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Quinoline- and isoquinoline-sulfonamide derivatives of LCAP as potent CNS multi-receptor—5-HT_{1A}/5-HT_{2A}/5-HT_7 and D_2/D_3/D_4—agents: The synthesis and pharmacological evaluation

Paweł Zajdel [a,*], Krzysztof Marciniec [b], Andrzej Maślankiewicz [b], Grzegorz Satała [c], Beata Duszyńska [c], Andrzej J. Bojarski [c], Anna Partyka [d], Magdalena Jastrzębska-Więsek [d], Dagmara Wróbel [d], Anna Wesołowska [d], Maciej Pawłowski [a]

[a] Department of Medicinal Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
[b] Department of Organic Chemistry, Medical University of Silesia, 4 Jagiellońska Street, 41-200 Sosnowiec, Poland
[c] Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland
[d] Department of Clinical Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

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ABSTRACT

Two series of arylpiperazinyl–alkyl quinoline-, isoquinoline-, naphthalene-sulfonamides with flexible (**13**–**26**) and semi-rigid (**33**–**36**) alkylene spacer were synthesized and evaluated for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7 and selected compounds for D_2, D_3, D_4 receptors. The compounds with a mixed 5-HT and D receptors profile **16** (*N*-{4-[4-(3-chlorophenyl)-piperazin-1-yl]-butyl}-3-quinolinesulfonamide) and **36** (4-(4-{2-[4-(4-chloro-phenyl)-piperazin-1-yl]-ethyl}-piperidine-1-sulfonyl)-isoquinoline), displaying antagonistic activity at 5-HT_7, 5-HT_{2A}, D_2 postsynaptic sites, produced antidepressant-like effects in the forced swim test in mice and showed significant anxiolytic activity in the plus-maze test in rats. The lead compound **36**, a multi-receptor 5-HT_{2A}/5-HT_7/D_2/D_3/D_4 agent, also displayed significant antipsychotic properties in the MK-801-induced hyperlocomotor activity in mice.

1. Introduction

The emergence of depressive symptoms indicates pathology in the central nervous system (CNS), which affects the functioning of an individual in the society and significantly deteriorates the quality of life. Despite remarkable advances in the treatment of depression, a great many patients remain undertreated. Regardless of the mechanism of drug action, the inefficacy of antidepressant treatment still results from the delayed onset of action and certain side-effects, such as, emesis or sexual dysfunction.

Although the pathology of depression has not been fully understood yet, for many years the development of antidepressant drugs has been associated with alteration in norepinephrine/serotonin/dopamine systems. Among the multiple serotonin receptors that have been widely studied as a target for the development of antidepressant agents, 5-HT_{1A} and 5-HT_{2A} subtypes have a prominent position.[1]

Recently, the 5-HT_7 receptor (5-HT_7R) has emerged as a new target with a potential for the treatment of psychiatric disorders.[2] It has also been found that several antipsychotics and antidepressants display high affinity for 5-HT_7Rs.[3] Furthermore, the distribution of 5-HT_7Rs in relevant regions of the brain provides information on their significant involvement in the control of the CNS.[4] Their high density in the hypothalamus (particularly in the suprachiasmatic nucleus) has been associated with the control of circadian rhythms. Thalamic and cortical 5-HT_7Rs might be important to sleep and mood regulation; the presence of 5-HT_7Rs in the hippocampus highlights their potential involvement in learning and memory, as well as in emotional processes.[5] Engagement of 5-HT_7Rs in mood regulation seems very important regarding the co-existence of depression and anxiety disorders. In approximately 30% of patients with depression anxiety symptoms may occur, and in about 50% of stressed people depression is likely to develop.

In support of the involvement of 5-HT_7Rs in depression it is proposed that blockade of 5-HT_7Rs produces an anti-immobility effect in the forced swim test.[6,7] As was later reported by Abbas et al. and Sarkisyan et al., the antidepressant-like effect of the well-known

* Corresponding author. Tel.: +48 12 620 54 50; fax: +48 12 620 54 05.
E-mail address: mfzajdel@cyf-kr.edu.pl (P. Zajdel).

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antipsychotic drugs amisulpride and aripiprazole, respectively, was most likely mediated by their 5-HT$_7$R antagonism.[8,9] Amisulpride and aripiprazole further support the hypothesis that antipsychotic drugs not only alleviate the negative symptoms of schizophrenia, but also help conquer depression.[10]

Of the numerous, structurally diverse 5-HT$_7$ receptor ligands, several antagonists have been examined so far, for example SB-269970—a sulfonamide derivative,[11] sulfon derivatives (reported by Raubo et al.)[12] or DR-4004—a tetrahydrobenzindole analog[13] (Fig. 1).

Several 5-HT$_7$R ligands, for example SB-269970 and SB-656104, are currently used as references for in vitro and in vivo pharmacological studies, or are templates for the development of novel 5-HT$_7$ agents.[14,15] It should be stressed here that in several cases a search for selective 5-HT$_7$R agents was hampered by the low selectivity over 5-HT$_{1A}$Rs, which was due to the relatively close homology between 5-HT$_7$ and 5-HT$_{1A}$ sites.[16–18]

Recently Volk et al. have described a series of degraded tetrahydrobenzindole derivatives which are connected by different-length alkylene spacers with the halo-substituted arylpiperazine.[19] Those authors have found that, in contrast to 5-HT$_7$Rs, 5-HT$_{1A}$ sites are highly sensitive for electron-withdrawing substituents in the phenylpiperazine ring. The introduction of a chlorine or a fluorine atom into the 3- and 4-position of phenylpiperazine is beneficial to the acquisition of active 5-HT$_7$ ligands. Although these compounds display low affinity for 5-HT$_{1A}$ receptors, they are devoid of 5-HT$_{2A}$ receptor selectivity (compounds **I**, **II**, Fig. 2). Interestingly, these compounds display anxiolytic activity in such animal models as, the conflict drinking and the light–dark tests.

Our research team has recently reported on the design, synthesis and pharmacological evaluation of quinolinesulfonamides as new 5-HT$_7$R ligands. Among them, the N-alkylated quinolinesulfonamide containing a perhydroisoquinoline moiety in the amine core has been classified as a 5-HT$_7$ antagonist, and it displayed antidepressant activity in the forced swim test in mice (10 mg/kg) (Fig. 3).[20]

To carry on our research with a group of azinesulfonamide derivatives of long-chain arylpiperazines (LCAP), we designed a new series of quinoline- and isoquinoline-sulfonamides of 3- and 4-chlorophenylpiperazines containing a flexible and semi-rigid alkylene spacer. In the present paper we described their synthesis, a biological evaluation for 5-HT$_7$R and other related 5-HTRs as well as dopamine receptors and determined their therapeutic potential in the animal models of depression, anxiety and schizophrenia.

2. Chemistry

The synthesis of the investigated compounds is presented in Schemes 1 and 2. The starting quinoline- and isoquinoline-sulfonyl

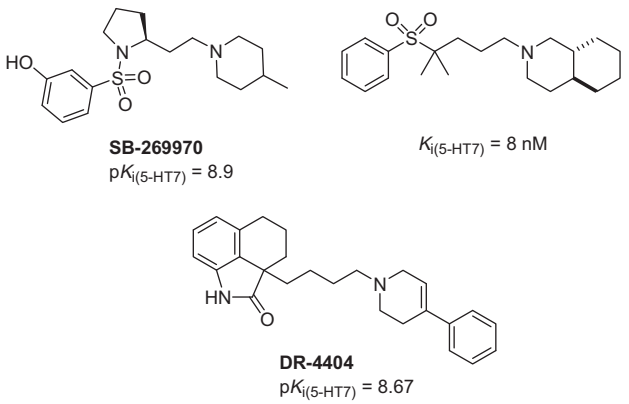


Figure 1. The structure of model 5-HT$_7$R ligands.

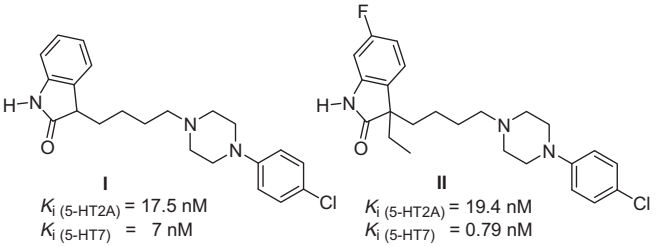
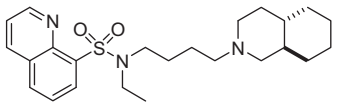


Figure 2. Representatives of the oxindole series of 5-HT$_7$R ligands.



PZ-376
$K_{i\,(5\text{-HT1A})}$ = 1099 nM $K_{i\,(5\text{-HT7})}$ = 13 nM

Figure 3. The structure of PZ-376 a 5-HT$_7$R antagonist.

chlorides were prepared from the corresponding haloquinolines or 4-bromoisoquinoline via their *S*-methyl analogs obtained in the reaction with an excess of sodium methanethiolate in the boiling DMF. *S*-Demethylation led to the respective azinethiolates which submitted to oxidative chlorination yielded the desired quinoline- and isoquinoline-sulfonyl chlorides.[21] The coupling of primary amines with azinesulfonyl chlorides or commercially available 1- and 2-naphthalenesulfonyl chlorides in methylene chloride in the presence of Hunig's base gave the respective sulfonamides **13**–**26**.

The synthesis of the semi-rigid derivatives **33**–**36** started by preparing the piperidine-derived secondary amines **31**–**32** (Scheme 2). The *N*-Boc-protected piperidine- methanol (**27**) and ethanol (**28**) were activated with tosyl chloride. Then the intermediates formed reacted with 4-chlorophenylpiperazine to give the respective tertiary amines **31** and **32**. After Boc removal, the compounds reacted with azinesulfonyl chlorides to yield the tertiary azinesulfonamides **33**–**36**. All the sulfonamides were converted into water-soluble hydrochloride salts.

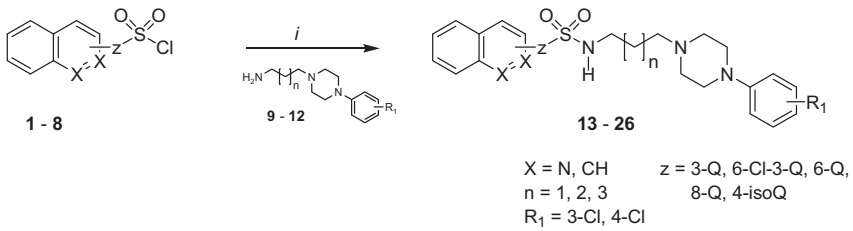
3. Pharmacology

3.1. In vitro evaluation

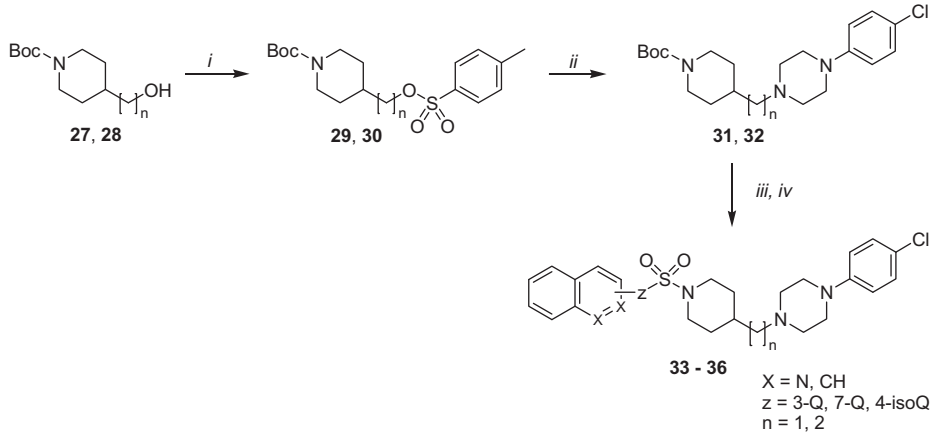
Radioligand binding assays were employed for determining the affinity and the selectivity profile of the synthesized compounds for native 5-HT$_{1A}$, 5-HT$_{2A}$, and dopamine D$_2$ as well as for cloned human 5-HT$_6$ and 5-HT$_7$ receptors. This was accomplished by displacement of [^{3}H]-8-OH-DPAT from rat hippocampus for 5-HT$_{1A}$R, [^{3}H]-ketanserin from rat cortex for 5-HT$_{2A}$R, and [^{3}H]-spiperone from rat striatum for D$_2$ receptors.[22,23] Displacement of [^{3}H]-LSD and [^{3}H]-5-CT from the cloned human receptor stably expressed in HEK293 cells was used in 5-HT$_6$R and 5-HT$_7$R binding experiments.[20,24] In case of D$_2$ receptors rat striatum and [^{3}H]-spiperone were used, whereas displacement of [^{3}H]-methylspiperone from the cloned human receptor stably expressed in HEK293 cells was used in D$_3$R and D$_4$R binding experiments.[25,26]

3.2. Functional evaluation

The functional activity of the two selected compounds **16** and **36** on intracellular cAMP levels, studied in CHO cells which stably expressed the human 5-HT$_7$ receptor, was determined at CEREP (Le Bois l'Eveque, 86600 Celle L'Evescault, France) according to the previously published methods.[27] The functional activity of the



Scheme 1. The synthesis of the new, flexible quinoline- and isoquinoline-sulfonamides **13**–**24**, and naphthalenesulfonamides **25**, **26**. Reagents and conditions: (i) DIEA, CH_2Cl_2, 0 °C→rt.



Scheme 2. The synthesis of the azinesulfonamides **33**–**36**. Reagents and conditions: (i) TsCl, Py, 0 °C→rt; (ii) 4-chlorophenylpiperazine, Et_3N, THF/toluene, 20 h; (iii) TFA/CH_2Cl_2 (9/1, v/v); (iv) quinoline- or isoquinoline-sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C.

selected compounds, based on their receptor affinity for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors, was tested in the commonly used in vivo models of their antagonistic activity. To determine the postsynaptic 5-HT_{1A} receptor antagonistic effects of derivative **16**, its ability to inhibit the 8-OH-DPAT-induced lower lip retraction (LLR) in rats was tested.[28] Compounds **16**, **17**, **20**, **35** and **36** with moderate, but significant affinity for 5-HT_{2A} and D_2 receptors were selected to assess their ability to antagonize the (±)-DOI-induced head twitch response in mice[29] and the apomorphine-induced climbing behavior in mice, respectively (Table 2).

3.3. Behavioral evaluation

In the successive phase of our investigation, the most in vitro-active derivatives, that is **16**, **17**, **20**, **35** and **36** were tested in vivo in the behavioral tests commonly used to predict antidepressant-, anxiolytic- and/or antipsychotic-like activity. The potential antidepressant activity was evaluated in the forced swim test (FST) in mice.[30] To determine the anxiolytic-like activity of the tested compounds, the elevated plus-maze test in rats was used. The influence of the effective doses recorded in the forced swim and the elevated plus-maze tests was studied in spontaneous locomotor activity in mice and rats, respectively, in order to exclude the possibility of competing behaviors such as general locomotor activity. Moreover, spontaneous locomotor activity and the hyperactivity induced by MK-801 were used to evaluate the specific antipsychotic-like activity in mice. Imipramine, diazepam, haloperidol and clozapine were used as reference drugs.

4. Results and discussion

The obtained series of compounds helped to extend the study aimed at developing new azinesulfonamide derivatives of the long-chain arylpiperazines, the 5-HT (with a significant 5-HT_7 component) and dopamine receptor ligands, as potential psychotropic agents for the treatment of anxiety, depression, and/or schizophrenia.

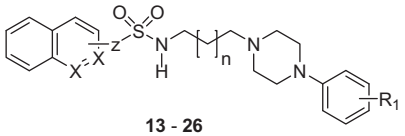
4.1. Structure–activity relationship studies

The newly synthesized quinoline- and isoquinoline-sulfonamides, connected by a polymethylene spacer (C_3–C_5) to 3- and 4-chloro-phenylpiperazines (**13**–**24**), displayed diversified affinity for the 5-HT receptors tested (Table 1). At first we examined the impact of linker length between the sulfonamide bond and the amine moiety on 5-HT_7R affinity. A tetramethylene spacer proved to be optimal for sulfonamides containing 3- and 4-chloro-substituted phenylpiperazines (PhP) (**13** vs **16** and **14** vs **20** and **24**).

Then we compared the data obtained for 3-chloro derivatives with those previously reported for their 2-methoxy analogs (**III**–**V**).[20] This modification increased the affinity for 5-HT_7Rs (up to 2-folds) and decreased the affinity for 5-HT_{1A}Rs. Next, we investigated an influence of the shift of the chlorine atom from 3- to 4-position. In opposite to Volk et al.,[19] who found that in a group of tetrahydrobenzindole derivatives the chlorine atom in 4-position decreased the affinity for 5-HT_{1A}Rs but maintained the affinity for 5-HT_7Rs at a high nanomolar range, in our experiments such modification decreased the affinity for both 5-HT_{1A} and 5-HT_7Rs.

It is noteworthy that among 3-Cl-PhP derivatives (**16**–**19**), the nitrogen position in the quinolinyl fragment impacted on the affinity for 5-HT_7R; we observed the same rank order (8-quinolinyl > 3-quinolinyl > 6-quinolinyl) as in the case of the previously reported 2-methoxyPhP derivatives.[20] In opposite, within a series of 4-chloro-PhPs (**20**–**22**), quinolinyl fragment marginally influenced affinity for 5-HT_7R. However, change of quinolinyl fragment for 4-isoquinolinyl one yielded compound **23** that displayed 4–5 folds higher affinity for 5-HT_7R (K_i = 68 nM).

Table 1
Binding of the quinoline- and isoquinoline-sulfonamides (**III**–**V**, **13**–**24**) and naphthalenesulfonamides (**25**, **26**) for 5-HT receptors



13 - 26

X = N, CH

Compd	Azinyl/naphthyl	*n*	R_1	K_i ± SEM (nM)			
				5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
III[a]	3-Quinolinyl	2	2-OCH_3	3.7 ± 0.6	NT[b]	NT	79 ± 8
IV[a]	6-Quinolinyl	2	2-OCH_3	40 ± 5	NT	NT	132 ± 18
V[a]	8-Quinolinyl	2	2-OCH_3	53 ± 7	NT	NT	55 ± 4
13	3-Quinolinyl	1	3-Cl	NT	206 ± 17	5043 ± 620	126 ± 7
14	3-Quinolinyl	1	4-Cl	NT	NT	6548 ± 1053	604 ± 57
15	4-Isoquinolinyl	1	4-Cl	888 ± 37	NT	2757 ± 328	113 ± 16
16	3-Quinolinyl	2	3-Cl	52 ± 6	22 ± 4	139 ± 11	56 ± 2
17	6-Cl-3-quinolinyl	2	3-Cl	208 ± 18	56 ± 7	155 ± 8	88 ± 6
18	6-Quinolinyl	2	3-Cl	68 ± 9	42 ± 3	397 ± 23	69 ± 4
19	8-Quinolinyl	2	3-Cl	74 ± 6	152 ± 18	3765 ± 222	25 ± 1
20	3-Quinolinyl	2	4-Cl	771 ± 92	56 ± 3	339 ± 23	310 ± 17
21	6-Quinolinyl	2	4-Cl	2030 ± 310	44 ± 6	1535 ± 213	335 ± 28
22	7-Quinolinyl	2	4-Cl	868 ± 66	63 ± 9	1725 ± 109	381 ± 31
23	4-Isoquinolinyl	2	4-Cl	1003 ± 124	211 ± 25	750 ± 43	68 ± 2
24	3-Quinolinyl	3	4-Cl	5801 ± 750	114 ± 8	2209 ± 150	334 ± 20
25	1-Naphthyl	2	3-Cl	397 ± 53	268 ± 34	127 ± 8	36 ± 3
26	2-Naphthyl	2	3-Cl	497 ± 29	22 ± 1	147 ± 13	50 ± 7

[a] Data taken from Ref. 20.
[b] NT—not tested.

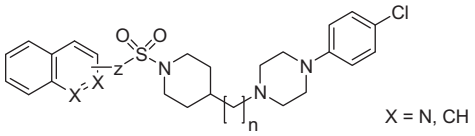
Following other authors, who described the effects of replacement of the naphthyl ring with nitrogen-containing aromatic heterocycles in different groups of carboxamide derivatives of LCAPs on the compounds solubility[31] or receptor affinity,[32] we tried to determine the impact of that modification on the 5-HT_7R and other related receptors binding profile. For this purpose we synthesized 1-naphthyl and 2-naphthyl sulfonamides, connected by a tetramethylene spacer to 3-Cl-PhP, as reference compounds. Compound **25**, a 1-naphthyl derivative, was classified as a 5-HT_7 receptor ligand (K_i = 36 nM) with moderate, 10-fold selectivity over 5-HT_{1A}Rs. On the other hand, the 2-naphthyl derivative (**26**) was classified as a dual 5-HT_{2A}/5-HT_7 receptor ligand. Neither the replacement of a 1-naphthyl ring with an 8-quinolinyl one (the α-position analog **19**) nor 2-naphthyl ring with 3-quinolinyl and 6-quinolinyl counterparts (the β-position analogs **16** and **18**, respectively) has greatly influenced affinity for 5-HT_7Rs. At the same time this modification increased the affinity of quinolinesulfonamides **14**–**17** for 5-HT_{1A}Rs. Thus, it has further confirmed that the nitrogen atom in the distal aromatic ring is unfavorable for the interaction with 5-HT_{1A}Rs; the rank order is as follows: 3-quinolinyl > 6-quinolinyl > 8-quinolinyl.

Interestingly, the affinity of 3-Cl-PhP and 4-Cl-PhP derivatives for 5-HT_{2A}Rs depended upon a kind of substitution of azinyl and naphthyl fragments. The β-position-substituted quinolinesulfonamides **16**–**18** and **20**–**22**, and 2-naphthenesulfonamide **26** displayed higher affinity for 5-HT_{2A} sites than their α-position–substituted counterparts **19**, **23**, and **26**.

Following Vermulen et al.,[14] we previously observed in a series of quinolinesulfonamides that N-alkylation of the sulfonamide group increased 5-HT_7/5-HT_{1A}R selectivity.[20] Hence in our present study we decided to replace the flexible tetramethylene spacer between the sulfonamide and the basic nitrogen atom with semi-rigid 4-alkylene–piperidine analogs. Such modification was carried out only for a series of 4-Cl-PhP derivatives which displayed the lowest affinity for 5-HT_{1A}Rs (Table 2). Compounds **33** and **34**, containing a sulfonamide bond embedded in 4-methylenepiperidine, displayed up to twice as high affinity for 5-HT_7Rs compared to their flexible analogs **20** and **22**. At the same time, that modification decreased the affinity for 5-HT_{2A}Rs (10–107 times). To further examine the semi-rigid spacer aiming at increasing the selectivity for 5-HT_7 sites, we decided to increase the distance between the piperidine moiety and the piperazine fragment equivalent to the pentamethylene spacer. The latter modification proved to be favorable for the binding of compounds **35** and **36** to 5-HT_7 receptors and selectivity over 5-HT_{1A} sites (Table 5). It thus seems that incorporation of the sulfonamide moiety into the piperidine fragment and the increase of the distance between sulfonamide and the basic nitrogen atom were beneficial to 5-HT_7/5-HT_{1A} selectivity. At the same time, elongation of the flexible fragment increased affinity for 5-HT_{2A}Rs and compounds **35** and **36** lost selectivity over these sites. Furthermore, on contrary to the favorable replacement of the 3-quinolinyl fragment with the 4-isoquinolinyl one for 5-HT_7R in the case of flexible sulfonamides (**20** vs **23**), this modification did not significantly change the receptor profile of semi-rigid compounds (**35** vs **36**).

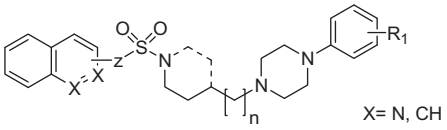
It is well known that 5-HTRs may indirectly modulate dopamine release. The recent data also presents that arenecarboxamides of the LCAP derivatives were systematically evaluated as potent D_3 receptor ligands,[33] of which the 4-quinolinecarboxamide derivative SB-277011 was classified as a potent D_3 antagonist. It was further found that quinolinecarboxamides were classified as mixed D_3, 5-HT_{1A} and 5-HT_{2A} ligands and displayed antipsychotic properties.[34] Having based on the above data, in a next move we wished to verify selectivity of the library representatives over dopamine D_2, D_3 and D_4 receptors (Table 3). Of the selected flexible quinolinesulfonamides, compound **16** (a 3-Cl-PhP derivative) displayed high affinity for D_2 and D_3 receptors, while its 4-Cl-PhP analog **20** showed low affinity for the dopamine receptors studied. Surprisingly, the partly rigidified 4-Cl-PhP derivatives **35** and **36** displayed high affinity for D_2, D_3 and D_4 receptors. Of the tested compounds, the 4-isoquinolinyl analog displayed the highest affinity for D_3 and D_4 sites.

Table 2
Binding of rigidified quinoline- and isoquinoline-sulfonamides **33**–**36** for 5-HT receptors



Compd	Azinyl	*n*	K_i ± SEM (nM)				Ratio $S_{1A/7}$
			5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7	
33	3-Quinolinyl	1	462 ± 62	6004 ± 520	10570 ± 860	145 ± 9	3
34	7-Quinolinyl	1	338 ± 46	761 ± 93	17150 ± 1200	207 ± 11	1.6
35	3-Quinolinyl	2	8300 ± 920	31 ± 5	24840 ± 3200	40 ± 2	207
36	4-Isoquinolinyl	2	3210 ± 518	59 ± 9	16650 ± 2115	47 ± 3	68

Table 3
Binding data of the selected quinoline- and isoquinoline-sulfonamides for dopamine receptors



Compd	Azinyl	Spacer	*n*	R_1	K_i (nM)		
					D_2[a]	D_3[b]	D_4[b]
16	3-Quinolinyl	Flexible	1	3-Cl	40 ± 6	70/94	30/63
20	3-Quinolinyl	Flexible	1	4-Cl	163 ± 12	46/82	37/78
35	3-Quinolinyl	Rigidified	2	4-Cl	56 ± 7	52/90	63/90
36	4-Isoquinolinyl	Rigidified	2	4-Cl	60 ± 9	67/93	70/98

[a] Full-binding experiment.
[b] Screening procedure—displacement % at $10^{-7}/10^{-6}$.

Table 4
Effects of the tested compounds on the (±)-DOI-induced head twitches response (A) and on the apomorphine-induced climbing behavior (B) in Swiss albino mice

Compd	A ID_{50}[a] (mg/kg ip)	B ID_{50}[a] (mg/kg ip)
16	10.09 (9.34–10.84)	29.40 (wide range)
17	4.75 (3.59–5.90)	NT[c]
20	19.55 (17.06–22.03)	60.10 (57.84–62.36)
35	5.12 (4.19–6.04)	24.99 (23.60–26.37)
36	20.26 (17.82–22.70)	25.49 (23.58–27.40)
Ketanserin[b]	0.12 (0.07–0.20)	—

[a] ID_{50}—the dose inhibiting the head twitches or climbing behavior in mice by 50%; confidence limits (95%) given in parentheses; apomorphine (3 mg/kg) evokes climbing behavior of ca. 100 s duration.
[b] Data taken from Ref. 35.
[c] NT—not tested.

4.2. Functional evaluation

The promising compounds **16** and **36** were selected for functional evaluation for 5-HT_7R. The obtained results with CHO cells, which stably expressed the human 5-HT_7 receptor, indicated that the compounds behaved like antagonists at the h5-HT_7R, with K_B = 110 nM for **16** and K_B = 90 nM for **36**. In addition it was further found, that compound **16** (10–20 mg/kg) dose-dependently and significantly attenuated the 8-OH-DPAT–induced LLR in rats (data not shown). Compounds **16** (2.5–20 mg/kg), **17** (2.5–30 mg/kg), **20** (10–30 mg/kg), **35** (2.5–10 mg/kg) and **36** (10–30 mg/kg) dose-dependently inhibited the (±)DOI-induced head twitches in mice (Table 4A). The compounds **16** (10–30 mg/kg), **35** (20–30 mg/kg) and **36** (20–30 mg/kg) decreased the apomorphine-induced climbing behavior in a dose-dependent and statistically significant manner (Table 4B). Concluding, the results of our in vitro and in vivo functional study showed that the investigated compounds displayed antagonistic activity at postsynaptic 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2 receptors.

4.3. Behavioral evaluation

A number of preclinical studies suggest some role of 5-HT receptors, in particular 5-HT_{1A}, 5-HT_{2A}, 5-HT_6 and 5-HT_7 ones, in many psychiatric disorders. Clinical and preclinical studies have shown that antidepressant-like effects are mainly produced by postsynaptic 5-HT_{1A} and/or 5-HT_{2A} receptor antagonists. The recent development of selective 5-HT_6 and 5-HT_7 receptor antagonists gives further substantial support for the interrelationship between serotonin and depression and/or anxiety.[4,36] The affinity of the new compounds for 5-HT_6R was not significant and ranged from 127 to 17150 nM. However, the high affinity of some azine-sulfonamides tested, for 5-HT_7Rs with a mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 (**16**, **20**) or 5-HT_{2A}/5-HT_7 (**17**, **35**, **36**) receptor profile and antagonistic activity at the postsynaptic sites, prompted us to evaluate their activity in the FST in mice.

The obtained results indicated that **16** (5–20 mg/kg), **17** (5–20 mg/kg) and **35** (10–30 mg/kg) significantly reduced the immobility time of mice by 37%, 44% and 54%, when administered in doses which had no effect of their own on spontaneous locomotor activity (Fig. 4). Compounds **20** (10–30 mg/kg) and **35** (10–30 mg/kg) were practically inactive in that test; however, in the case of **20**, a tendency to decrease immobility could be observed; although the obtained results were not statistically significant. The antidepressant-like activity of **16**, **17**, and **36** was comparable to that of imipramine, a conventional antidepressant used as a positive control, which decreased the immobility time of mice by 26.5% and 52% after administration in doses of 10 and 20 mg/kg, respectively.

Another important finding of this study is that both selected compounds **16** and **36** exhibited characteristic properties of anxiolytic drugs in the plus-maze test in rats. Compound **16** administered in a highest dose (20 mg/kg) significantly increased the time spent in the open arms. Other parameters measured in that model were also increased; however, the obtained results did not reach statistical significance. Moreover, an anxiolytic-like effect of **16** was not specific since its effective dose (20 mg/kg) modified the general activity of rats increasing the number of total entries into open and closed arms and X ambulation (data not shown) (Table 5). Compound **36** produced anxiolytic-like activity after administration in all doses tested, that is 2.5, 5 and 10 mg/kg. This derivative administered in a dose of 10 mg/kg induced a specific anxiolytic-like effect, having significantly increased the time spent in the open arms, the percentage of the time spent therein and the number of entries into the open arms not affecting the parameters

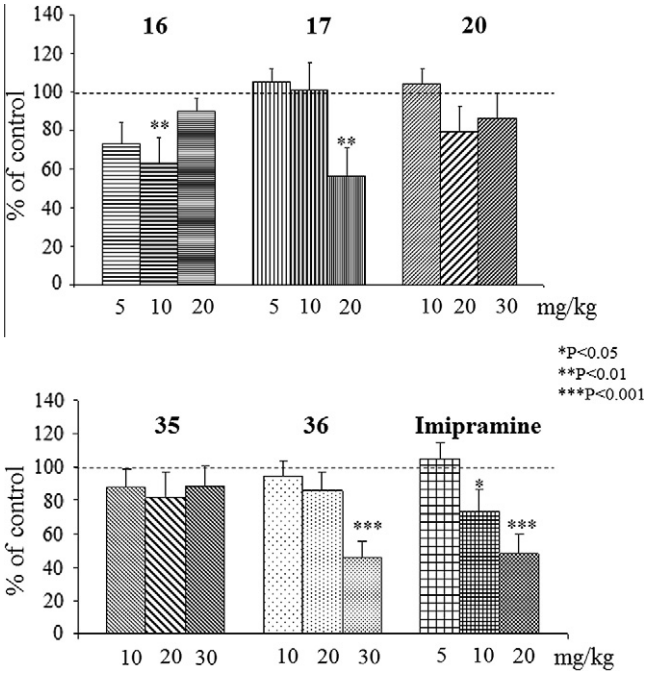


Figure 4. Effects of the tested compounds on the immobility time in the forced swim test in mice.

of general locomotor activity. Its dose of 5 mg/kg producing anxiolytic effect did not reach statistically significant level (Table 5). When compound **36** was given in a dose of 2.5 mg/kg, it evoked non-specific anxiolytic-like activity because it simultaneously modified the general activity of rats by increasing the total number of entries, the total distance and Y ambulation (data not shown). Diazepam (1.25 and 2.5 mg/kg), used as a reference drug, significantly increased the time spent in the open arms, the percentage of the time spent in the open arms, and the number of entries into the open arms (Table 5).

In view of the fact, that some antidepressants can modulate the dopaminergic system, and that compounds **16** and **36** display significant affinity for dopamine D_2, D_3, and/or D_4 receptors (Table 3), in a successive stage we attempted to determine the antipsychotic potential in the MK-801-induced hyperactivity test. Such dual

Table 6
Effects of compounds **16**, **36**, haloperidol and clozapine on the hiperlocomotor activity induced by MK-801 in CD-1 mice

Compd dose (mg/kg)	Number of crossings during 60 min mean ± S.E.M.
Vehicle + vehicle	3514.5 ± 296.5
Vehicle + MK-801 (0.2)	7577.6 ± 968.2[b]
16 (2.5) + MK-801 (0.2)	6606.6 ± 759.2[a]
16 (5) + MK-801 (0.2)	6416.9 ± 422.8[a]
16 (10) + MK-801 (0.2)	6551.3 ± 617.7[a]
	F(4,43) = 5.578
	P <0.01
Vehicle + vehicle	2717.4 ± 513.9
Vehicle + MK-801 (0.2)	9519.6 ± 1097.9[b]
16 (20) + MK-801 (0.2)	6701.0 ± 1325.0[b]
	F(2,26) = 10.028
	P <0.001
Vehicle + vehicle	2717.4 ± 513.9
Vehicle + MK-801 (0.2)	9785.0 ± 714.7[b]
36 (2.5) + MK-801 (0.2)	9349.1 ± 1100.6[a]
36 (5) + MK-801 (0.2)	5618.8 ± 609.5[A]
36 (10) + MK-801 (0.2)	5509.0 ± 812.4[A]
36 (20) + MK-801 (0.2)	3852.1 ± 720.4[B]
36 (30) + MK-801 (0.2)	3838.5 ± 607.6[B]
	F(6,72) = 14.1260
	P <0.0001
Vehicle + vehicle	690.8 ± 117.2
Vehicle + MK-801 (0.2)	6139.3 ± 755.4[b]
Haloperidol (0.5) + MK-801 (0.2)	4231.8 ± 828.9[b]
Haloperidol (1) + MK-801 (0.2)	844.0 ± 183.5[B]
	F(3,35) = 22.124
	P <0.0001
Vehicle + vehicle	902.2 ± 123.9
Vehicle + MK-801 (0.2)	4838.0 ± 639.5[b]
Clozapine (1.25) + MK-801 (0.2)	2781.7 ± 730.6
Clozapine (2.5) + MK-801 (0.2)	2503.9 ± 654.1[A]
	F(3,35) = 7.347
	P <0.001

[a] *P* <0.01 versus respective vehicle + vehicle group.
[b] *P* <0.001 versus respective vehicle + vehicle group.
[A] *P* <0.01 versus respective vehicle + MK-801 group (Bonferroni's test).
[B] *P* <0.001 versus respective vehicle + MK-801 group (Bonferroni's test).

Table 5
Effects of the compounds **16**, **36**, and diazepam in the elevated plus-maze test in rats

Compd dose (mg/kg)	Time spent in open arms (s) mean ± S.E.M.	Percentage of time spent in open arms mean ± S.E.M.	Open arm entries mean ± S.E.M.	Percentage of open arm entries mean ± S.E.M.
Vehicle	27.9 ± 6.0	11.5 ± 3.0	5.8 ± 1.1	25.7 ± 2.7
16 (2.5)	81.9 ± 25.4	31.0 ± 9.2	6.7 ± 1.1	34.2±1.8
16 (5)	81.7 ± 9.7	33.4 ± 3.6	10.9 ± 1.7[a]	34.4 ± 2.2
16 (10)	98.9 ± 23.3	41.0 ± 8.9	7.4 ± 1.2	33.6 ± 5.0
16 (20)	120.8 ± 46.8[a]	41.7 ± 8.9	2.9 ± 0.8	58.1 ± 11.4[c]
	F(4,33) = 2.942	*F*(4,33) = 3.075	*F*(4,33) = 5.005	*F*(4,33) = 5.422
	P <0.05	*P* <0.05	*P* <0.01	*P* <0.01
Vehicle	20.2 ± 3.2	7.9 ± 1.4	4.1 ± 0.9	23.1 ± 3.7
36 (2.5)	62.3 ± 10.3[b]	25.0 ± 4.2[b]	10.9 ± 2.4[a]	36.4 ± 7.3
36 (5)	49.2 ± 9.7	19.4 ± 3.9	8.7 ± 0.9	32.9 ± 2.8
36 (10)	72.8 ± 6.9[c]	29.3 ± 2.5[c]	11.3 ± 1.1[a]	40.3 ± 3.5
	F(3,24) = 8.013	*F*(3,24) = 8.364	*F*(3,24) = 4.957	*F*(3,24) = 2.478
	P <0.001	*P* <0.001	*P* <0.01	ns
Vehicle	29.9 ± 4.8	13.0 ± 2.3	9.3 ± 1.2	26.3 ± 2.1
Diazepam (1.25)	82.0 ± 18.3[b]	29.7 ± 9.7[a]	8.6 ± 2.2	63.7 ± 11.3[b]
Diazepam (2.5)	71.7 ± 10.9[a]	28.5 ± 3.9[a]	13.1 ± 1.3	46.6 ± 3.1
Diazepam (5)	48.0 ± 9.4	21.8 ± 7.1	13.4 ± 3.0	35.4 ± 4.8
	F(3,24) = 4.799	*F*(3,24) = 4.089	*F*(3,24) = 1.492	*F*(3,24) = 6.316
	P <0.01	*P* <0.05	ns	*P* <0.01

[a] *P* <0.05 versus respective vehicle + vehicle group (Bonferroni's post-hoc test).
[b] *P* <0.01 versus respective vehicle + vehicle group (Bonferroni's post-hoc test).
[c] *P* <0.001 versus respective vehicle + vehicle group (Bonferroni's post-hoc test); ns—non-significant.

Table 7
Effects of the compounds **16**, **36**, haloperidol and clozapine on the spontaneous locomotor activity in CD-1 mice

Compd dose (mg/kg)	Number of crossings during 60 min mean ± S.E.M.
Vehicle + vehicle	2018.9 ± 377.7
16 (2.5) + vehicle	1836.6 ± 255.2
16 (5) + vehicle	1456.4 ± 108.9
16 (10) + vehicle	1430.9 ± 194.2
	$F(3,32) = 1.1844$ ns
Vehicle + vehicle	2790.2 ± 698.5
36 (10) + vehicle	2080.0 ± 348.5
36 (20) + vehicle	1531.8 ± 380.4
36 (30) + vehicle	1415.6 ± 333.2
	$F(3,36) = 1.830$ ns
Vehicle + vehicle	1650.1 ± 220.3
Haloperidol (0.25) + vehicle	1803.6 ± 270.0
Haloperidol (0.5) + vehicle	826.8 ± 149.9[a]
Haloperidol (1) + vehicle	439.6 ± 123.1[b]
	$F(3,36) = 10.754$
	$P < 0.0001$
Vehicle + vehicle	1185.3 ± 292.6
Clozapine (1.25) + vehicle	867.0 ± 197.1
Clozapine (2.5) + vehicle	1069.0 ± 318.9
	$F(2,24) = 0.363$ ns
Vehicle + vehicle	1197.9 ± 153.8
Clozapine (5) + vehicle	446.3 ± 175.0[a]
	$F(1,18) = 10.405$
	$P < 0.01$

[a] $P < 0.05$ versus respective vehicle + vehicle group (Bonferroni's post-hoc test).
[b] $P < 0.01$ versus respective vehicle + vehicle group (Bonferroni's post-hoc test); ns—non-significant.

activity seems very interesting, since several antipsychotic drugs (e.g., amisulpride, aripiprazole) with a multi-receptor profile decrease depression and/or obsessive–compulsive disorder symptoms.[10]

Thus compound **36** with a multi-receptor 5-HT_{2A}/5-HT_7/D_2/D_3/D_4 profile, given in doses of 5–30 mg/kg, significantly inhibited the MK-801-evoked hyperactivity in mice (Table 6). Its effect seemed to be specific, since **36** administered in effective doses had no influence on spontaneous the locomotor activity of mice (Table 7). On the other hand, derivative **16** produced no effect on either the hyperactivity induced by MK-801 or spontaneous locomotor activity in mice (Tables 6 and 7). By comparison, haloperidol and clozapine dose-dependently and significantly attenuated the hyperactivity evoked by MK-801; however, only clozapine produced specific antipsychotic-like activity in that test, while haloperidol produced such an effect in doses which per se decreased spontaneous locomotor activity (Tables 6 and 7).

Some interesting results of the pharmacological studies with quinoline- and isoquinoline-sulfonamides of the long-chain arylpiperazine derivatives have drawn our attention. First of all, affinity for the 5-HT_7R proved to be an essential component for behavioral activity of tested compounds in the mice models of depression and anxiety. The most potent mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 receptor ligand **16** and the 5-HT_{2A}/5-HT_7 receptor ligand **36** displayed potential antidepressant and anxiolytic activity in the FST and the plus-maze test, respectively. Compound **36**, which also targeted D_2, D_3 and D_4 sites, was found to have a major additional effect in MK-801-induced hyperlocomotor activity test in mice, a robust model of the negative and cognitive symptoms of schizophrenia.[37,38] Undoubtedly, the unique properties of compound **36** resulted from the type of the azine moiety.

5. Conclusions

In the present study we described the synthesis of two series of quinoline-, isoquinoline-sulfonamides containing a flexible (**13**–**26**) and semi-rigid (**33**–**36**) alkylene spacer, their receptor affinity, and the functional profile; finally, we carried out their behavioral evaluation. The study allowed us to identify some potent, mixed 5-HT_{2A}/5-HT_7 and 5-HT_{1A}/5-HT_{2A}/5-HT_7 receptor ligands with additional affinity for dopamine D_2, D_3, and/or D_4 receptors. Our lead compound **36**, a 4-isoquinolinesulfonamide with a semi-rigid alkylene spacer, classified as a multi-receptor 5-HT_{2A}/5-HT_7/D_2/D_3/D_4 ligand, produced significant psychotropic activity: antidepressant, anxiolytic, and antipsychotic. That compound seems very promising regarding the complexity of CNS disorders and the limitations of monotherapy. Selective drugs may prove beneficial to some specific symptoms of mentioned CNS diseases, to certain subpopulations of patients or both. However, some network analyses of the brain and its dysfunctions suggest that agents with multiple and complementary modes of action are more likely to show broad-based efficacy in relation to the core and comorbid symptoms of depression/anxiety/schizophrenia.

6. Experimental

6.1. Materials and methods

Organic transformations were carried out at ambient temperature, unless indicated otherwise. Organic solvents (from Aldrich and Chempur) were of reagent grade and were used without purification. All other reagents were from Aldrich and Alfa Aesar.

Purity of the synthesized compounds was confirmed by TLC performed on Merck silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany). Spots were detected by their absorption under UV light ($\lambda = 254$ nm).

Analytical HPLC were run on a Waters Alliance HPLC instrument, equipped with a ChromolithSpeedROD column (4.6 × 50 mm). Standard conditions were eluent system A (water/0.1% TFA), system B (acetonitrile/0.1% TFA). A flow rate of 5 mL/min and a gradient of (0–100)% B over 3 min were used. Detection was performed on a PDA detector. Retention times (t_R) are given in minutes.

^{1}H NMR spectra were recorded at 300 MHz (Varian BB 200 spectrometer) using TMS (0.00 ppm) and chloroform-d_1, J values are in hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet).

Mass spectrometry analyses: Samples were prepared in acetonitrile/water (10/90 v/v) mixture. The LC/MS system consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All the analyses were carried out using a Acquity UPLC BEH C18, 50 × 2.1 mm reversed-phase column. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 5 min was used. Eluent A: water/0.1% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. Nitrogen was used for both nebulizing and drying gas. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 100 to 700 m/z; 8 scans were summed up to produce the final spectrum.

Elemental analyses were found within ± 0.4% of the theoretical values.

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

Column chromatography separations were carried out on column with Merck Kieselgel 60.

The following abbreviations were used: DIEA—diisopropylethylamine; DMF—dimethylformamide; TFA—trifluoroacetic acid. The other abbreviations used were recommended by the IUPAC-IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9–37).

6.2. General procedure for the preparation of compounds 13–26 (Method A)

The starting *N*-(ω-aminoalkyl)-3-chlorophenylpiperazines (**9**, **10**) and *N*-(ω-aminoalkyl)-4-chlorophenylpiperazines (**11**, **12**) were synthesized according to the Gabriel method. A mixture of the appropriate *N*-(ω-aminoalkyl)-3-chlorophenylpiperazine or *N*-(ω-aminoalkyl)-4-chlorophenylpiperazine (1.2 mmol) in CH_2Cl_2 (7 mL), and DIEA (2.4 mmol) was cooled down (ice bath), and azinesulfonyl (**1**–**6**) or naphthalenesulfonyl chloride (**7**, **8**) (1.3 mmol) was added at 0 °C in one portion. The reaction mixture was stirred for 2–6 h under cooling. Then, the solvent was evaporated and the sulfonamides were separated by column chromatography using SiO_2 and a mixture of CH_2Cl_2/MeOH = 9/1 or 9/0.7, as an eluting system. Free bases were then converted into the hydrochloride salts by treatment of their solution in anhydrous ethanol with 4 M HCl in dioxane.

6.2.1. *N*-{3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propyl}-3-quinolinesulfonamide (13)

The compound was prepared by the general method A in 72% yield as yellow oil; t_R = 1.21; MS calcd for $[M+H]^+$: $C_{22}H_{25}ClN_4O_2S$ *m*/*z*: 444.2, found 445.2. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.63 (br s, 4H), 2.60–2.63 (m, 4H), 3.02–3.06 (m, 2H), 3.23–3.26 (m, 4H), 6.77–6.83 (td, 1H), 6.86–6.87 (m, 1H), 7.15–7.19 (t, 1H) 7.63–7.68 (m, 1H), 7.82–7.89 (m, 2H), 8.16–8.19 (d, 1H), 8.55 (br s, 1H), 8.63–8.64 (d, 1H), 9.22–9.23 (d, 1H). Compound **13**·HCl mp 172.1–174.2 °C. Anal. ($C_{22}H_{25}ClN_4O_2S$·HCl) C, H, N.

6.2.2. *N*-{3-[4-(4-Chlorophenyl)-piperazin-1-yl]-propyl}-3-quinolinesulfonamide (14)

The compound was prepared by the general method A in 77% yield as white powder, mp 190.6–191.5 °C; t_R = 1.23; MS calcd for $[M+H]^+$: $C_{22}H_{25}ClN_4O_2S$ *m*/*z*: 444.2, found 445.2. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.68–1.76 (m, 2H), 2.48–2.52 (m, 2H), 2.58–2.61 (m, 4H), 3.15–3.21 (m, 6H), 6.81–6.87 (m, 2H), 7.19–7.26 (m, 3H), 7.19–7.67 (m, 1H), 7.86–7.96 (m, 2H), 8.18–8.21 (d, *J* = 8.46, 1H), 8.68–8.69 (d, *J* = 2.31, 1H), 9.23–9.24 (d, *J* = 2.31, 1H). Compound **14**·HCl mp 186–187 °C. Anal. ($C_{22}H_{25}ClN_4O_2S$·HCl) C, H, N.

6.2.3. *N*-{3-[4-(4-Chlorophenyl)-piperazin-1-yl]-propyl}-4-isoquinolinesulfonamide (15)

The compound was prepared by the general method A in 72% yield as white powder, mp 170.3–171.7 °C; t_R = 1.09; MS calcd for $[M+H]^+$: $C_{22}H_{25}ClN_4O_2S$ *m*/*z*: 444.2, found 445.3. 1H NMR ($CDCl_3$) δ (ppm) 1.67–1.73 (m, 2H), 2.46–2.50 (m, 2H), 2.56–2.59 (t, *J* = 4,87, 4H), 3.16–3.20 (m, 7H), 6.81–6.86 (m, 2H), 7.18–7.23 (m, 2H), 7.53–7.57 (m, 1H), 7.92–7.98 (m, 2H), 8.21–8.25 (m, 1H), 8.64 (s, 1H), 9.03–9.05 (m, 1H). Compound **15**·HCl mp 145.2–145.7 °C. Anal. ($C_{22}H_{25}ClN_4O_2S$·HCl) C, H, N.

6.2.4. *N*-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-3-quinolinesulfonamide (16)

The compound was prepared by the general method A in 72% yield as white oil, which solids upon standing; t_R = 1.41; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.63 (br s, 4H), 2.39–2.43 (t, 2H), 2.60–2.64 (m, 4H), 3.03–3.07 (m, 2H), 3.24–3.27 (m, 4H), 6.77–6.83 (td, 1H, *J* = 6.2 Hz, *J* = 1.1 Hz), 6.86–6.87 (m, 1H), 7.15–7.2 (t, 1H) 7.63–7.68 (m, 1H), 7.82–7.89 (m, 2H), 8.16–8.19 (d, 1H), 8.55 (br s, 1H), 8.63–8.64 (d, 1H), 9.22–9.23 (d, 1H, *J* = 2.3 Hz). Compound **16**·HCl mp 183.5–185.0 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.5. *N*-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-6-chloro-3-quinolinesulfonamide (17)

The compound was prepared by the general method A in 72% yield as oli; t_R = 1.56; MS calcd for $[M+H]^+$: $C_{23}H_{26}Cl_2N_4O_2S$ *m*/*z*: 492.1, found 493.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.64 (br s, 4H), 2.41–2.44 (t, 2H), 2.62–2.65 (m, 4H), 3.03–3.05 (m, 2H), 3.25–3.28 (m, 4H), 6.76–6.79 (dd, 1H), 6.84–6.89 (m, 2H), 7.16–7.21 (t, 1H) 7.76–7.80 (m, 2H), 8.09–8.12 (dd, 2H), 8.52–8.53 (d, 1H, *J* = 2.1 Hz), 9.19–9.20 (dd, 1H, *J* = 4.1 Hz, *J* = 2.0 Hz). Compound **17**·HCl mp 168.9–169.9 °C. Anal. ($C_{23}H_{26}Cl_2N_4O_2S$·HCl) C, H, N.

6.2.6. *N*-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-6-quinolinesulfonamide (18)

The compound was prepared by the general method A in 72% yield as yellow oil; t_R = 1.19; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.63 (br s, 4H), 2.39–2.43 (t, 2H), 2.60–2.64 (m, 4H), 3.03–3.07 (m, 2H), 3.24–3.27 (m, 4H), 6.77–6.83 (td, 1H, *J* = 6.2 Hz, *J* = 1.1 Hz), 6.86–6.87 (m, 1H), 7.15–7.2 (t, 1H) 7.63–7.68 (m, 1H), 7.82–7.89 (m, 2H), 8.16–8.19 (d, 1H), 8.55 (br s, 1H), 8.63–8.64 (d, 1H), 9.22–9.23 (d, 1H, *J* = 2.3 Hz). Compound **18**·HCl mp 205.2–206.6 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.7. *N*-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-8-quinolinesulfonamide (19)

The compound was prepared by the general method A in 72% yield as yellow oil; t_R = 1.40; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.48–1.52 (m, 4H), 2.26–2.31 (t, 2H), 2.48–2.50 (m, 4H), 2.91–2.92 (m, 2H), 3.12–3.15 (m, 4H), 6.46 (br s, 1H), 6.74–6.85 (m, 3H), 7.13–7.18 (t, 1H, *J* = 8.2 Hz), 7.54–7.58 (q, 1H), 7.64–7.69 (q, 1H), 8.04–8.08 (dd, 1H, *J* = 8.2 Hz, *J* = 1.5 Hz), 8.27–8.30 (d, 1H, *J* = 8.2 Hz, *J* = 1.8 Hz), 8.43–8.46 (dd, 1H, *J* = 7.4 Hz, *J* = 1.3 Hz), 9.01–9.03 (dd, 1H, *J* = 4.4 Hz, *J* = 1.8 Hz). Compound **19**·HCl mp 212.4–213.2 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.8. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-3-quinolinesulfonamide (20)

The compound was prepared by the general method A in 69% yield as yellow oil; t_R = 1.39; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. Compound **20**·HCl: ^{1}H NMR (DMSO) δ (ppm) 1.42–1.47 (m, 2H), 1.70 (br s, 2H), 2.82–2.88 (m, 2H), 2.99–3.07 (m, 4H), 3.41–3.47 (m, 2H), 3.69–3.74 (m, 4H), 6.97–7.00 (m, 2H), 7.25–7.28 (m, 2H), 7.75–7.80 (td, 1H), 7.93–7.99 (td, 1H), 8.07–8.15 (dd, 1H), 8.24–8.26 (dd, 1H), 8.89–8.90 (dd, 1H), 9.20–9.21 (dd, 1H), 10.82 (br s, 1H). Compound **20**·HCl Mp 190.1–192.3 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.9. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-6-quinolinesulfonamide (21)

The compound was prepared by the general method A in 68% yield as yellow oil; t_R = 1.20; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.57–1.65 (br s, 4H), 2.37–2.42 (t, 2H), 2.59–2.63 (m, 4H), 3.02–3.06 (m, 2H), 3.16–3.20 (m, 4H), 6.79–6.85 (dt, 2H), 7.19–7.24 (dt, 2H), 7.47–7.51 (q, 1H), 8.01–8.04 (dd, 1H), 8.09–8.12 (dd, 1H), 8.17 (s, 1H), 8.20 (s, 1H), 8.35–8.36 (d, 1H), 9.02–9.04 (dd, 1H). Compound **21**·HCl mp 204.1–205.4 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.10. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-7-quinolinesulfonamide (22)

The compound was prepared by the general method A in 68% yield as pale yellow oil; t_R = 1.24; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m*/*z*: 458.2, found 459.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.60–1.62 (m, 4H), 2.38–2.42 (t, 2H), 2.60–2.64 (m, 4H), 3.04–

3.08 (t, 2H), 3.19–3.23 (m, 4H), 6.80–6.85 (dt, 2H), 7.17–7.23 (dt, 2H), 7.51–7.55 (q, 1H), 7.88–7.94 (m, 1H), 8.19–8.23 (m, 1H), 8.61 (s, 1H), 9.01–9.03 (dd, 1H). Compound **22**·HCl mp 223.9–224.6 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.11. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-4-isoquinolinesulfonamide (23)

The compound was prepared by the general method A in 66% yield as white powder, mp 132.8–133.2 °C; t_R = 1.19; MS calcd for $[M+H]^+$: $C_{23}H_{27}ClN_4O_2S$ *m/z*: 458.2, found 459.3. ^1H NMR ($CDCl_3$) δ (ppm) 1.60–1.62 (s, 4H), 2.41–2.43 (m, 2H), 2.64–2.67 (m, 4H), 3.03 (s, 2H), 3.25–3.28 (m, 4H), 6.83–6.88 (m, 2H), 7.19–7.27 (m, 2H), 7.68–7.78 (m, 2H), 8.06–8.09 (m, 1H), 8.50 (s, 1H), 8.64–8.67 (m, 1H), 9.09 (s, 1H), 9.38 (s, 1H). Compound **23**·HCl mp 198.1–198.8 °C. Anal. ($C_{23}H_{27}ClN_4O_2S$·HCl) C, H, N.

6.2.12. *N*-{5-[4-(4-Chlorophenyl)-piperazin-1-yl]-pentyl}-3-quinolinesulfonamide (24)

The compound was prepared by the general method A in 72% yield as a pale oil; t_R = 1.31; MS calcd for $[M+H]^+$: $C_{24}H_{29}ClN_4O_2S$ *m/z*: 472.2, found 473.3. ^1H NMR ($CDCl_3$) δ (ppm) 1.25–1.38 (m, 4H), 1.42–1.59 (m, 4H), 2.29–2.36 (m, 2H), 2.51–2.54 (m, 4H), 3.06–3.15 (m, 5H), 6.79–6.84 (m, 2H), 7.16–7.22 (m, 2H), 7.67–7.72 (m, 1H), 7.86–7.95 (m, 2H), 8.18–8.21 (d, 1H, *J* = 8.72), 8.69–8.70 (d, 1H, *J* = 2.05), 9.25–9.26 (d, 1H, *J* = 2.31). Compound **24**·HCl mp 145.3–146.0 °C. Anal. ($C_{24}H_{29}ClN_4O_2S$·HCl) C, H, N.

6.2.13. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-1-naphthalenesulfonamide (25)

The compound was prepared by the general method A in 78% yield as pale yellow oil; t_R = 1.57; MS calcd for $[M+H]^+$: $C_{24}H_{28}ClN_3O_2S$ *m/z*: 457.2, found 458.4. ^1H NMR ($CDCl_3$) δ (ppm) 1.39–1.44 (m, 2H), 1.67 (b s, 2H), 2.76–2.83 (m, 2H), 3.01–3.10 (m, 6H), 3.41–3.35 (m, 2H), 3.81–3.85 (m, 2H), 6.84–6.87 (dd, 1H), 6.91–6.95 (dd, 1H), 7.01–7.03 (br, 1H), 7.22–7.27 (t, 1H), 7.64–7.73 (m, 2H), 7.79–7.84 (m, 2H), 8.03–8.06 (dd, 1H), 8.12–8.17 (m, 2H), 8.43 (dd, 1H). Compound **25**·HCl mp 199.7–200.4 °C. Anal. ($C_{24}H_{28}ClN_3O_2S$·HCl) C, H, N.

6.2.14. *N*-{4-[4-(4-Chlorophenyl)-piperazin-1-yl]-butyl}-2-naphthalenesulfonamide (26)

The compound was prepared by the general method A in 72% yield as yellow oil; t_R = 1.55; MS calcd for $[M+H]^+$: $C_{24}H_{28}ClN_3O_2S$ *m/z*: 457.2, found 457.9. ^1H NMR ($CDCl_3$) δ (ppm) 1.35–1.38 (m, 2H), 1.53–1.56 (m, 2H), 2.77–2.83 (m, 2H), 2.98 (b s, 4H), 3.06–3.10 (m, 2H), 3.38–3.46 (m, 2H), 3.81–3.85 (m, 2H), 6.84–6.87 (dd, 1H), 6.92–6.97 (dd, 1H), 7.02–7.04 (t, 1H), 7.21–7.28 (t, 1H), 7.62–7.75 (m, 2H), 8.02–8.13 (m, 4H), 8.21–8.27 (dd, 1H), 8.62–8.66 (dd, 1H). Compound **26**·HCl mp 218.6–219.9 °C. Anal. ($C_{24}H_{28}ClN_3O_2S$·HCl) C, H, N.

6.3. Synthesis of 1-(4-chloro-phenyl)-4-piperidin-4-ylmethyl-piperazine (31) and 1-(4-chlorophenyl)-4-piperidin-4-ylethyl-piperazine (32)

Boc-piperidinemethanol (**27**) or Boc-piperidineethanol (**28**) (23 mmol) dissolved in pyridine (50 mL) and cooled down under nitrogen. Then a tosyl chloride (27,87 mmol) was added portionwise. The was carried out for 3 h on bath-ice, then warm to room temperature and stirred for additional 10 h. The reaction mixture was extracted with CH_2Cl_2 (150 mL) and the organic phase was washed with 1 M $KHSO_4$ (4 × 50 mL), water, brine and dried over Na_2SO_4. The tosyl derivatives (**29**, **30**) were separated by column chromatography using SiO_2 and CH_2Cl_2 followed by CH_2Cl_2/MeOH = 9/0.1. Next tosyl derivatives (2.44 mmol) were reacted with 4-chlorophenylpiperazine (2 mmol) by heating them in the presence of TEA (21.5 mmol) the boiling mixture of THF/toluene (5 mL/10 mL) for 20 h. After solvent evaporation the crude product was purified on silica gel column chromatography (CH_2Cl_2/MeOH 9/0.5, v/v) to yield Boc-protected piperidine **31** and **32**. For the next stage the products were converted into their TFA salts and were isolated as white foams.

6.3.1. 4-(Toluene-4-sulfonyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (29)

The compound was prepared in 65% yield as white powder; t_R = 1.95; MS calcd for $[M+H]^+$: $C_{18}H_{27}NO_5S$ *m/z*: 370.2, found 370.4; mp 84.0–84.2 °C. ^1H NMR ($CDCl_3$) δ (ppm) 1.02–1.16 (m, 2H), 1.43 (s, 9H), 1.62–1.66 (m, 2H), 1.77–1.86 (m, 1H), 2.45 (s, 3H), 2.60–2.69 (t, *J* = 12.6 Hz, 2H), 3.84–3.86 (d, *J* = 6.41 Hz, 2H), 4.06–4.10 (d, *J* = 11.8 Hz, 2H), 7.36–7.73 (d, *J* = 7.9 Hz, 2H), 7.76–7.79 (dd, *J* = 6.7 Hz, 2H).

6.3.2. 4-[2-(Toluene-4-sulfonyloxy)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (30)

The compound was prepared in 65% yield as white powder; t_R = 1.99; MS calcd for $[M+H]^+$: $C_{19}H_{29}NO_5S$ *m/z*: 383.2, found 384.1; mp 61.2–61.6 °C. ^1H NMR ($CDCl_3$) δ (ppm) 0.97–1.09 (m, 2H), 1.44 (s, 9H), 1.46–1.60 (m, 5H), 2.45 (s, 3H), 2.60–2.65 (m, 2H), 4.01–4.09 (m, 4H), 7.34–7.36 (d, *J* = 9.2 Hz, 2H), 7.78–7.81 (dd, *J* = 6.7 Hz, *J* = 1.79 Hz, 2H).

6.3.3. 4-[4-(4-Chloro-phenyl)-piperazin-1-ylmethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (31)

The compound was prepared in 64% yield as white powder; t_R = 1.34; MS calcd for $[M+H]^+$: $C_{21}H_{32}ClN_3O_2$ *m/z*: 393.2, found 394.4; mp 115.9–116.2 °C. ^1H NMR ($CDCl_3$) δ (ppm) 1.02–1.15 (m, 2H), 1.45 (s, 9H), 1.60–1.67 (m, 1H), 1.69–176 (m, 2H), 2.21–2.31 (d, *J* = 6.9 Hz, 2H), 2.53–2.56 (m, 4H), 2.65–2.74 (m, 2H), 3.12–3.16 (m, 4H), 4.08–4.11 (d, *J* = 9.7 Hz, 2H), 6.80–6.85 (m, 2H), 7.17–7.22 (m, 2H). Anal. ($C_{21}H_{32}ClN_3O_2$) C, H, N.

6.3.4. 4-[4-(4-Chloro-phenyl)-piperazin-1-ylethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (32)

The compound was prepared in 67% yield as white powder; t_R = 1.36; MS calcd for $[M+H]^+$: $C_{22}H_{34}ClN_3O_2$ *m/z*: 407.2, found 408.3; mp 92.2–92.4 °C. ^1H NMR ($CDCl_3$) δ (ppm) 1.12–1.18 (m, 2H), 1.20–1.32 (m, 1H), 1.45 (s, 11H), 1.64–1.68 (d, *J* = 12.3 Hz, 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 (d, *J* = 8.9 Hz, 2H), 7.18–7.26 (m, 2H) Anal. ($C_{22}H_{34}ClN_3O_2$) C, H, N.

6.4. General procedure for the preparation of compounds and 33–36 (Method B)

A mixture of 1-(4-chlorophenyl)-4-piperidin-4-ylmethyl-piperazine (**31**) or 1-(4-chloro-phenyl)-4-piperidin-4-ylethyl-piperazine (**32**) (1.2 mmol) in CH_2Cl_2 (7 mL), and DIEA (3.6 mmol) was cooled down (ice bath), and azinesulfonyl (1.3 mmol) was added at 0 °C in one portion. The reaction mixture was stirred for 2–6 h under cooling. Then, the solvent was evaporated and the sulfonamides were separated by column chromatography using SiO_2 and a mixture of CH_2Cl_2/MeOH = 9/0.5, as an eluting system. Free bases were then converted into the hydrochloride salts by treatment of their solution in anhydrous ethanol with 4 M HCl in dioxane.

6.4.1. 3-{4-[4-(4-Chlorophenyl)-piperazin-1-ylmethyl]-piperidine-1-sulfonyl}-quinoline (33)

The compound was prepared by the general method B starting from **31** in 62% yield as white powder mp 179.5–180.2 °C; t_R = 1.30; MS calcd for $[M+H]^+$: $C_{25}H_{29}ClN_4O_{2S}$ *m/z*: 484.2, found 485.3. ^1H NMR ($CDCl_3$) δ (ppm) 1.26–1.42 (m, 3H), 1.84–1.88 (d,

2H), 2.18–2.20 (d, 2H), 2.34–2.42 (m, 2H), 2.47–2.49 (m, 4H), 3.01–3.07 (t, 4H), 3.90–3.94 (d, 2H), 6.78–6.81 (m, 2H), 7.16–7.19 (m, 2H), 7.68–7.73 (m, 1H), 7.88–7.99 (m, 2H), 8.20–8.22 (d, 1H), 8.61–8.62 (d, J = 2.1 Hz, 1H), 9.18–9.19 (d, J = 2.3 Hz, 1H). Compound **33**·HCl mp 244.2–254.8 °C. Anal. ($C_{25}H_{29}ClN_4O_2S$·HCl) C, H, N.

6.4.2. 7-{4-[4-(4-Chlorophenyl)-piperazin-1-ylmethyl]-piperidine-1-sulfonyl}-quinoline (34)

The compound was prepared by the general method B starting from amine **31** in 71% yield as white powder mp 134.5–135.0 °C; t_R = 1.26; MS calcd for $[M+H]^+$: $C_{25}H_{29}ClN_4O_2S$ m/z: 484.2, found 485.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.19–1.43 (m, 3H), 1.81–1.85 (d, J = 11.54, 2H), 2.17–2.20 (d, J = 6.41, 2H), 2.31–2.39 (m, 2H), 2.47 (s, 4H), 3.08 (s, 4H), 3.89–3.93 (d, J = 12.0 Hz, 2H), 7.15–7.19 (m, 2H), 7.26–7.27 (m, 2H), 7.55–7.59 (m, 1H), 7.85–7.89 (m, 1H), 7.97–8.00 (d, J = 8.72, 1H), 8.24–8.27 (d, J = 7.7 Hz, 1H), 8.60 (s, 1H), 9.05–9.07 (m, 1H). Compound **34**·HCl mp 251.2–252.0 °C. Anal. ($C_{25}H_{29}ClN_4O_2S$·HCl) C, H, N.

6.4.3. 3-(4-{2-[4-(4-Chlorophenyl)-piperazin-1-yl]-ethyl}-piperidine-1-sulfonyl)-quinoline (35)

The compound was prepared by the general method B starting from amine **32** in 71% yield as white powder mp 174.0–174.9 °C; t_R = 1.34; MS calcd for $[M+H]^+$: $C_{26}H_{31}ClN_4O_2S$ m/z: 498.2, found 499.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.25–1.48 (m, 4H), 1.69 (s, 1H), 1.76–1.79 (m, 2H), 2.32–2.40 (m, 4H), 2.51–2.54 (t, J = 4.8 Hz, 4H), 3.10–3.13 (m, 4H), 3.87–3.91 (d, J = 11.8 Hz, 2H), 6.78–6.83 (m, 2H), 7.15–7.25 (m, 2H), 7.68–7.73 (m, 1H), 7.87–7.98 (m, 2H), 8.19–8.22 (d, J = 8.21, 1H), 8.60–8.61 (d, J = 2.0 Hz, 1H), 9.17–9.18 (d, J = 2.0 Hz, 1H). Compound **35**·HCl mp 251.3–252.0 °C. Anal. ($C_{26}H_{31}ClN_4O_2S$·HCl) C, H, N.

6.4.4. 4-(4-{2-[4-(4-Chlorophenyl)-piperazin-1-yl]-ethyl}-piperidine-1-sulfonyl)-isoquinoline (36)

The compound was prepared by the general method B starting from amine **32** in 73% yield as white powder mp 144.5–145.3 °C; t_R = 1.31; MS calcd for $[M+H]^+$: $C_{26}H_{31}ClN_4O_2S$ m/z: 498.2, found 499.3. ^{1}H NMR ($CDCl_3$) δ (ppm) 1.25–1.32 (m, 4H), 1.40–1.45 (m, 2H), 1.65 (s, 1H), 1.72–1.76 (d, J = 10.3 Hz, 2H), 2.32–2.37 (m, 2H), 2.51–2.56 (m, 4H), 3.10–3.13 (m, 4H), 3.89–3.93 (d, J = 12.0 Hz, 2H), 6.78–6.83 (m, 2H), 7.16–7.21 (m, 2H), 7.72–7.77 (m, 1H), 7.85–7.91 (m, 1H), 8.08–8.11 (d, J = 8.2 Hz, 1H), 8.70–8.73 (d, J = 8.7 Hz, 1H), 9.07 (s, 1H), 9.40 (s, 1H). Compound **36**·HCl mp 221.8–222.3 °C. Anal. ($C_{26}H_{31}ClN_4O_2S$·HCl) C, H, N.

6.5. In vitro pharmacology

6.5.1. Radioligand binding experiments

Compounds were tested in competition binding experiments for native 5-HT_{1A}, 5-HT_{2A} receptors, and dopamine D_2 as well as for cloned human 5-HT_6 and 5-HT_7 receptors, according to the previously published procedures.[20,22,23,27] The binding parameters are summarized in Table 1-SM. Following incubation, the receptor preparations were rapidly filtered under vacuum through GF/B glass fiber filters; the filters were washed extensively with an ice cold buffer using a harvester. Bound radioactivity was measured by scintillation counting using a liquid scintillation cocktail. Compounds (7–9 concentrations) were tested in triplicate. The inhibition constants (K_i) were calculated from the Cheng–Prushoff equation.[39] Results are expressed as means of at least three separate experiments.

6.5.2. Effects on adenylate cyclase activity

Adenylate cyclase activity is expressed as the percentage of the maximal effect obtained with 300 nM serotonin. The compounds were tested in 5 concentrations at 10^{-4}–10^{-9} in the h5-HT_7 antagonist effect. For the antagonists, the apparent dissociation constants (K_B) were calculated using the modified Cheng Prusoff equation ($K_B = IC_{50}/(1+(A/EC_{50}A))$, where A = concentration of reference agonist in the assay, and $EC_{50}A = EC_{50}$ value of the reference agonist).

6.6. In vivo pharmacology

6.6.1. Subjects

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 (accredited animal facility Jagiellonian University Medical College Kraków, Poland) and mice were kept in groups of ten to Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm). Male Wistar rats weighing 205–225 g obtained from accredited animal facility Jagiellonian University Medical College (Kraków, Poland) were housed in polycarbonate Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm) in groups of four. All 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 Kraków.

6.6.2. Experimental procedures

All the experiments were conducted in the light phase between 09.00 and 14.00 h. Each experimental group consisted of 5–10 animals/dose, and the animals were used only once in each test. The experiments were performed by an observer unaware of the treatment administered.

6.6.3. Lower lip retraction (LLR) in rats

The LLR was assessed according to the method of Berendsen et al.[28] The rats were individually placed in cages (30 × 25 × 25 cm) and were scored three times (at 15, 30 and 45 min after the tested compound or 8-OH-DPAT administration as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The sum at maximum score, amounted to 3 for each rat. The effect of the investigated compound on the LLR induced by 8-OH-DPAT (1 mg/kg) was assessed in a separate experiment. The investigated compound was administered 45 min before 8-OH-DPAT, and the animals were scored at 15, 30 and 45 min after 8-OH-DPAT administration.

6.6.4. Head twitch response in Swiss albino mice

In order to habituate mice to the experimental environment, each animal was randomly transferred to a 12 (diameter × 20 cm (height) glass cage, lined with sawdust 20 min before the treatment. Head twitches in mice were induced by (±)DOI (2.5 mg/kg). Immediately after treatment, the number of head twitches was counted during 20 min.[29] ID_{50} (the dose inhibiting the head twitches in mice by 50%) was calculated using Graph Pad Prism 5 Software.

6.6.5. Apomorphine-induced climbing behavior in Swiss albino mice

For observation, mice were placed in separate cages with walls made of metal bars. Apomorphine (3 mg/kg) was injected 10 min after the drugs. Twenty minutes after injection of apomorphine, time of climbing was determined for 2 min. Climbing time was defined as the period during which the animal held the 2, 3 or 4 paws on the wall. ID_{50} (the dose inhibiting climbing behavior in mice by 50%) was calculated using Graph Pad Prism 5 Software.

6.6.6. Forced swim test in Swiss albino mice

The experiment was carried out according to the method of Porsolt et al.[30] Briefly, mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 6 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.

6.6.7. Locomotor activity in mice

The locomotor activity was recorded with an Opto M3 multichannel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). The Swiss albino mice were individually placed in plastic cages (22 × 12 × 13 cm), and then the crossings of each channel (ambulation) were counted from 2 to 6 min, that is the time equal to the observation period in the forced swim test and during 30-min experimental sessions. 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.

6.6.8. MK-801-induced hyperlocomotor in CD-1 mice

The locomotor activity was recorded according to the method described above.

6.6.9. Elevated plus-maze in rats

Plus-maze apparatus (an automated device produced by Campden Instruments Ltd, UK) made of durable, high density, non porous black plastic, elevated to a height of 50 cm, consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 cm, and 30 cm high walls), arranged so that the two arms of each type were opposite each other. Floor of the plus-maze was made of infrared transparent material what means that there are no visible sensors. The plus-maze was placed in a darkened room, and the center of the apparatus was illuminated with a 25 W electric bulb hanging 100 cm above. Plus-maze apparatus was connected to PC software by control chassis. Each rat was gently placed in the center of the plus-maze, facing one of the closed arms, immediately after a 5-min adaptation period in a plastic black box (60 × 60 × 35 cm). During a 5-min test period, automated Motor Monitor System recorded the number of entries into the closed and open arms, the time spent in either type of the arms. After each trial the maze was wiped clean.

6.6.10. General locomotor activity in rats

The experiment was performed using plus-maze apparatus (an automated device produced by Campden Instruments Ltd, UK) (please see above). During a 5-min test period (i.e., the time equal to the observation period in the elevated plus-maze test), automated Motor Monitor System recorded the total entries, the total distance, X and Y ambulations; all parameters giving evidence to general locomotor activity, competing behavior which should be excluded in case of effective anxiolytic doses.

6.6.11. Drugs

The following drugs were used: imipramine (hydrochloride, Sigma), diazepam (Polfa, Poland), MK-801 (hydrogen maleate, Sigma) and tested compounds: **16**, **17**, **20**, **35**, **36**. Imipramine and MK-801 were dissolved in distilled water; remaining compounds were suspended in a 1% aqueous solution of Tween 80 immediately before administration. All the compounds were administered intraperitoneally at a volume of 10 ml/kg (mice) or 2 ml/kg (rats) and were given 30 min before the test, excluding diazepam and MK-801 which were injected 60 and 15 min, respectively, before testing. Control animals received a vehicle injection according to the same schedule.

6.6.12. Statistics

All the data are presented as the mean ± SEM. The statistical significance of the results was evaluated by a one-way ANOVA, followed by Bonferroni Comparison Test.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.039.

References and notes

1. Carr, G. V.; Lucky, I. *Psychopharmacology* **2011**, *213*, 265.
2. Hedlund, P. B.; Sutcliffe, J. G. *Trends Pharmacol. Sci.* **2004**, *25*, 418.
3. Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J., Jr.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403.
4. Hedlund, P. B. *Psychopharmacology* **2009**, *206*, 345.
5. Roberts, A. J.; Hedlund, P. B. *Hippocampus* **2011**. doi:10.1002/hipo.20938.
6. Wesołowska, A.; Nikiforuk, A.; Stachowicz, K.; Tatarczyńska, E. *Neuropharmacology* **2006**, *51*, 578.
7. Hedlund, P. B.; Huitron-Resendiz, S.; Henriksen, S. J.; Sutcliffe, J. G. *Biol. Psychiatry* **2005**, *58*, 831.
8. Abbas, A. I.; Hedlund, P. B.; Huang, X. P.; Tran, T. B.; Meltzer, H. Y.; Roth, B. L. *Psychopharmacology* **2009**, *205*, 119.
9. Sarkisyan, G.; Roberts, A. J.; Hedlund, P. B. *Behav. Brain Res.* **2010**, *209*, 99.
10. Leucht, S.; Corves, C.; Arbter, D.; Engel, R. R.; Li, C.; Davis, J. M. *Lancet* **2009**, *373*, 31.
11. Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; Forbes, I. T.; Jennings, A. J.; King, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunders, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thomas, D. R. *J. Med. Chem.* **2000**, *43*, 342.
12. Raubo, P.; Beer, M. S.; Hunt, P. A.; Huscroft, I. T.; London, C.; Stanton, J. A.; Kulagowski, J. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1255.
13. Kikuchi, C.; Ando, T.; Watanabe, T.; Nagaso, H.; Okuno, M.; Hiranuma, T.; Koyama, M. *J. Med. Chem.* **2002**, *45*, 2197.
14. Vermeulen, E. S.; van Smeden, M.; Schmidt, A. W.; Sprouse, J. S.; Wikström, H. V.; Grol, C. J. *J. Med. Chem.* **2004**, *47*, 5451.
15. Leopoldo, M.; Lacivita, E.; Berardi, F.; Perrone, R.; Hedlund, P. B. *Pharmacol. Ther.* **2011**, *129*, 120.
16. Zajdel, P.; Subra, G.; Bojarski, A. J.; Duszyńska, B.; Pawłowski, M.; Martinez, J. *Bioorg. Med. Chem.* **2005**, *13*, 3029.
17. Medina, R. A.; Sallander, J.; Benhamu, B.; Porras, E.; Campillo, M.; Pardo, L.; Lopez-Rodriguez, M. L. *J. Med. Chem.* **2009**, *52*, 2384.
18. Zajdel, P.; Subra, G.; Bojarski, A. J.; Duszyńska, B.; Martinez, J.; Pawłowski, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4827.
19. Volk, B.; Barkóczy, J.; Hegedús, E.; Udvari, S. Z.; Gacsályi, I.; Mezei, T.; Pallagi, K.; Kompagne, H.; Lévay, G.; Egyed, A.; Hársing, L. G., Jr.; Spedding, M.; Simig, G. *J. Med. Chem.* **2008**, *51*, 2522.
20. Zajdel, P.; Marciniec, K.; Maślankiewicz, A.; Paluchowska, M.; Satała, G.; Partyka, A.; Jastrzebska-Więsek, M.; Wróbel, M.; Wesołowska, A.; Duszyńska, B.; Bojarski, A. J.; Pawłowski, M. *Bioorg. Med. Chem.* **2011**, *19*, 6750.
21. Marciniec, K.; Maślankiewicz, A.; Pawłowski, M.; Zajdel, P. *Heterocycles* **2007**, *71*, 1975.
22. Bojarski, A. J.; Cegła, M. T.; Charakchieva-Minol, S.; Mokrosz, M. J.; Maćkowiak, M.; Misztal, S.; Mokrosz, J. L. *Pharmazie* **1993**, *48*, 289.
23. Bojarski, A. J.; Paluchowska, M. H.; Duszyńska, B.; Kłodzińska, A.; Tatarczyńska, E.; Chojnacka-Wójcik, E. *Bioorg. Med. Chem.* **2005**, *13*, 2293.
24. Paluchowska, M. H.; Bugno, R.; Tatarczyńska, E.; Nikiforuk, A.; Lenda, T.; Chojnacka-Wójcik, E. *Bioorg. Med. Chem.* **2007**, *15*, 7116.
25. MacKenzie, R. G.; VanLeeuwen, D.; Pugsley, T. A.; Shih, Y. H.; Demattos, S.; Tang, L.; Todd, R. D.; O'Malley, K. L. *Eur. J. Pharmacol.* **1994**, *266*, 79.
26. Van Tol, H. H.; Wu, C. M.; Guan, H. C.; Ohara, K.; Bunzow, J. R.; Civelli, O.; Kennedy, J.; Seeman, P.; Niznik, H. B.; Jovanovic, V. *Nature* **1992**, *358*, 149.
27. Adham, N.; Zgombick, J. M.; Bard, J.; Branchek, T. A. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 508.

28. Berendsen, H. H.; Jenck, F.; Broekkamp, C. L. *Pharmacol. Biochem. Behav.* **1989**, *33*, 821.
29. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. *Pharmacol. Biochem. Behav.* **1990**, *36*, 901.
30. Porsolt, R. D.; Bertin, A.; Jalfre, M. *Arch. Int. Pharmacodyn. Ther.* **1977**, *229*, 327.
31. Chen, J.; Ding, K.; Levant, B.; Wang, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 443.
32. Graulich, A.; Leonard, M.; Resimont, M.; Huang, X.-P.; Roth, B. L.; Liegeois, J.-F. *Aust. J. Chem.* **2010**, *63*, 56.
33. Lober, S.; Hubner, H.; Tschammer, N.; Gmeiner, P. *Trends Pharmacol. Sci.* **2011**, *32*, 148.
34. Butini, S.; Gemma, S.; Campiani, G.; Franceschini, S.; Trotta, F.; Borriello, M.; Ceres, N.; Ros, S.; Coccone, S. S.; Bernetti, M.; De Angelis, M.; Brindisi, M.; Nacci, V.; Fiorini, I.; Novellino, E.; Cagnotto, A.; Mennini, T.; Sandager-Nielsen, K.; Andreasen, J. T.; Scheel-Kruger, J.; Mikkelsen, J. D.; Fattorusso, C. *J. Med. Chem.* **2009**, *52*, 151.
35. Zajdel, P.; Subra, G.; Bojarski, A. J.; Duszyńska, B.; Tatarczyńska, E.; Nikiforuk, A.; Chojnacka-Wójcik, E.; Pawłowski, M.; Martinez, J. *Bioorg. Med. Chem.* **2007**, *15*, 2907.
36. Wesołowska, A. *Pharmacol. Rep.* **2010**, *62*, 564.
37. Carlsson, M.; Carlsson, A. *Trends Neurosci.* **1990**, *13*, 272.
38. Jentsch, J. D.; Roth, R. H. *Neuropsychopharmacology* **1999**, *20*, 201.
39. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.