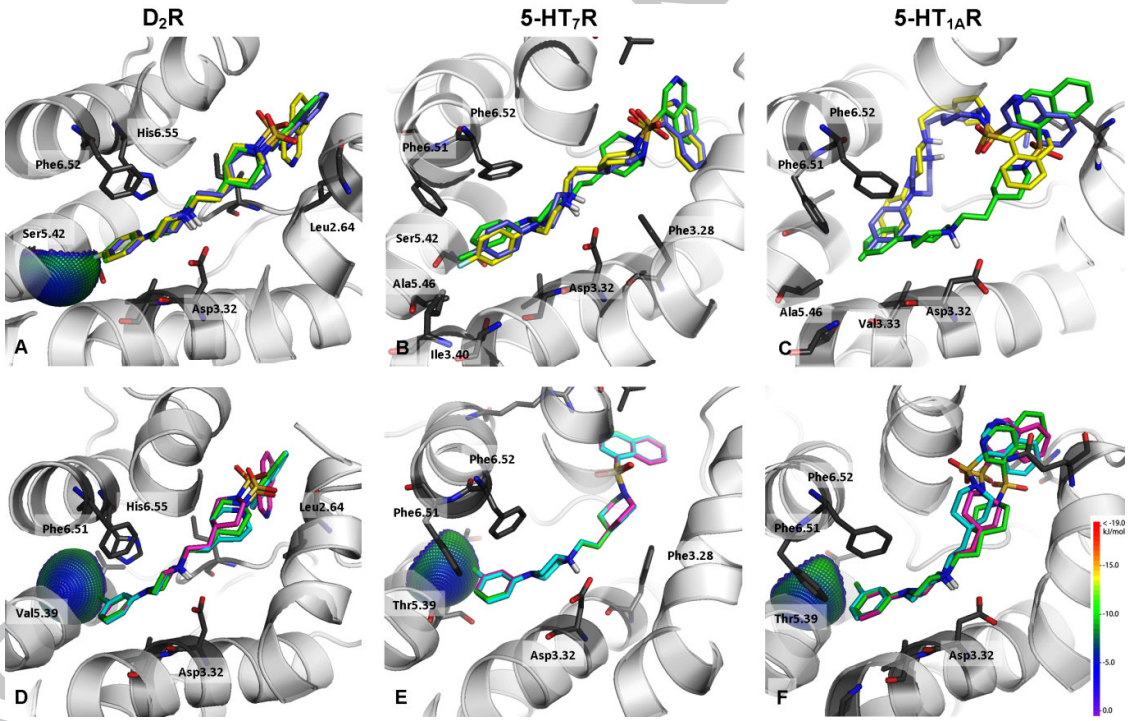



**Figure 4. A–C:** Binding mode of compounds **11** (green), **III** (violet) and **14** (yellow) for $D_2$, 5-$HT_7$ and 5-$HT_{1A}$Rs, respectively; **D–F:** Binding mode of compounds **11** (green), **16** (cyan) and **18** (magenta) for $D_2$, 5-$HT_7$ and 5-$HT_{1A}$Rs respectively. The chlorine-oxygen theoretical interaction spheres illustrate the projected qualities of the formed L-R halogen bonds.

Interestingly, the shift of the chlorine from 4- to 3-position increased the affinity for 5-$HT_{1A}$ and $D_2$ sites (**12** *vs* **15** and **III** *vs* **16**). At the same time, this modification decreased the affinity for 5-$HT_{2A}$Rs by 3–4 fold. In consequence, compounds **15** and **16** were classified as $D_2$/5-$HT_{1A}$/5-$HT_7$ receptor ligands. In contrast, an introduction of 3-fluorine atom at phenylpiperazine decreased affinity for all of the tested receptors (**17** *vs* **15**). These findings were confirmed by molecular modeling experiments revealing that only the presence of chlorine atom in 3-position stabilized the L-R complex through the formation of halogen bond with Val5.39 residue of $D_2$Rs (X-bond distance = 2.95 Å, σ-hole angle = 171.87°) and with Thr5.39 residues of 5-$HT_7$ and 5-$HT_{1A}$Rs (X-bond distance = 2.88 and 3.83 Å, σ-hole angle = 155.80° and 137.46°, respectively) (Figure 4D–F). It was found that increasing the electron withdrawing effect of the substituent in 3-position at the phenylpiperazine moiety by the replacement of chlorine atom with the $CF_3$ group decreased affinity up to 3 fold for $D_2$ and 5-$HT_7$Rs (**15** *vs* **19**). Although introduction of a second chlorine atom in 2-position at the phenylpiperazine, yielding compound **21** and **22**, created an additional halogen bond with His6.55 (X-bond distance = 2.81 Å, σ-hole angle = 149.45°, Figure 5A), this modification did not affect the receptor binding profile in comparison to 3-chloro counterpart (**21** *vs* **15** or **22** *vs* **16**). Thus, compounds **21** and **22** were classified as $D_2$/5-$HT_{1A}$/5-$HT_7$ multi-receptor ligands.

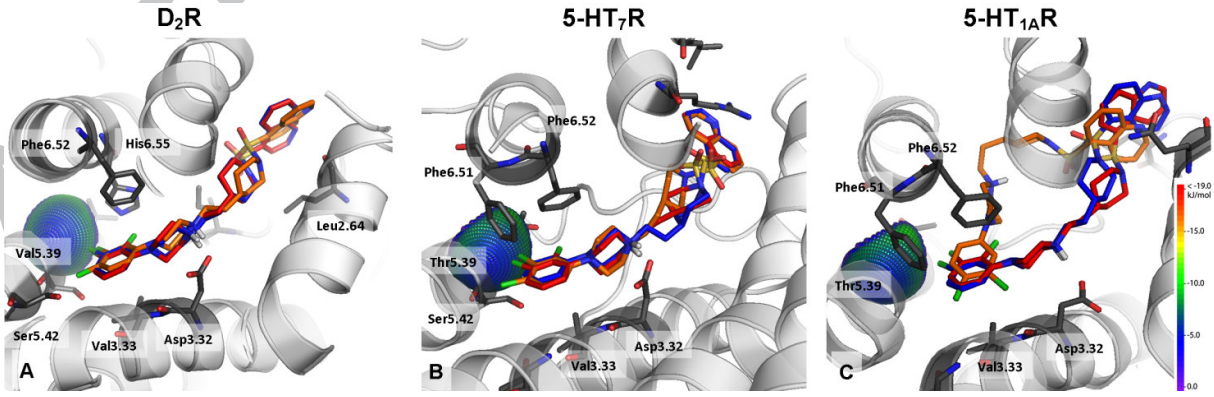



**Figure 5. A–C:** A superposition of the poses of compounds **15** (blue), **21** (red) and **23** (orange) against putative halogen binding pocket interaction spheres for $D_2$, 5-$HT_7$ and 5-$HT_{1A}$Rs,

respectively. The chlorine-oxygen theoretical interaction spheres illustrate the projected qualities of the formed L-R halogen bonds.

Further explanation results from the fact, that chlorine atom in 2-position of compound **21** was not directly engaged in stabilization of ligand – 5-$HT_{1A}$ and 5-$HT_7$ complexes. Consequently, chlorine atom in 3-position stabilized the L–5-$HT_{1A}$ and 5-$HT_7$R complexes through the formation of halogen bonds with Thr5.39 (X-bond distance = 3.20 Å, σ-hole angle = 157.84° for 5-$HT_{1A}$R, and X-bond distance = 3.15 Å, σ-hole angle = 169.93° for 5-$HT_7$R, respectively) (Figure 5B-C). In contrary, introduction of the second chlorine atom in 4-position, giving 3-4-dichlorosubstituted analogs **23** and **24**, drastically decreased the affinity for 5-$HT_{1A}$ and $D_2$Rs, yet maintaining a high affinity for 5-$HT_7$ site (**21** *vs* **23** and **22** *vs* **24**). This was confirmed by stabilization of the L-5-$HT_7$R complex by compound **23** through the formation of halogen bond with Thr5.39 (X-bond distance = 3.31 Å; σ-hole angle = 172.12°) and Ser5.42 (X-bond distance = 2.84 Å, σ-hole angle = 147.15°, Figure 5C).

**Table 2.** The antagonist activity of selected compounds for $D_2$, 5-$HT_{1A}$ and 5-$HT_7$Rs.

| Compd | % inh[a] | % inh[b] | % inh[c] |
|---|---|---|---|
| | $D_2$ | 5-$HT_{1A}$ | 5-$HT_7$ |
| **16** | 85 | 69 | 73 |
| **21** | 88 | 63 | 69 |
| **22** | 79 | 64 | 75 |

[a] inhibition of control agonist response (dopamine 30 nM) at $10^{-6}$M; performed at Eurofins Cerep
[b] inhibition of control agonist response (8-OH-DPAT 100 nM) at $10^{-6}$M; performed at Eurofins Cerep
[c] inhibition of control agonist response (serotonin 300 nM) at $10^{-6}$M; performed at Eurofins Cerep

In the next step, compounds displaying a mixed dopamine/5-HT activity were selected for extended *in vitro* functional evaluation. It was found that tested compounds **16**, **21** and **22** behaved as moderate antagonist at the human 5-$HT_{1A}$ and 5-$HT_7$Rs and were classified as potent antagonist

at the $D_2$Rs (Table 2). Interestingly, in contrast to flexible azinesulfonamides of LCAPs with 2,3-dichlorophenylpiperazine moiety (e.g., compound **II**, Figure 2), which behaved as 5-$HT_{1A}$R partial agonists, compounds **21** and **22** were classified as 5-$HT_{1A}$R antagonists.

### 6.2. Behavioral evaluation

In view of the receptor profile and functional activity of the selected derivatives their potential antipsychotic- and/or antidepressant-like properties were evaluated in behavioral tests in mice.

Acute administration of NMDA receptor antagonists, such as dizocilpine (MK-801), results in behavioral syndrome which includes hyperlocomotion, stereotypic behaviors, and social and cognitive deficits. These symptoms may be analogous to symptoms of schizophrenia in humans.[29] Behavioral abnormalities induced in rodents by MK-801 are prevented by antipsychotic drugs.[30] The binding data revealed that compounds **16**, **21** and **22** displayed significant $D_2$ receptor affinity, thus their antipsychotic activity was studied in the MK-801-induced locomotor hyperactivity test in CD-1 mice. Compounds **16** (30 mg/kg) and **21** (10 and 20 mg/kg) significantly attenuated the MK-801-evoked increase of mice locomotor activity and the effect was similar to that produced by reference compound **III**. On the other hand, compound **22** administered at doses of 5–20 mg/kg produced no effects on the hyperactivity induced by MK-801. By comparison, the reference antipsychotic drug, aripiprazole, at a dose of 0.5 mg/kg remarkably suppressed the stimulating effect of dizocilpine (Figure 6). It is noteworthy that **16**, **21** and aripiprazole given in antipsychotic active doses remained without significant influence on the spontaneous locomotor activity of animals, thus their effects in the MK-801-induced hyperactivity test seem to be specific (Table 1-SI).

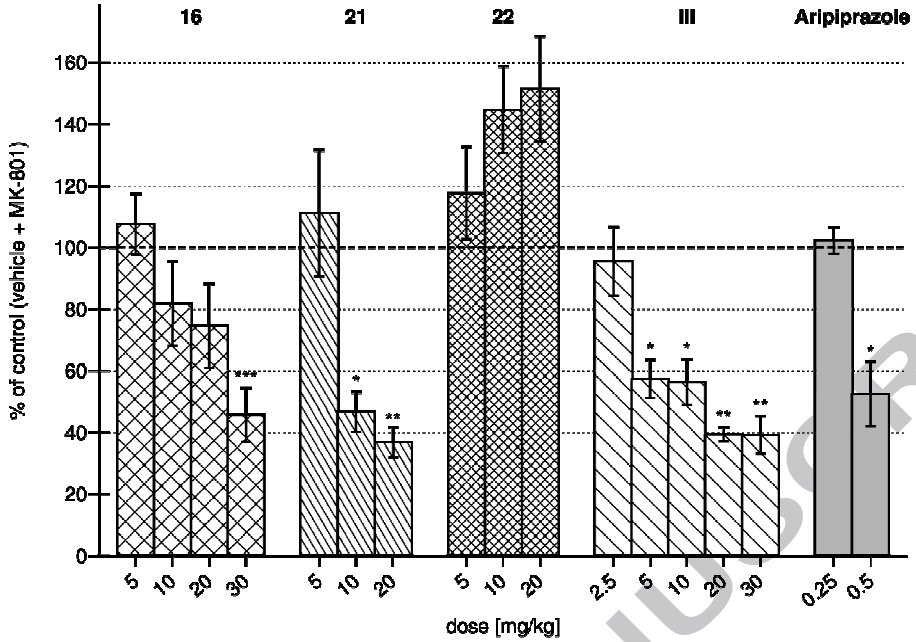



**Figure 6.** Effects of compounds **16**, **21**, **22**, reference compound **III** and aripiprazole on the hyperlocomotor activity induced by MK-801 in CD-1 mice. Data are presented as the mean ± standard error of the mean of N = 8–10 animals per group.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the respective vehicle group, followed by the Bonferroni's comparison test after a significant analysis of variance.

Next, we used the forced swim test to evaluate antidepressant-like activity of the tested derivatives. The obtained results indicate that compounds **16** and **21** exhibit characteristic of antidepressant drugs. In fact, **16** given at doses of 1.25 and 2.5 mg/kg ($F(4,46) = 8.3915$, $p < 0.0001$), and **21** at a dose of 0.625 mg/kg ($F(3,39) = 3.7577$, $p < 0.05$) produced distinct antidepressant-like effects in that test, significantly reducing by 36%, 32% and 23% the immobility time of animals (Figure 7).

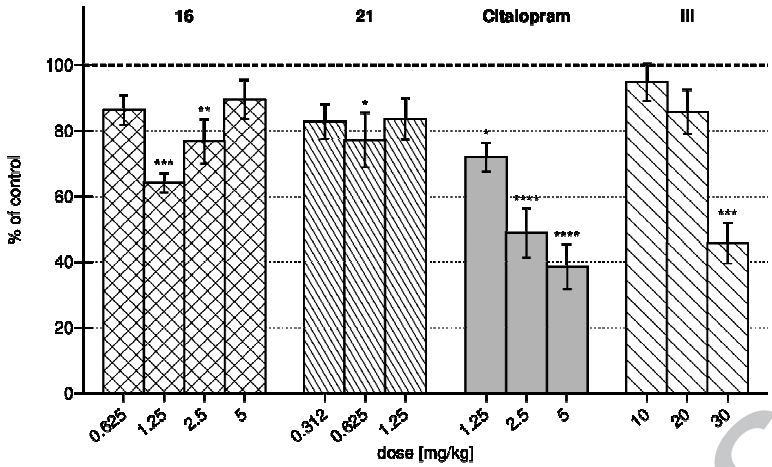



**Figure 7.** Effects of the compounds **16**, **21**, citalopram and compound **III** on the immobility time of mice in the forced swim test. Data are presented as the mean ± standard error of the mean of N = 7–12 animals per group.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the respective vehicle group, followed by the Bonferroni's comparison test after a significant analysis of variance.

Importantly, all of the tested compounds displayed their antidepressant-like activity at doses 12–48 times lower than the active dose of the reference compound **III**. It is worth to note that their potency was similar to that of the clinically-used antidepressant citalopram, that administered at dose of 1.25 mg/kg shortened the immobility time of mice by 28% ($F(3, 28) = 19.238$, $p < 0.0001$). Moreover, the antidepressant-like effects produced by all studied compounds seem to be specific, since the effective doses of **16**, **21** as well as citalopram had no influence on the spontaneous locomotor activity of mice during initial 2–6 min session (i.e. at the time identical to the observation period in the forced swim test) (data not shown).

It thus seems, that starting from compound **III**, classified as mixed $D_2$/5-$HT_{2A}$/5-$HT_7$ antagonist, which showed antipsychotic-like properties in MK-801-induced hyperlocomotor activity (MED = 5 mg/kg, *i.p*), and antidepressant-like activity in FST (MED = 30 mg/kg, *i.p.*), structural modification in halogen substitution pattern led to compound **21** ($D_2$/5-$HT_{1A}$/5-$HT_7$ antagonist), which apart of its antipsychotic properties (MED = 10 mg/kg, *i.p.*) produced an antidepressant-like activity at lower doses in FST (MED = 0.625 mg/kg).

## 7. Conclusions

A small series of 15 azinesulfonamides of phenylpiperazine derivatives, based on compound **III** with semi-rigid alkylene spacer, was synthesized and evaluated as multimodal dopamine/serotonin receptor ligands. Structure-activity relationship studies supported with molecular modeling approach revealed that the affinity of evaluated compounds for $D_2$, 5-$HT_{1A}$ and 5-$HT_7$ receptors was highly dependent upon formation of halogen interactions. The study allowed to identify compound **21** which behaved as mixed $D_2$/5-$HT_{1A}$/5-$HT_7$ receptor antagonist. Preliminary pharmacological *in vivo* evaluation showed that compound **21** was active in MK-801-evoked hyperactivity test in mice, and produced antidepressant-like activity in a mouse model of depression. Further studies in the area of CNS agents with multiple mode of action might confirm their broad-based efficacy in the treatment of comorbid symptoms of schizophrenia/depression/anxiety.

## 8. Experimental

### 8.1. 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, $^{13}$C NMR and $^{19}$F NMR spectra were obtained in Varian BB 200 spectrometer using TMS (0.00 ppm) as an internal standard in $CDCl_3$ or DMSO-$d_6$ and were recorded at 300, 75 and 282 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), td (triplet of doublets), ddd (doublet of doublet of doublets), m (multiplet).

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.

### 8.1.1. General procedure for the synthesis of intermediates 2–9

Boc-piperidineethanol **1** (23 mmol) was dissolved in pyridine (50 mL) and cooled down under nitrogen. Then a tosyl chloride (27 mmol) was added portion-wise. The reaction was carried out for 3 h on bath-ice, then warm to room temperature and stirred for additional 10 h. The reaction mixture was next extracted with $CH_2Cl_2$ (150 mL) and the organic phase was washed with 1M $KHSO_4$ (4 x 50 mL), water, brine, dried over $Na_2SO_4$, filtered and evaporated under pressure. The obtained tosyl derivative were subsequently used without any further purification. Next, to a solution of the differently substituted 4-phenylpiperazine (2 mmol) and TEA (20 mmol,) in a mixture of THF/toluene (5 mL/10 mL), tosyl derivatives (2.5 mmol,) was added and the reaction was stirred under reflux for 20 h. After solvent evaporation the crude product was purified on silica gel column chromatography ($CH_2Cl_2$/MeOH) to yield Boc-protected piperidines **2–9**.

### 8.1.2. Characterization data for intermediates 2–9

#### 8.1.2.1. *Tert*-butyl-4-[2-(4-phenylpiperazin-1-yl)ethyl]piperidine-1-carboxylate (2)

White solid, 450 mg (yield 58%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 98%, $t_R$ = 4.86, $C_{22}H_{35}N_3O_2$, MW 373.53, Monoisotopic Mass 373.27, $[M+H]^+$ 374.4. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.06–1.21 (m, 2H), 1.45 (s, 9H), 1.46–1.55–1.59 (m, 3H), 1.67–1.72 (m, 2H), 2.39–2.46 (m, 2H), 2.56–2.62 (m, 4H), 2.69 (t, *J* = 12.0 Hz, 2H), 3.17–3.24 (m, 4H), 4.00–4.10 (m, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 7.22–7.30 (m, 2H). Mp for $C_{22}H_{34}ClN_3O_2$: 148.3–149.8°C.

**8.1.2.2. *Tert*-butyl-4-{2-[4-(4-chlorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (3)**

White solid, 420 mg (yield 52%) following cchromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 98%, $t_R = 5.49$, $C_{22}H_{34}ClN_3O_2$, MW 407.98, Monoisotopic Mass 407.23, $[M+H]^+$ 408.6. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.12–1.18 (m, 2H), 1.20–1.32 (m, 3H), 1.45 (s, 9H), 1.64–1.68 (m, 2H), 2.41–2.45 (m, 2H), 2.60–2.72 (m, 6H), 3.15–3.18 (m, 4H), 4.05–4.23 (m, 2H), 6.81–6.85 (m, 2H), 7.18–7.26 (m, 2H). Mp for $C_{22}H_{34}ClN_3O_2$: 155.2–156.8°C.

**7.1.2.3. *Tert*-butyl-4-{2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (4)**

White solid, 440 mg (yield 57%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 100%, $t_R = 4.78$, $C_{22}H_{34}FN_3O_2$, MW 391.52, Monoisotopic Mass 391.26, $[M+H]^+$ 392.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.12–1.20 (m, 2H), 1.37–1.50 (m, 12H), 1.64–1.69 (m, 2H), 2.45 (t, $J = 7.44$ Hz, 2H), 2.58–2.72 (m, 6H), 3.13 (t, $J = 4.88$ Hz, 4H), 4.05–4.08 (m, 2H), 6.84–6.88 (m, 2H), 6.92–6.98 (m, 2H). Mp for $C_{22}H_{34}FN_3O_2$: 179.1–180.2°C.

**7.1.2.4. *Tert*-butyl-4-{2-[4-(3-chlorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (5)**

Yellow oil, 420 mg (yield 52%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 98%, $t_R = 5.17$, $C_{22}H_{34}ClN_3O_2$, MW 407.98, Monoisotopic Mass 407.23, $[M+H]^+$ 408.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.10–1.25 (m, 2H), 1.45–1.49 (m, 12H), 1.64–1.69 (m, 2H), 2.40–2.44(m, 2H), 2.58–2.61 (m, 4H), 2.63–2.72 (m, 2H), 3.23–3.26 (m, 4H), 4.05–4.08 (m, 2H), 7.04–7.10 (m, 2H), 7.31–7.35 (m, 2H). Mp for $C_{22}H_{34}ClN_3O_2 \cdot HCl$: 187.2–189.8°C.

**7.1.2.5. *Tert*-butyl-4-{2-[4-(3-fluorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (6)**

White solid, 480 mg (yield 59%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R = 4.63$, $C_{22}H_{34}FN_3O_2$, MW 391.52, Monoisotopic Mass 391.26, $[M+H]^+$ 392.2. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.07–1.21 (m, 2H), 1.46 (s, 9H), 1.47–

1.52 (m, 2H), 1.63–1.72 (m, 3H), 2.38–2.45 (m, 2H), 2.55–2.60 (m, 4H), 2.69 (t, $J$ = 12.0 Hz, 2H), 3.17–3.23 (m, 4H), 4.03–4.12 (m, 2H), 6.48–6.52 (m, 1H), 6.55–6.62 (m, 1H), 6.67 (dd, $J$ = 8.2, 2.3 Hz, 1H), 7.14–7.22 (m, 1H). Mp for $C_{22}H_{34}FN_3O_2$: 182.4–183.2°C.

#### 7.1.2.6. *Tert*-butyl-4-(2-{4-[3-(trifluoro)phenyl]piperazin-1-yl}ethyl)piperidine-1-carboxylate (7)

White solid, 430 mg (yield 52%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.3); UPLC/MS purity 99%, $t_R$ = 5.49, $C_{22}H_{34}F_3N_3O_2$, MW 441.53, Monoisotopic Mass 441.26, $[M+H]^+$ 442.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.08–1.25 (m, 2H), 1.45–1.48 (m, 12H), 1.65–1.69 (m, 2H), 2.43 (t, $J$ = 7.4 Hz, 2H), 2.58–2.61 (m, 4H), 2.68 (t, $J$ = 12.0 Hz, 2H), 3.24 (t, $J$ = 4.8 Hz, 4H), 4.05–4.07 (m, 2H), 7.04–7.10 (m, 3H), 7.35 (t, $J$ = 7.9 Hz, 1H). Mp for $C_{22}H_{34}F_3N_3O_2$: 178.2–179.8°C.

#### 7.1.2.7. *Tert*-butyl-4-{2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (8)

White solid, 500 mg (yield 59%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R$ = 5.78, $C_{22}H_{33}Cl_2N_3O_2$, MW 442.42, Monoisotopic Mass 441.19, $[M+H]^+$ 442.2. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.12–1.20 (m, 2H), 1.45–1.50 (m, 12H), 1.65–1.69 (m, 2H), 2.44 (t, $J$ = 7.4 Hz, 2H), 2.63–2.73 (m, 6H), 3.07–3.11 (m, 4H), 4.03–4.07 (m, 2H), 6.92–6.98 (m, 1H), 7.10–7.17 (m, 2H). Mp for $C_{22}H_{33}Cl_2N_3O_2$: 202.5–204.3°C.

#### 7.1.2.8. *Tert*-butyl-4-{2-[4-(3,4-dichlorophenyl)piperazin-1-yl]ethyl}piperidine-1-carboxylate (9)

White solid, 480 mg (yield 57%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.3); UPLC/MS purity 100%, $t_R$ = 5.67, $C_{22}H_{33}Cl_2N_3O_2$, MW 442.42,

Monoisotopic Mass 441.19, $[M+H]^+$ 442.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.12–1.18 (m, 2H), 1.45–1.49 (m, 12H), 1.64–1.68 (m, 2H), 2.42 (t, *J* = 7.69 Hz, 2H), 2.55–2.58 (m, 4H), 2.68 (t, *J* = 12.5 Hz, 2H), 3.15 (t, *J* = 5.1 Hz, 4H), 4.02–4.06 (m, 2H), 6.73 (dd, *J* = 8.9, 2.8 Hz, 1H), 6.94 (d, *J* = 2.8 Hz, 1H), 7.24–7.28 (m, 1H). Mp for $C_{22}H_{33}Cl_2N_3O_2$: 205.5–206.9°C.

### 7.1.3. General procedure for the synthesis of final compounds 10–24

In the next stage intermediates **2–9** were converted into their TFA salts by treatment with a mixture of TFA/$CH_2Cl_2$ (4 mL /1 mL). The excess of reagent and solvent were removed under reduced pressure and left under vacuum overnight. Then, a mixture of the proper secondary amine (0.76 mmol) in $CH_2Cl_2$ (8 mL), and TEA (3.04 mmol) was cooled down (ice bath), and the selected azinesulfonyl chloride (0.91 mmol) was added in one portion. The reaction mixture was stirred for 2-6 hours under cooling. Then, the solvent was evaporated and the sulfonamides were purified by column chromatography using $CH_2Cl_2$/MeOH as eluting system. Final compounds were obtained as hydrochloride salts by treatment with a solution of 1.25 M HCl in MeOH.

### 7.1.4. Characterization data for final compounds 10–24

#### 7.1.4.1. 5-({4-[2-(4-phenylpiperazin-1-yl)ethyl]piperidin-1-yl}sulfonyl)quinoline (10)

White solid, 300 mg (yield 87%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 100%, $t_R$ = 4.19, $C_{26}H_{32}N_4O_2S$, MW 464.62, Monoisotopic Mass 464.22, $[M+H]^+$ 465.1. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.26–1.47 (m, 5H), 1.74 (d, *J* = 10.6 Hz, 2H), 2.30–2.37 (m, 2H), 2.51–2.55 (m, 4H), 3.12–3.19 (m, 4H), 3.85 (t, *J* = 11.7 Hz, 2H), 6.81–6.93 (m, 3H), 7.21–7.28 (m, 2H), 7.55 (q, *J* = 2.8 Hz, 1H), 7.77–7.82 (m, 1H), 8.22 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.32 (dd, *J* = 8.46, 1.2 Hz, 1H), 9.00 (dd, *J* = 4.1, 1.5 Hz, 1H), 9.13 (ddd, *J* = 8.7, 1.0, 0.7 Hz, 1H). Mp for $C_{26}H_{32}N_4O_2S{\cdot}2HCl$: 186.4–188.0°C.

#### 7.1.4.1. 4-({4-[2-(4-phenylpiperazin-1-yl)ethyl]piperidin-1-yl}sulfonyl)isoquinoline (11)

White solid, 280 mg (yield 84%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 100%, $t_R$ = 4.48, $C_{26}H_{32}N_4O_2S$, MW 464.62,

Monoisotopic Mass 464.22, $[M+H]^+$ 465.5. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.28–1.48 (m, 5H), 1.75 (d, $J$ = 9.9 Hz, 2H), 2.31–2.38 (m, 2H), 2.51–2.55 (m, 4H), 2.56–2.62 (m, 2H), 3.12–3.18 (m, 4H), 3.90 (d, $J$ = 12.3 Hz, 2H), 6.81–6.92 (m, 3H), 7.21–7.28 (m, 2H), 7.71–7.78 (m, 1H), 7.84–7.91 (m, 1H), 8.09 (d, $J$ = 7.6 Hz, 1H), 8.72 (d, $J$ = 8.8 Hz, 1H), 9.08 (s, 1H), 9.40 (s, 1H). Mp for $C_{26}H_{32}N_4O_2S$·2HCl: 187.0–188.1°C.

#### 7.1.4.2. 5-({4-(2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (12)

White solid, 310 mg (yield 88%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 100%, $t_R$ = 4.77, $C_{26}H_{31}ClN_4O_2S$, MW 499.07, Monoisotopic Mass 498.19, $[M+H]^+$ 499.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.25–1.30 (m, 3H), 1.39–1.45 (m, 2H), 1.71–1.75 (m, 2H), 2.33 (t, $J$ = 7.4 Hz, 2H), 2.46–2.53 (m, 6H), 3.11 (t, $J$ = 4.8 Hz, 4H), 3.81–3.85 (m, 2H), 6.78–6.83 (m, 2H), 7.15–7.20 (m, 2H), 7.55 (q, $J$ = 2.8 Hz, 1H), 7.77–7.82 (m, 1H), 8.22 (dd, $J$ = 7.4, 1.2 Hz, 1H), 8.32 (dd, $J$ = 8.46, 1.2 Hz, 1H), 9.00 (dd, $J$ = 4.1, 1.5 Hz, 1H), 9.13 (ddd, $J$ = 8.7, 1.0, 0.7 Hz, 1H). Mp for $C_{26}H_{31}ClN_4O_2S$·2HCl: 197.1–199.2°C.

#### 7.1.4.3. 5-({4-(2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (13)

White solid, 270 mg (yield 77%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/1); UPLC/MS purity 100%, $t_R$ = 4.38, $C_{26}H_{31}FN_4O_2S$, MW 482.61, Monoisotopic Mass 482.22, $[M+H]^+$ 483.4. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.25–1.30 (m, 3H), 1.42–1.52 (m, 2H), 1.72–1.75 (m, 2H), 2.35 (t, $J$ = 7.7 Hz, 2H), 2.42–2.53 (m, 6H), 3.09 (t, $J$ = 4.6 Hz, 4H), 3.83 (d, $J$ = 12.3 Hz, 2H), 6.84–6.87 (m, 2H), 6.92–6.97 (m, 2H), 7.55 (q, $J$ = 4.3 Hz, 1H), 7.77–7.82 (m, 1H), 8.23 (dd, $J$ = 7.4, 1.2 Hz, 1H), 8.35 (dd, $J$ = 8.4, 1.2 Hz, 1H), 9.01 (dd, $J$ = 4.3, 2.2 Hz, 1H), 9.12 (dd, $J$ = 8.7, 2.2 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 29.2, 31.3, 32.4, 45.7, 46.5, 50.8, 53.4, 115.4, 115.8, 116.1, 118.4 (d, $J$ (C–F) = 8.3 Hz), 124.2, 124.8, 131.0, 131.4, 131.8, 134.0, 138.7, 143.3, 146.6, 148.8, 155.5. $^{19}$F NMR (282 MHz, DMSO-$d_6$) δ -123.9. Anal. calcd for

$C_{26}H_{31}FN_4O_2S·2HCl$: C: 56.21, H: 5.99, N: 10.09, S: 5.77; Found C: 56.23, H: 6.23, N: 9.85, S: 5.80. Mp for $C_{26}H_{31}FN_4O_2S·2HCl$: 187.6–189.8°C.

#### 7.1.4.4. 4-({4-(2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)isoquinoline (14)

White solid, 310 mg (yield 88%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 98%, $t_R = 4.62$, $C_{26}H_{31}FN_4O_2S$, MW 482.61, Monoisotopic Mass 482.22, $[M+H]^+$ 483.4. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.21–1.39 (m, 3H), 1.42–1.47 (m, 2H), 1.66–1.76 (m, 2H), 2.32–2.37 (m, 2H), 2.32–2.60 (m, 6H), 3.06–3.10 (m, 4H), 3.90 (d, $J = 12.3$ Hz, 2H), 6.82–6.88 (m, 2H), 6.90–6.99 (m, 2H), 7.74 (td, $J = 6.9$, 1.2 Hz, 1H), 7.88 (td, $J = 7.4$, 1.5 Hz, 1H), 8.08 (dd, $J = 8.2$, 0.7 Hz, 1H), 8.71 (dd, $J = 8.4$, 0.7 Hz, 1H), 9.07 (s, 1H), 9.40 (s, 1H). Mp for $C_{26}H_{31}FN_4O_2S·2HCl$: 189.5–191.6°C.

#### 7.1.4.5. 5-({4-(2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (15)

White solid, 230 mg (yield 65%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.3); UPLC/MS purity 98%, $t_R = 5.27$, $C_{26}H_{31}ClN_4O_2S$, MW 499.07, Monoisotopic Mass 498.19, $[M+H]^+$ 499.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.25–1.38 (m, 3H), 1.43–1.46 (m, 2H), 1.72–1.76 (m, 2H), 2.41 (t, $J = 7.1$ Hz, 2H), 2.54–2.68 (m, 4H), 2.74 (t, $J = 12.0$ Hz, 2H), 3.17 (t, $J = 4.6$ Hz, 4H), 3.96–4.00 (m, 2H), 6.65–6.79 (m, 1H), 6.82 (t, $J = 2.0$ Hz, 1H), 7.17 (t, $J = 8.2$ Hz, 2H), 7.54 (q, $J = 8.7$ Hz, 1H), 7.77–7.83 (m, 1H), 8.23 (dd, $J = 7.4$, 1.2 Hz, 1H), 8.34 (dd, $J = 8.4$, 1.2 Hz, 1H), 9.01 (dd, $J = 4.1$, 1.5 Hz, 1H), 9.13 (dd, $J = 8.9$, 1.2 Hz, 1H). Mp for $C_{26}H_{31}ClN_4O_2S·2HCl$: 167.9–170.1°C.

#### 7.1.4.6. 4-({4-(2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)isoquinoline (16)

White solid, 210 mg (yield 60%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R = 5.38$, $C_{26}H_{31}ClN_4O_2S$, MW 499.07, Monoisotopic Mass 498.19, $[M+H]^+$ 499.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.23–1.30 (m, 3H), 1.42–1.45 (m, 2H), 1.73–1.76 (m, 2H), 2.35 (t, $J = 7.1$ Hz, 2H), 2.50–2.59 (m, 6H), 3.16 (t, $J = 5.1$ Hz, 4H), 3.91 (d, $J = 12.3$ Hz, 2H), 6.73–6.80 (m, 2H), 6.84 (t, $J = 2.3$ Hz, 1H), 7.18 (t, $J = 8.2$ Hz, 1H), 7.77 (td, $J = 8.2$, 1.0 Hz, 1H), 7.88 (td, $J = 8.4$, 1.5 Hz, 1H), 8.09 (d, $J = 8.2$ Hz, 1H), 8.72 (dd, $J = 7.6$, 1.0 Hz, 1H), 9.07 (s, 1H), 9.41 (s, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ 29.4, 31.3, 32.7, 45.2, 46.7, 50.6, 53.5, 114.6, 115.7, 119.6, 121.7, 128.4, 128.5, 129.4, 130.7, 131.1, 132.6, 134.4, 135.4, 149.4, 151.2, 153.9. Anal. calcd for $C_{26}H_{31}ClN_4O_2S \cdot 2HCl \cdot H_2O$:C: 52.93, H: 5.98, N: 9.50, S: 5.43; Found C: 52.57, H: 5.93, N: 9.36, S: 5.36. Mp for $C_{26}H_{31}ClN_4O_2S \cdot 2HCl \cdot H_2O$: 187.8–190.2°C.

#### 7.1.4.7. 5-({4-(2-[4-(3-Fluorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (17)

White solid, 350 mg (yield 89%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R = 4.25$, $C_{26}H_{31}FN_4O_2S$, MW 482.61, Monoisotopic Mass 482.22, $[M+H]^+$ 483.2. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.22–1.34 (m, 3H), 1.38– 1.47 (m, 2H), 1.73 (d, $J = 9.9$ Hz, 2H), 2.29–2.37 (m, 2H), 2.46–2.57 (m, 6H), 3.11–3.19 (m, 4H), 3.85 (d, $J = 12.3$ Hz, 2H), 6.47–6.58 (m, 2H), 6.64 (dd, $J = 8.5$, 2.1 Hz, 1H), 7.12–7.21 (m, 1H), 7.54 (q, $J = 8.7$ Hz, 1H), 7.77–7.83 (m, 1H), 8.23 (dd, $J = 7.4$, 1.2 Hz, 1H), 8.34 (dd, $J = 8.4$, 1.2 Hz, 1H), 9.01 (dd, $J = 4.1$, 1.5 Hz, 1H), 9.13 (dd, $J = 8.9$, 1.2 Hz, 1H). Mp for $C_{26}H_{31}FN_4O_2S \cdot 2HCl$: 188.5–190.3°C.

#### 7.1.4.8. 4-({4-(2-[4-(3-Fluorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)isoquinoline (18)

White solid, 300 mg (yield 86%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R = 4.72$, $C_{26}H_{31}FN_4O_2S$, MW 482.61, Monoisotopic Mass 482.22, $[M+H]^+$ 483.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.27–1.34 (m, 3H), 1.39–1.47 (m, 2H), 1.70–1.80 (m, 2H), 2.30–2.37 (m, 2H), 2.49–2.54 (m, 4H), 2.54–2.61 (m, 2H), 3.13–3.18 (m, 4H), 3.86–3.95 (m, 2H), 6.47–6.59 (m, 2H), 6.64 (dd, $J = 7.9$, 2.1 Hz, 1H), 7.17 (td, $J = 8.2$, 7.0 Hz, 1H), 7.75 (ddd, $J = 8.2$, 7.0, 1.2 Hz, 1H), 7.88 (ddd, $J = 8.5$, 7.0, 1.5 Hz, 1H), 8.09 (d, $J = 8.2$ Hz, 1H), 8.69–8.74 (m, 1H), 9.07 (s, 1H), 9.41 (s, 1H). Mp for $C_{26}H_{31}FN_4O_2S \cdot 2HCl$: 188.2–190.0°C.

**7.1.4.9. 5-({4-(2-(4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl)ethyl)piperidin-1-yl}sulfonyl) quinoline (19)**

White solid, 230 mg (yield 65%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 100%, $t_R = 5.11$, $C_{27}H_{31}F_3N_4O_2S$, MW 532.62, Monoisotopic Mass 532.21, $[M+H]^+$ 533.3. $^1H$ NMR (300 MHz, $CDCl_3$) δ 1.25–1.31 (m, 3H), 1.46–1.49 (m, 2H), 1.72–1.75 (m, 2H), 2.30–2.36 (m, 2H), 2.38–2.54 (m, 6H), 3.22–3.26 (m, 4H), 3.81–3.86 (m, 2H), 7.02–7.05 (m, 3H), 7.33 (t, $J = 7.4$ Hz, 1H), 7.54 (q, $J = 8.7$ Hz, 1H), 7.77–7.83 (m, 1H), 8.23 (dd, $J = 7.4$, 1.2 Hz, 1H), 8.34 (dd, $J = 8.4$, 1.2 Hz, 1H), 9.01 (dd, $J = 4.1$, 1.5 Hz, 1H), 9.13 (dd, $J = 8.9$, 1.2 Hz, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ 29.4, 31.3, 32.4, 45.1, 45.7, 50.5, 53.8, 116.3, 117.6, 121.2, 124.5, 128.6, 129.2, 129.7, 130.1, 131.2, 131.6, 132.1, 134.3, 144.0, 149.7, 158.1. Anal. calcd for $C_{27}H_{31}F_3N_4O_2S \cdot 2HCl \cdot H_2O$: C: 50.55, H: 5.81, N: 8.73, S: 5.00; Found C: 50.90, H: 5.78, N: 8.79, S: 5.28. Mp for $C_{27}H_{31}F_3N_4O_2S$ $2HCl \cdot H_2O$: 179.4–181.2°C.

**7.1.4.10.4-({4-(2-(4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl)ethyl)piperidin-1-yl}sulfonyl) isoquinoline (20)**

White solid, 290 mg (yield 82%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 98%, $t_R = 5.42$, $C_{27}H_{31}F_3N_4O_2S$, MW 532.62,

Monoisotopic Mass 532.21, $[M+H]^+$ 533.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.23–1.30 (m, 3H), 1.42–1.44 (m, 2H), 1.72–1.76 (m, 2H), 2.30–2.36 (m, 2H), 2.49–2.60 (m, 6H), 3.11–3.14 (m, 4H), 3.88–3.93(m, 2H), 6.69 (dd, *J* = 8.9, 3.0 Hz, 2H), 6.91 (d, *J* = 2.8 Hz, 1H), 7.23–7.27 (m, 1H), 7.74 (td, *J* = 6.9, 1.0 Hz, 1H), 7.88 (td, *J* = 6.9, 1.2 Hz, 1H), 8.09 (dd, *J* = 8.4, 0.7 Hz, 1H), 8.71 (dd, *J* = 8.7, 0.7 Hz, 1H), 9.07 (s, 1H), 9.41 (s, 1H). Mp for $C_{27}H_{31}F_3N_4O_2S \cdot 2HCl$: 174.8–176.8°C.

**7.1.4.11.5-({4-(2-[4-(2,3-Dichlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (21)**

White solid, 310 mg (yield 88%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.7); UPLC/MS purity 100%, $t_R$ = 5.23, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, Monoisotopic Mass 532.15, $[M+H]^+$ 533.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.23–1.30 (m, 3H), 1.43–1.49 (m, 2H), 1.72–1.75 (m, 2H), 2.37–2.41 (m, 2H), 2.50–2.57 (m, 6H), 3.03–3.10 (m, 4H), 3.81–3.85 (m, 2H), 6.91–6.94 (dd, *J* = 6.6, 2.8 Hz, 1H), 7.10–7.25 (m, 2H), 7.55 (q, *J* = 4.1 Hz, 1H), 7.77–7.83 (m, 1H), 8.24 (dd, *J* = 7.1, 1.2 Hz, 1H), 8.33 (dd, *J* = 8.4, 1.2 Hz, 1H), 9.01 (dd, *J* = 4.3, 1.8 Hz, 1H), 9.15 (dd, *J* = 8.7, 1.5 Hz, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 31.7, 33.0, 33.6, 45.9, 51.2, 53.3, 55.8, 118.5, 122.5, 124.6, 124.8, 127.4, 127.6, 130.4, 133.4, 133.6, 134.0, 135.6, 148.6, 151.1, 151.2. Anal. calcd for $C_{26}H_{30}Cl_2N_4O_2S \cdot 2HCl \cdot 2H_2O$: C: 48.61, H: 5.65, N: 8.72, S: 4.99; Found C: 48.85, H: 5.59, N: 8.42, S: 5.24.Mp for $C_{26}H_{30}Cl_2N_4O_2S \cdot 2HCl \cdot 2H_2O$: 173.9–175.2°C.

**7.1.4.12.4-({4-(2-[4-(2,3-Dichlorophenyl)iperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl) isoquinoline (22)**

White solid, 310 mg (yield 88%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 100%, $t_R$ = 5.44, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, Monoisotopic Mass 532.15, $[M+H]^+$ 533.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.23–1.26 (m, 3H), 1.40–1.45 (m, 2H), 1.73–1.77 (m, 2H), 2.35–2.40 (m, 2H), 2.53–2.57 (m, 6H), 3.03–3.10 (m, 4H),

3.89–3.93 (m, 2H), 6.91–6.96 (dd, $J$ = 6.6, 3.0 Hz, 2H), 7.12–7.24 (m, 2H), 7.73 (td, $J$ = 8.2, 1.2 Hz, 1H), 7.87 (td, $J$ = 8.4, 1.5 Hz, 1H), 8.08 (dd, $J$ = 8.2, 1.0 Hz, 1H), 8.72 (dd, $J$ = 8.7, 1.0 Hz, 1H), 9.07 (s, 1H), 9.40 (s, 1H). Mp for $C_{26}H_{30}Cl_2N_4O_2S{\cdot}2HCl$: 198.9–201.9°C.

**7.1.4.13.5-({4-(2-[4-(3,4-Dichlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl)quinoline (23)**

White solid, 220 mg (yield 63%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); UPLC/MS purity 99%, $t_R$ = 5.23, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, Monoisotopic Mass 532.15, $[M+H]^+$ 533.3. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 1.23–1.30 (m, 3H), 1.41–1.47 (m, 2H), 1.71–1.75 (m, 2H), 2.31–2.36 (m, 2H), 2.46–2.50 (m, 6H), 3.13–3.19 (m, 4H), 3.81–3.85 (m, 2H), 6.70 (dd, $J$ = 8.9, 2.8 Hz, 1H), 6.92 (d, $J$ = 2.8 Hz, 1H), 7.23–7.26 (m, 1H), 7.55 (q, $J$ = 4.1 Hz 1H), 7.77–7.82 (m, 1H), 8.22 (dd, $J$ = 8.4, 1.2 Hz, 1H), 9.34 (dd, $J$ = 8.4, 1.2 Hz, 1H), 8.99 (dd, $J$ = 4.1, 1.54 Hz, 1H), 9.13 (dd, $J$ = 8.9, 1.0 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 29.2, 31.3, 32.4, 45.1, 45.7, 50.4, 53.5, 116.2, 117.4, 121.2, 124.0, 124.7, 130.7, 131.1, 131.5, 1321.1, 132.3, 133.8, 137.3, 144.8, 149.7, 149.7. Anal. calcd for $C_{26}H_{30}Cl_2N_4O_2S{\cdot}2HCl{\cdot}2H_2O$: C: 48.61, H: 5.65, N: 8.72, S: 4.99; Found C: 48.92, H: 5.61, N: 8.37, S: 4.89. Mp for $C_{26}H_{30}Cl_2N_4O_2S{\cdot}2HCl{\cdot}2H_2O$: 172.5–174.1°C.

**7.1.4.14.4-({4-(2-[4-(3,4-Dichlorophenyl)piperazin-1-yl]ethyl)piperidin-1-yl}sulfonyl) isoquinoline (24)**

White solid, 270 mg (yield 77%) following chromatographic purification over silica gel with $CH_2Cl_2$/MeOH (9/0.5); LC/MS purity 98%, $t_R$ = 5.43, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, Monoisotopic Mass 532.15, $[M+H]^+$ 533.3. $^1$H NMR (300 MHz, $CDCl_3$) δ 1.23–1.30 (m, 3H), 1.40–1.44 (m, 2H), 1.72–1.76 (m, 2H), 2.34 (t, $J$ = 7.1 Hz, 2H), 2.49–2.59 (m, 6H), 3.12 (t, $J$ = 5.1 Hz, 4H), 3.88–3.92(m, 2H), 6.70 (dd, $J$ = 8.9, 2.8 Hz, 1H), 6.91 (d, $J$ = 2.8 Hz, 1H), 7.73 (s, 1H), 7.74 (td, $J$ = 8.2,

1.02 Hz, 1H), 7.89 (td, *J* = 8.4, 1.2 Hz, 1H), 8.09 (dd, *J* = 8.2 ,1.2 Hz, 1H), 8.71 (dd, *J* = 8.4, 0.7 Hz, 1H), 9.07 (s, 1H), 9.40 (s, 1H). Mp for $C_{26}H_{30}Cl_2N_4O_2S$ 2HCl: 163.3–165.4°C.

### 7.2. Molecular modeling

The 3-dimensional structures of the ligands were prepared using LigPrep v3.6,[31] and the appropriate ionization states at pH = 7.4 were assigned using Epik v3.4.[32] The Protein Preparation Wizard was used to assign the bond orders, appropriate amino acid ionization states and to check for steric clashes. The receptor grid was generated (OPLS_2005 force field) by centering the grid box with a size of 12 Å on Asp3.32. Docking was performed by procedure involves quantum-polarized ligand docking (QPLD) to obtain the QM-derived ligand atomic charges in the protein environment at the B3PW91/cc-pVTZ level and the MM/GBSA algorithm to calculate the binding free energies, which performance was recently evaluated for docking of halogenated ligands.[18]

### 7.3. *In vitro* pharmacology

#### 7.3.1. Radioligand binding assay protocol

Radioligand binding assays were employed to determine the affinity and selectivity profiles of the synthesized compounds in competition binding experiments for human serotonin, 5-$HT_{7b}$, 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and $D_{2L}$ receptors, which were all stably expressed in HEK293 cells. According to the previously published procedures, the experiments were carried out using [$^3$H]-5-CT (39.2 Ci/mmol), [$^3$H]-8-OH-DPAT (187 Ci/mmol), [$^3$H]-Ketanserin (66.9 Ci/mmol), [$^3$H]-LSD (85.2 Ci/mmol) and [$^3$H]-Raclopride (74.4 Ci/mmol) for 5-$HT_7$, 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ and $D_2$ receptors, respectively. 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-$HT_6$R: 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 µl in 96-well microtitre plates for 1 h at 37°C, except for 5-$HT_{1A}$R that was incubated for 1 h at room temperature. 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. Non-specific binding is defined with10 µM of 5-HT in 5-$HT_{1A}$R and 5-$HT_7$R binding experiments, whereas10 µM of methiothepine or 1 µM of (+)butaclamol were used in 5-$HT_6$R 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. Results were expressed as means of at least three separate experiments. Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on described protocols (Perkin Elmer). Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on described protocols.

#### 7.3.2 Functional cAMP assay protocol

The functional properties of the selected compounds were evaluated in functional cAMP cellular assays, performed at Eurofins Cerep using CHO cells which stably the human 5-$HT_7$Rs and HEK293 cells which stably expressed the human 5-$HT_{1A}$ and $D_2$Rs. Experimental conditions for these assays are described online at www.cerep.fr.

### 7.4. *In vivo* pharmacology

#### 7.4.1. Subject

The experiments were performed on male Swiss albino mice (22–26 g) purchased from a licensed breeder Staniszewska (Ilkowice, Poland) or male CD-1 mice from accredited animal facility Jagiellonian University Medical College Kraków, Poland. Mice were kept in groups of ten to Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm). The animals were kept in an environmentally controlled rooms (ambient temperature 22 ± 2°C; relative humidity 50–60%; 12:12

light:dark cycle, lights on at 8:00). They were allowed to acclimatize with the environment for one week before commencement of the experiments. Standard laboratory food (Ssniff M-Z) and filtered water were freely available. All the experimental procedures were approved by the I Local Ethics Commission at the Jagiellonian University in Krakow.

### 7.4.2. Experimental procedures

All the experiments were conducted in the light phase between 09.00 and 14.00 hours. Each experimental group consisted of 7–10 animals/dose. The animals were used only once. The experiments were performed by an observer unaware of the treatment administered.

### 7.4.3. MK-801-induced hyperlocomotor activity in CD-1 mice

The locomotor activity was recorded with an Opto M3 multi-channel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). The CD-1 mice were individually placed in plastic cages (22 × 12 × 13 cm) for 30 min habituation period, and then ambulation were counted during 1 h with data recording every 5 min. The cages were cleaned up with 70% ethanol after each mouse.

### 7.4.4. Forced swim test in Swiss albino mice

The experiment was carried out according to the method of Porsolt *et al*.[23] Swiss albino mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 10 cm of water maintained at 23–25°C, and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

### 7.4.5. Spontaneous locomotor activity protocol

The locomotor activity was recorded according to the method described above. The test was performed using the antidepressant- or antipsychotic-active doses of the tested compounds. The CD-1 mice were individually placed in plastic cages and ambulation were counted during 1 h with data recording every 5 min. The Swiss albino mice were individually placed in plastic cages and

the crossings of each channel (ambulation) were counted from 2 to 6 min, i.e. the time equal to the observation period in the forced swim test. The cages were cleaned up with 70% ethanol after each mouse.

### 7.4.6. Drugs

The following drugs were used: citalopram (hydrochloride Adamed Pharmaceuticals, Pienkow, Poland), (+)-MK-801 (hydrogen maleate, Sigma-Aldrich, UK). Citalopram, MK-801 and tested compounds were dissolved in distilled water immediately before administration. Compounds **16**, **21** and **22** was administered intraperitoneally (*i.p.*) 60 min, before the test, citalopram was injected *i.p.* 30 min, and MK-801 15 min before testing. All the compounds were given at a volume of 10 ml/kg b.w. Control animals received a vehicle injection according to the same schedule.

### 7.4.7. Statistics

All the data are presented as the mean ± S.E.M. The statistical significance of the results was evaluated by a one-way ANOVA, followed by Bonferroni's Comparison Test. $p<0.05$ were considered statistically significant.

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**Supplementary data**

Supporting Information Available: MS, $^{1}$H NMR, $^{13}$C NMR and $^{19}$F NMR spectra for representative final compounds. This material is available free of charge via the Internet at

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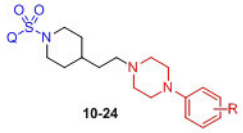
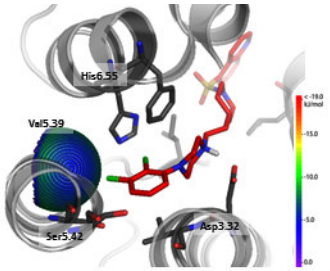
31. LigPrep version 3.6.

32. Epik version 3.4.

**Table of content**




Q = 4-isoquinolyl, 5-quinolyl
R = H, 4-Cl, 4-F, 3-Cl, 3-$CF_3$, 2,3-diCl, 3,4-diCl





$K_i$ ($D_2$) = 36 nM; $K_i$ (5-$HT_{1A}$) = 45 nM; $K_i$ (5-$HT_7$) = 55 nM;
MED (MK-801) = 10 mg/kg *i.p.*
MED (FST) = 0.625 mg/kg *i.p.*

**Library Synthesis** → **Molecular Docking** → ***In vitro* and *in vivo* pharmacological evaluation**