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Original article

Synthesis and pharmacological evaluation of new 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-dione derivatives. Novel 5-HT_{1A} receptor agonist with potential antidepressant and anxiolytic activity

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ABSTRACT

The synthesis of 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-dione derivatives with an arylpiperazinylpropyl moiety (**12**–**23**) and their in vitro and in vivo pharmacological properties and molecular characteristics were described. The investigated compounds exhibited high affinity for 5-HT_{1A} (**13**–**22**) and 5-HT_{2A} (**18**, **20**, **21**, **23**) receptors and diversified pharmacological profile. Compounds **17**, **20** and **22** showed antagonistic, partial agonistic and agonistic activity, respectively, toward 5-HT_{1A} receptor and they were investigated as potential antidepressants and/or anxiolytics. The most interesting compound **22** (1-[3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl]-3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione), a pre- and postsynaptic 5-HT_{1A} receptor agonist produced an antidepressant-like effect, which was more pronounced than that of imipramine in the forced swim test in mice, without affecting locomotor activity. Moreover, compound **22** produced a weak anxiolytic-like effect in the four-plate test in mice. Molecular docking studies of compound **22** to the homology model of the 5-HT_{1A} receptor showed that a 3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione moiety played an important role in stabilizing the ligand–receptor complex.

1. Introduction

The role of serotonin (5-HT) in the central nervous system and its importance for the pathogenesis and treatment of mood disorders are unquestionable. 5-HT is believed to act via an interaction with 14 identified 5-HT receptor subtypes, of which 5-HT_{1A} one is the most extensively studied because of its specific connotation of affective disorders [1,2]. Despite a great number of currently marketed antidepressants and anxiolytics, continual efforts are made to develop new drugs with greater efficacy (especially in non-responders), an earlier onset of therapeutic improvement and less intensive side-effects [3]. Among the numerous potential drug candidates currently explored, 5-HT_{1A} receptor agonists are a group of high interest, which is evidenced by not only the number of scientific studies [4–6], but also the fact that some of them are currently in different phases of clinical trials for the treatment of depression and anxiety (OPC-14523, II phase, Pharmos [7]; MN-305, II/III phase, MediciNova; AP-521, II phase, Asahi Kasei Corporation; Vilazodone, III phase, PGxHealth, LLC [8]; Gepirone ER, III phase, Fabre-Kramer [9]) as well as Alzheimer's disease (Xaliproden, III phase, Sanofi-Aventis [10]).

In the course of our studies with active 5-HT_{1A} and 5-HT_{2A} ligands, a series of β-tetralinohydantoin (3′,4′-dihydro-1′H-spiro[imidazolidine-4,2′-naphthalene]-2,5-dione) derivatives were synthesized. Among them, a few compounds were especially interesting due to their 5-HT_{1A} receptor agonistic activity and potential anxiolytic/antidepressant properties [11,12]. As a continuation of our research, in the present experiment we synthesized a series with a novel terminal amide part containing 5-(cyclo)alkyl-5-phenylimidazolidine-2,4-diones (5-cyclopropyl-5-phenyl (**15**–**17**) and 5-methyl-5-phenylimidazolidine-2,4-diones (**12**–**14**)), or their counterparts with 5-spiroimidazolidino-2,4-diones (2′,3′-dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**18**–**20**) and 3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-diones (**21**–**23**)). We chose those terminal amide parts to examine the influence of structural changes at the 5-position of the imidazolidine ring on receptor affinity and pharmacological profile. The planned compounds possess an asymmetric carbon atom at

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terminal amide part which can exist in two stereoisomeric forms R or S. The racemic compounds were chosen for the synthesis with the aim to evaluate in vitro affinity at central serotonin and dopamine receptors. To explore the effect of structural modifications at terminal amide part, 5-HT_{1A}, 5-HT_{2A} and dopamine D_2 receptor affinities and in vivo intrinsic activities were determined for the newly synthesized compounds. Three compounds (**17**, **20**, **22**) with a diversified 5-HT_{1A} functional profile were chosen and examined using common behavioural tests for predicting antidepressant and/or anxiolytic like activity in mice. Furthermore, to ascertain the potential ligand binding mode of the studied compounds within the 5-HT_{1A} receptor, the most interesting one (**22**) was docked to the homology model of that receptor, and molecular interactions, potentially important to its pharmacological activity, were described.

2. Chemistry

The compounds were synthesized according to Scheme 1. The appropriate 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-diones (**4**–**7**) were prepared from a ketone by means of the Bucherer–Bergs reaction with modifications described by Goodson et al. [11,13]. Compounds **12**–**23** were obtained by a two-step procedure involving alkylation of imidazolidino-2,4-diones at N3 position (intermediate compounds **8**–**11**) with 1-bromo-3-chloropropane and condensation of intermediates with the appropriate arylpiperazine (final bases **12**–**23**). The structures of the compounds were established using spectral ^{1}H NMR spectra and an elemental analysis. The detailed spectral data of each molecule are presented in experimental section. For further pharmacological in vitro and in vivo studies, bases **12**–**23** were transformed into water-well-soluble hydrochlorides.

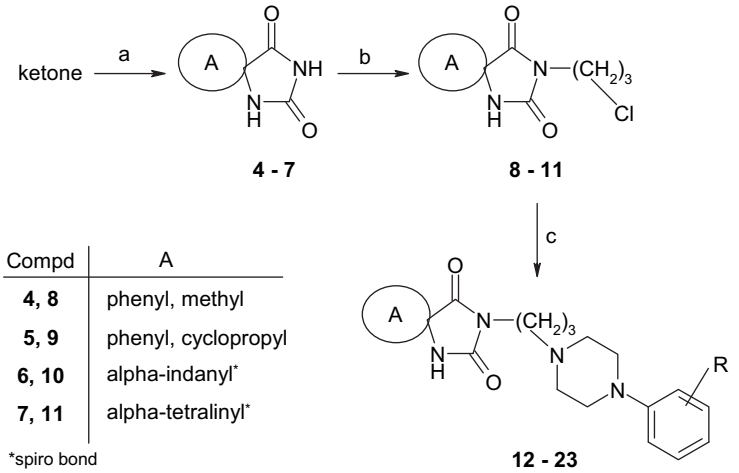
3. Pharmacology

3.1. In vitro tests

The affinities of all the synthesized compounds (**12**–**23**) for central serotonin 5-HT_{1A} and 5-HT_{2A} and dopamine D_2 receptors were evaluated on the basis of their ability to displace [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), [^{3}H]-ketanserin and [^{3}H]-spiperone, respectively. Radioligand binding studies were conducted on rat brain using the cerebral cortex (5-HT_{1A}, 5-HT_{2A}) and the striatum (D_2) [14–16].

3.2. In vivo tests

Compounds with the highest affinities for 5-HT_{1A} (**13**, **14**, **15**, **16**, **17**, **19**, **20**, **22**) and 5-HT_{2A} (**18**, **20**, **21**, **23**) receptors were investigated in in vivo models to establish their 5-HT_{1A} and/or 5-HT_{2A} intrinsic activity.



Compd	A
4, 8	phenyl, methyl
5, 9	phenyl, cyclopropyl
6, 10	alpha-indanyl*
7, 11	alpha-tetralinyl*

*spiro bond

Compd	A	R	Compd	A	R
12	phenyl, methyl	H	**18**	α-indanyl*	H
13	phenyl, methyl	2-OCH_3	**19**	α-indanyl*	2-OCH_3
14	phenyl, methyl	3-Cl	**20**	α-indanyl*	3-Cl
15	phenyl, cyclopropyl	H	**21**	α-tetralinyl*	H
16	phenyl, cyclopropyl	2-OCH_3	**22**	α-tetralinyl*	2-OCH_3
17	phenyl, cyclopropyl	3-Cl	**23**	α-tetralinyl*	3-Cl

*spiro bond

Reagents, reaction conditions:

(a) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol;

(b) $Br(CH_2)_3Cl$, K_2CO_3, acetone, reflux;

(c) 1-phenylpiperazine derivative, anhydrous ethyl alcohol, reflux;

Scheme 1. The synthesis pathways of the investigated compounds.

It was already proved that the hypothermia induced by 8-hydroxy-2-(di-n-propylamino)tetralin) (8-OH-DPAT, a 5-HT_{1A} receptor agonist) and abolished by (*N*-{2[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635, a selective 5-HT_{1A} receptor antagonist) in mice was mediated by activation of presynaptic 5-HT_{1A} receptors [17,18]. Thus the ability of the tested compounds to produce that effect was regarded as presynaptic 5-HT_{1A} receptor agonistic activity. The compounds that either did not change mouse body temperature or abolished or attenuated the hypothermic effect of 8-OH-DPAT were regarded as presynaptic 5-HT_{1A} receptor antagonists. To determine the postsynaptic 5-HT_{1A} receptor agonistic effect of the tested compounds, their ability to induce lower lip retraction (LLR) in rats was tested. It had been commonly accepted that the 8-OH-DPAT-induced LLR in rats depended on stimulation of postsynaptic 5-HT_{1A} receptors [19,20]. Moreover, it was shown that the later symptom was sensitive to a 5-HT_{1A} receptor antagonist [18]. The ability of the tested compounds to inhibit the 8-OH-DPAT-induced LLR was regarded as postsynaptic 5-HT_{1A} receptor antagonistic activity. In order to determine the central 5-HT_{2A} receptor antagonistic properties of the tested compounds, their ability to inhibit the head twitches induced by the 5-HT_{2A} receptor agonist (±)-DOI was studied in mice [21,22]. The results of in vitro and in vivo studies on the newly synthesized compounds **12**–**23** are presented in Table 1.

The potential antidepressant activity of compound **22** was evaluated by the forced swim test in mice [23], and the potential anxiolytic properties of compounds **17**, **20**, **22** were determined by the four-plate test in mice [24]. To check possible occurrence of drug-induced changes in the locomotor activity of mice, which may have contributed to their behaviour in those two tests, the influence of compound **22** on locomotor activity was also examined.

4. Molecular modeling

The potential ligand binding mode of the investigated compounds within the 5-HT_{1A} receptor was studied using *in silico* modeling techniques, by means of the automated docking of the most interesting compound **22** to the homology, a rhodopsin-based model of the 5-HT_{1A} receptor.

5. Results and discussion

Since ligands at 5-HT_{1A}/5-HT_{2A}/D_2 receptor sites seem to be of therapeutic interest, extensive studies with chemically different structures are under way in search of new potential drugs. To continue our investigations on β-tetralinohydantoins [11,12], we designed, synthesized and tested in vitro and in vivo the activity of new 5-substituted imidazolidine-2,4-dione derivatives with the arylpiperazinepropyl moiety (**12**–**23**). The affinity of the synthesized compounds for 5-HT_{1A} receptors ranged from 11 to 457 nM. The majority of compounds (except **23**) with a 3-chloro or a 2-methoxy group in the phenylpiperazine ring displayed very high affinity for 5-HT_{1A} receptors (K_i < 50 nM), the best ligands being **16** and **17** with contained 5-cyclopropyl-5-phenylimidazolidine-2,4-dione (K_i = 11 nM and 13 nM, respectively) (Table 1). In general, introduction of a substituent into the phenylpiperazine moiety had a positive impact on the binding at 5-HT_{1A} receptor sites. In fact, the unsubstituted analogues (**12**, **15**, **18** and **21**) showed lower 5-HT_{1A} receptor affinity. It is also noteworthy that high and significant affinity for 5-HT_{2A} receptors, which ranged from 25 to 53 nM, was found for some of the 5-spiroimidazolidine-2,4-dione derivatives, unsubstituted (**18**, **21**) and 3-Cl substituted (**20**, **23**) in phenylpiperazine fragment.

Moreover, few selected compounds (**14**, **18**, **20**, **21**, **22**, **23**) were evaluated in respect of their affinity for dopaminergic D_2 receptors. Compounds **21** and **22** demonstrated very low affinity for D_2 receptors (K_i = 675 ± 82 nM and 965 ± 6 nM, respectively), whereas **14**, **18**, **20**, and **23** were practically devoid of any affinity (K_i > 1800 nM).

In the following study, selected compounds with the highest affinity for 5-HT_{1A} (K_i up to 70 nM) (**13**, **14**, **15**, **16**, **17**, **19**, **20**, **22**) and/or 5-HT_{2A} (**18**, **20**, **21**, **23**) receptors were further examined by functional in vivo assays. The results of our in vivo experiments clearly suggest that the investigated compounds show diversified (full agonistic, partial agonistic or antagonistic) intrinsic activity, agonistic or partial agonistic at 5-HT_{1A} receptors and have 5-HT_{2A} antagonistic properties (Table 1). Compounds with a 2-methoxyphenylpiperazine (**13**, **16**, **19** and **22**) induced effects characteristic of presynaptic 5-HT_{1A} receptor agonists (the hypothermia model in mice), but only **22**, containing α-tetralin in the terminal amide part revealed features of an agonist of postsynaptic 5-HT_{1A} receptors (the LLR model in rats); on the other hand, the remaining analogues were inactive (**13**, **16**) or devoid of intrinsic activity at those sites (**19**). Therefore it seems that the α-tetralin substituent in the amide fragment may contribute the postsynaptic 5-HT_{1A} agonism to those compounds. The results of functional studies with derivatives with the 3-chlorophenylpiperazine moiety (**14**, **17** and **20**) indicate that their presynaptic 5-HT_{1A} receptor activity is diversified, since **14** was inactive, **17** behaved like an antagonist and **20** like an agonist of those sites. On the other hand, those compounds (**14**, **17** and **20**) showed antagonistic activity against postsynaptic 5-HT_{1A}

Table 1
5-HT_{1A}, 5-HT_{2A} and D_2 receptor affinities, and functional in vivo 5-HT_{1A}/5-HT_{2A} receptor activity of compounds **12**–**23**.

Compd	K_i (nM)			5-HT_{1A} activity		5-HT_{2A} activity
	5-HT_{1A}	5-HT_{2A}	D_2	Presynaptic	Postsynaptic	
12	457 ± 142	69 ± 6	nd	nd	nd	nd
13	47 ± 1	730 ± 68	nd	Agonist	Non active	nd
14	29 ± 5	312 ± 21	3900 ± 600	Non active	Antagonist	nd
15	67 ± 12	100 ± 12	nd	Non active	Non active	nd
16	11 ± 1	710 ± 88	nd	Agonist	Non active	nd
17	13 ± 1	78 ± 1	nd	Antagonist	Antagonist	nd
18	98 ± 16	30 ± 2	15600 ± 300	nd	nd	Antagonist
19	49 ± 2	653 ± 100	nd	Agonist	Antagonist	nd
20	38 ± 1	53 ± 6	6200 ± 500	Agonist	Antagonist	Antagonist
21	88 ± 21	25 ± 5	675 ± 82	nd	nd	Antagonist
22	23 ± 5	284 ± 9	965 ± 6	Agonist	Agonist	nd
23	350 ± 123	35 ± 6	1800 ± 300	nd	nd	Antagonist

nd: not determined; full agonist – compound administrated at doses up to 20 mg/kg behaved as agonist of pre- and postsynaptic 5-HT_{1A} receptors; partial agonist – compound administrated at doses up to 20 mg/kg behaved as both presynaptic agonist and postsynaptic antagonist of 5-HT_{1A} receptors; non active – compound administrated at doses up to 20 mg/kg behaved as neither agonist nor antagonist of pre- and/or postsynaptic 5-HT_{1A} receptors.

receptors. The above observations indicate that depending on the combination of the structural features described in the present paper yielded compounds with diversified 5-HT_{1A} receptor properties, i.e. full agonistic (**22**, pre- and postsynaptic agonist), partial agonistic (**20**, presynaptic agonist and postsynaptic antagonist), or antagonistic (**17**, pre- and postsynaptic antagonist). It is noteworthy that **20** is also an antagonist of 5-HT_{2A} receptors.

It has been commonly accepted that 5-HT_{1A} receptor agonists, partial agonists and antagonists, as well as combined 5-HT_{1A}/5-HT_{2A} receptor antagonists exhibit antidepressant- and/or anxiolytic-like effects [25–30]. Taking into account the intrinsic activity of the investigated compounds, we selected compounds **22** (a full 5-HT_{1A} receptor agonist), **20** (a partial 5-HT_{1A} receptor agonist/5-HT_{2A} receptor antagonist) and **17** (a 5-HT_{1A} receptor antagonist) for further in vivo preclinical studies. The potential antidepressant (**22**) and anxiolytic (**17**, **20**, **22**) activity of the selected compounds was evaluated in the forced swim [23] and four-plate [24] tests in mice, respectively. Our present results showed that compound **22** given at doses of 10 and 20 mg/kg produced a distinct antidepressant-like effect in the forced swim test in mice. Moreover, it shortened mouse immobility time at a dose of 10 mg/kg which is lower than that of the reference antidepressant imipramine (Table 2). Its anti-immobility effect seems to be specific, since at doses effective in the forced swim test (10 and 20 mg/kg) that compound did not change the locomotor activity of mice during 6- or 30-min experimental sessions (Table 4). Imipramine showed a weak sedative effect in mice only when their locomotor activity was recorded for 30 min (Table 4). Moreover, compound **22** exhibited anxiolytic-like activity in the four-plate test in mice (Table 3); in terms of potency and active doses, its effect was weaker than that of diazepam used as a reference anxiolytic. Compounds **17** and **20** were ineffective in that test (Table 3). Compound **22** displayed an anxiolytic-like effect practically at one medium dose only, but produced antidepressant-like activity in a dose-dependent manner. A limited number of data concerning in vivo functional activity of compound **22** do not permit us to find an explanation for the loss of anxiolytic-like activity after administration of its higher doses. It is noteworthy that some anxiolytics produce U-shaped dose–response effects in animal models commonly used for the prediction of potential anxiolytic activity [31].

Molecular docking studies with compound **22** in the homology model of 5-HT_{1A} receptor revealed that the investigated compound interacted with the receptor at the binding site localized inside the heptahelical bundle on the extracellular side of the receptor. The ligand **22** was found to be placed along transmembrane helix (TMH) 3, its arylpiperazine moiety being located between TMHs 4–6 and the 3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione fragment localized in the vicinity of TMHs 7, 1 and 2. (Fig. 1) The main interaction anchoring the ligand in the receptor was a charge-reinforced hydrogen bond between the protonated nitrogen atom of the piperazine ring and Asp3.32 (Ballesteros's and Weinstein's nomenclature). This interaction is widely accepted to be crucial in the group of monoamine neurotransmitter receptors [32–35].

The phenyl ring substituted at the piperazine moiety was localized in the close vicinity of the aromatic cluster of TMH 6; it formed a CH-π interaction with Phe6.52, while its 2′-methoxy substituent formed a hydrogen bond with Ser5.42. The putative role of Phe6.52 and Ser5.42 in binding monoaminergic receptor ligands was postulated in literature [33,34,36,37], however other residues like: Phe6.51 and Phe3.28 or Ser/Thr5.43 were also proposed as counterparts for aromatic and H-bond accepting moieties of serotonin receptor ligands, respectively [38].

Table 2
The effects of **22** and imipramine in the forced swim test in mice.

Treatment	Dose (mg/kg)	Immobility time (s) Mean ± SEM
Vehicle	–	181.4 ± 4.9
22	5	161.2 ± 14.0
	10	121.4 ± 12.1[a]
	20	103.1 ± 11.5[a]
		$F(3,32) = 10.298$
		$p < 0.0001$
Vehicle	–	167.1 ± 6.7
Imipramine	10	149.1 ± 10.7
	20	107.8 ± 12.4[b]
		$F(2,27) = 8.760$
		$p < 0.01$

Compound **22** and imipramine were administered 30 min before the test. $n = 9$–10 mice per group.
[a] $p < 0.01$ *vs.* vehicle (Dunnett's test).

Table 3
The effects of **17**, **20**, **22** and diazepam in the four-plate test in mice.

Treatment	Dose (mg/kg)	Number of punished crossings mean ± SEM
Vehicle	–	3.8 ± 0.2
17	10	3.8 ± 0.4
	20	3.9 ± 0.3
	30	3.7 ± 0.3
		$F(3,36) = 0.0543$
		ns
Vehicle	–	4.2 ± 0.4
20	10	4.0 ± 0.3
	20	4.2 ± 0.2
	30	4.2 ± 0.2
		$F(3,36) = 0.125$
		ns
Vehicle	–	3.8 ± 0.2
22	5	3.9 ± 0.4
	10	5.9 ± 0.5[b]
	20	4.4 ± 0.3
		$F(3,36) = 6.065$
		$p < 0.01$
Vehicle[c]	–	3.5 ± 0.4
Diazepam	1.25	5.5 ± 0.5[a]
	2.5	6.8 ± 0.6[b]
	5	6.7 ± 0.6[b]
		$F(3,36) = 9.514$
		$p < 0.001$

Compound **22** was administered 30 min, while **20**, **17** – 60 min before the test. $n = 10$ mice per group.
ns: non-significant.
[a] $p < 0.05$.
[b] $p < 0.01$ *vs.* vehicle (Dunnett's test).
[c] Data taken from Ref. [34].

Table 4
The effects of **22** and imipramine on the locomotor activity of mice.

Treatment	Dose (mg/kg)	Locomotor activity: number of crossings during:	
		6 min	30 min
Vehicle	–	107.1 ± 8.5	249.2 ± 21.0
22	10	110.0 ± 7.0	212.9 ± 23.8
	20	111.6 ± 9.8	225.3 ± 18.6
		F(2,27) ± 0.072	F(2,27) ± 0.479
		ns	ns
Vehicle	–	94.8 ± 4.1	210.7 ± 17.7
Imipramine	10	94.4 ± 6.3	208.7 ± 27.6
	20	89.1 ± 6.1	141.6 ± 15.3[a]
		$F(2,27) = 0.321$	$F(2,27) = 3.541$
		ns	$p < 0.05$

Compound **22** and imipramine were administered 30 min before the test. $n = 10$ mice per group.
ns: non-significant.
[a] $p < 0.05$ *vs.* vehicle (Dunnett's test).

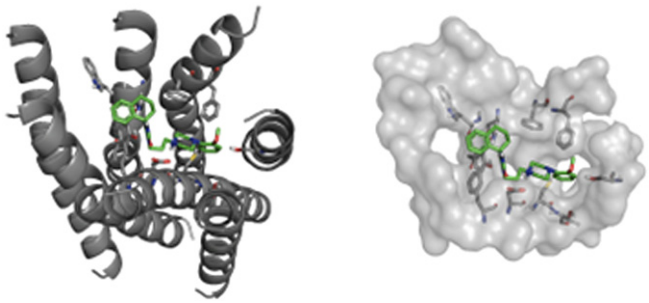


Fig. 1. The ligand binding mode of compound **22** in the 5-HT_{1A} receptor (a view from the extracellular side); amino acids entering into specific interactions with ligands are presented as "sticks". (A) "Cartoon" helices; (B) the surface of the binding site.

The substituted imidazolidine-2,4-dione moiety was found to form a bifurcated hydrogen bond between the carbonyl oxygen atom at 5-position of the imidazolidine ring, and Tyr7.43 and Asn7.39. The spiro-substituted tetralin moiety had favorable van der Waals's contacts with the hydrophobic residues of TMHs 7,1 and 2, especially Phe3.28, yet, without entering into any specific interaction.

The binding mode of compound **22** is in line with the binding mode of long chain arylpiperazines proposed by us earlier [39,40], and opposite to the binding mode of arylpiperazine derivatives with hydantoin moiety proposed by Lopez-Rodriguez et al. [41–43], considering orientation of the ligand in the binding site.

Since the investigated compounds possess an asymmetry center at the 4-(spiro) position of the imidazolidine ring, they can interact with the receptor in an R or S stereoisomer. To find out which stereoisomer is most probably responsible for the activity of the investigated compounds on the 5-HT_{1A} receptor, compound **22** was docked to the receptor model in both stereoisomeric forms. As a result, a more favorable binding mode was found for the R stereoisomer. As a consequence of above molecular modeling results, we are going to synthesize the R stereoisomer of the most promising compound **22** and further investigate its antidepressant activity.

6. Conclusions

A few new 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-dione derivatives with the arylpiperazinylpropyl moiety were designed and synthesized. The replacement of a flexible 5-alkyl-5-phenyl by a rigid 5-spiroring in the imidazolidine-2,4-dione moiety resulted in the diversification of intrinsic activity at the 5-HT_{1A} receptor binding site. The introduction of a (cyclo)alkyl-phenyl or spiro substituent to the amide moiety and 2-methoxy or 3-chloro group to the phenylpiperazine ring substantially changed the pharmacological in vivo properties of the compounds tested i.e. **17** became a pre- and postsynaptic 5-HT_{1A} antagonist and **22** was a pre- and postsynaptic 5-HT_{1A} agonist. Additionally, molecular docking showed that the cyclic amide part (3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione moiety) played an important role in stabilizing the 5-HT_{1A} receptor–ligand complex. Of the obtained compounds, **22** was the only one, that behaved like a potent agonist of pre- and postsynaptic 5-HT_{1A} receptors in the behavioural tests used. Moreover, compound **22** showed antidepressant and anxiolytic like activity in animal models and seemed to be devoid of any unfavourable motor effects. However, the present data need to be corroborated by further pharmacological studies in order to establish the position of compound **22** as a new full 5-HT_{1A} agonist and to determine the mechanism of its potential antidepressant activity. The study shows that its in vitro affinity and functional profile is interesting, especially with respect to its potential antidepressant and anxiolytic effects. In this connection, new butyl derivatives of 5-spiroimidazolidine-2,4-dione are being currently developed.

7. Experimental protocols

7.1. Chemistry

Melting points (m.p.) were determined in open capillaries on the Electrothermal 9100 melting point apparatus (Jencons) and were uncorrected. Elemental analysis was carried out using an elemental Vario EI III Elementar analyzer (Hanau, Germany). An ascending thin-layer chromatography method was performed on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany) using the following solvents: benzene/ethyl acetate/acetone (10:5:1), acetone/isopropanol/chloroform (20:10:1). Spots were detected by their absorption under UV light ($\lambda = 254$ nm). Analytical HPLC was conducted on a Waters HPLC instrument with Waters 485 Tunable Absorbance Detector UV, equipped with a Symetry column (C18, 3.5 μm, 4.6 × 30 mm). A flow rate of 5 ml/min and a 3 min gradient of water/0.1% TFA and acetonitrile/0.1% TFA (from 0 to 100%) were used, detection at 214 nm. The purity of the investigated compounds **12**–**23** ranged from 95 to 99 per cent.

^{1}H NMR spectra were recorded with a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 300 MHz in a $CDCl_3$ solution, with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown as δ values (ppm), the *J* values are expressed in Hertz (Hz). Signal multiplicities are represented as s (singlet), d (doublet), t (triplet), q (quintet) or m (multiplet).

The substituted 1-phenylpiperazine derivatives, 1-bromo-3-chloropropane, α-indanone, α-tetralone, methyl(phenyl)ketone, cyclopropyl(phenyl)ketone and other chemicals were commercially available (Aldrich or Fluka).

7.1.1. General procedure for preparation of imidazolidine-2,4-diones (**4**–**7**)

All imidazolidine-2,4-diones (**4**–**7**) were prepared from the ketone by Bucherer–Bergs reaction with modifications described by Goodson et al. [13].

A solution of ketone (330 mmol) and ammonium carbonate (1 mol) in ethanol (330 ml) and water (220 ml) was warmed to 50 °C, at which time potassium cyanide (350 mmol) dissolved in 50 ml of water was dropped in over a period of 15 min. The mixture was heated under a reflux at 56–60 °C for more than 20 h. The reflux condenser was then replaced by an air condenser and the temperature raised to 80 °C for 1 h to remove the excess ammonium carbonate. Then the reaction solution was cooled and acidified. The precipitated solid was filtered off, washed with water, and recrystallized from a mixture of ethanol and water, giving **4**–**7** hydantoin.

*7.1.1.1. 5-Methyl-5-phenylimidazolidine-2,4-dione (**4**).* Following the general procedure **4** was obtained from acetophenone (50° EtOH). Yield: 51%; m.p. 180–182 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.52 (s, 1H), 8.15 (s, 1H), 7.55–7.30 (m, 5H), 1.85 (s, 3H). Anal. calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.52; H, 5.45; N, 14.92.

*7.1.1.2. 5-Cyclopropyl-5-phenylimidazolidine-2,4-dione (**5**).* Following the general procedure **5** was obtained from cyclopropyl(phenyl) methanone (50° EtOH). Yield: 66%; m.p. 184–185 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.78 (s, 1H), 8.30 (s, 1H), 7.60–7.50 (m, 2H), 7.45–7.30 (m, 3H), 1.75–1.52 (m, 1H), 0.60–0.24 (m, 4H). Anal. calcd

for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96. Found: C, 65.12; H, 4.92; N, 12.97.

*7.1.1.3. 2′,3′-Dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**6**).* This compound was previously described [13].

*7.1.1.4. 3′,4′-Dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione (**7**).* This compound was previously described [13].

*7.1.2. General procedure for synthesis of 3-(3-chloropropyl)imidazolidine-2,4-dione derivatives (**8**–**11**)*

A suspension of appropriate imidazolidine-2,4-dione (50 mmol) and anhydrous K_2CO_3 (150 mmol) was refluxed in acetone (160 ml) under intensive stirring for 30 min. Afterwards a solution of 1-bromo-3-chloropropane (55 mmol) in acetone (50 ml) was instilled during the period of 20 min. The reaction was carried on for 8–14 h. The hot reaction mixture was then filtered off, the solvent was evaporated and the oily residue was purified by crystallization from a 40° or 50° ethanol.

*7.1.2.1. 3-(3-Chloropropyl)-5-methyl-5-phenylimidazolidine-2,4-dione (**8**).* Following the general procedure **8** was obtained from **4** (40° EtOH). Yield: 98%; m.p. 107–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53–7.33 (m, 5H), 6.58 (s, 1H), 3.70–3.63 (t, $J = 6.8$ Hz, 2H), 3.53–3.47 (t, $J = 6.5$ Hz, 2H), 2.16–2.03 (q, $J = 6.6$ Hz, 2H), 1.83 (m, 3H). Anal. calcd for $C_{13}H_{15}N_2O_2Cl$: C, 58.54; H, 5.67; N, 10.50. Found: C, 58.27; H, 5.60; N, 10.46.

*7.1.2.2. 3-(3-Chloropropyl)-5-cyclopropyl-5-phenylimidazolidine-2,4-dione (**9**).* Following the general procedure **9** was obtained from **5** (40° EtOH). Yield: 86%; m.p. 96–101 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58–7.53 (m, 2H), 7.45–7.26 (m, 3H), 5.78 (s, 1H), 3.72–3.64 (t, $J = 7$ Hz, 2H), 3.55–3.49 (t, $J = 7$ Hz, 2H), 2.18–2.05 (q, $J = 7$ Hz, 2H), 1.74–1.59 (m, 1H), 0.75–0.52 (m, 3H), 0.37–0.28 (m, 1H). Anal. calcd for $C_{15}H_{17}N_2O_2Cl$: C, 61.54; H, 5.85; N, 9.57. Found: C, 61.27; H, 5.70; N, 9.37.

*7.1.2.3. 1-(3-Chloropropyl)-2′,3′-dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**10**).* Following the general procedure **10** was obtained from **6** (40° EtOH). Yield: 70%: m.p. 101–102 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33–7.15 (m, 4H), 5.98 (s, 1H), 3.67–3.61 (t, $J = 6.8$ Hz, 2H), 3.55–3.50 (t, $J = 6.6$ Hz, 2H), 3.29–3.18 (m, 1H), 3.08–2.98 (m, 1H) 2.72–2.64 (m, 1H), 2.28–2.22 (m, 1H), 2.18–2.06 (q, $J = 6.8$ Hz, 2H). Anal. calcd for $C_{14}H_{15}N_2O_2Cl$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.34; H, 5.64; N, 10.03.

*7.1.2.4. 1-(3-Chloropropyl)-3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione (**11**).* Following the general procedure **11** was obtained from **7** (50° EtOH). Yield: 81%; m.p. 150–152 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.05 (m, 4H), 5.92 (s, 1H), 3.65–3.59 (t, $J = 7$ Hz, 2H), 3.24–3.18 (q, $J = 7.2$ Hz, 2H), 2.66–2.54 (t, $J = 7$ Hz, 2H), 2.40–2.29 (m, 2H), 2.15–1.96 (m, 4H). Anal. calcd for $C_{15}H_{17}N_2O_2Cl$: C, 61.54; H, 5.85; N, 9.57. Found: C, 61.62; H, 5.66; N, 9.30.

*7.1.3. General procedure for synthesis of compounds **12**–**23***

A mixture of appropriate derivatives of 3-(3-chloropropyl)-hydantoin (5 mmol), the substituted 1-phenylpiperazine (1 mmol) in anhydrous ethanol (30 ml) was refluxed for 28–30 h. After cooling, the solvent was evaporated and the residue was treated with water (50 ml); and the solution was extracted with $CHCl_3$ (3 × 15 ml). The combined organic phases were dried over anhydrous Na_2SO_4. Then the solution was filtered off, the solvent was evaporated and the crude product was purified by crystallization from anhydrous ethanol.

*7.1.3.1. 5-Methyl-5-phenyl-3-[3-(4-phenylpiperazin-1-yl)propyl]-imidazolidine-2,4-dione (**12**).* Following the general procedure **12** was obtained from **8** (EtOH). Yield: 53%; m.p. 140–144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54–7.25 (m, 7H), 6.95–6.85 (m, 3H), 6.22 (s, 1H), 3.65–3.60 (t, $J = 7$ Hz, 2H) 3.20–3.17 (t, $J = 5$ Hz, 4H), 2.60–2.57 (t, $J = 5$ Hz, 4H), 2.46–2.41 (t, $J = 7$ Hz, 2H), 1.93–1.83 (m, 5H). Anal. calcd for $C_{23}H_{28}N_4O_2$: C, 70.38; H, 7.19; N, 14.27. Found: C, 70.25; H, 7.00; N, 14.36.

*7.1.3.2. 5-Methyl-5-phenyl-3-{3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-imidazolidine-2,4-dione (**13**).* Following the general procedure **13** was obtained from **8** (EtOH). Yield: 46%; m.p. 156–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.33 (m, 5H), 7.03–6.81 (m, 4H), 5.56 (s, 1H), 3.88–3.82 (s, 3H), 3.64–3.55 (t, $J = 7$ Hz, 2H) 3.10–3.01 (m, 4H), 2.62–2.54 (m, 4H), 2.43–2.35 (t, $J = 7$ Hz, 2H), 1.89–1.80 (m, 5H). Anal. calcd for $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26. Found: C, 67.70; H, 7.24; N, 13.15.

*7.1.3.3. 5-Methyl-5-pheny-3-{3-[4-(3-chlorophenyl)piperazin-1-yl]-propyl}-imidazolidine-2,4-dione (**14**).* Following the general procedure **14** was obtained from **8** (EtOH). Yield: 45%; m.p. 142–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52–7.27 (m, 5H), 7.03–6.82 (m, 4H), 5.86 (s, 1H), 3.68–3.61 (t, $J = 6.8$ Hz, 2H) 3.22–3.17 (m, 4H), 2.66–2.55 (m, 4H), 2.52–2.44 (t, $J = 6.8$ Hz, 2H), 1.89–1.81 (m, 5H). Anal. calcd for $C_{23}H_{27}N_4O_2Cl$: C, 64.70; H, 6.37; N, 13.12. Found: C, 64.46; H, 6.14; N, 13.09.

*7.1.3.4. 5-Cyclopropyl-5-phenyl-3-[3-(4-phenylpiperazin-1-yl)propyl]imidazolidine-2,4-dione (**15**).* Following the general procedure **15** was obtained from **9** (EtOH). Yield: 41%; m.p. 131–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61–7.25 (m, 7H), 6.96–6.85 (m, 3H), 5.92 (s, 1H), 3.66–3.61 (t, $J = 7$ Hz, 2H), 3.22–3.19 (m, 4H), 2.63–2.59 (m, 4H), 2.48–2.43 (t, $J = 7$ Hz, 2H), 1.92–1.87 (q, $J = 7$ Hz, 2H), 1.73–1.71 (m, 1H), 0.74–0.35 (m, 4H). Anal. calcd for $C_{25}H_{30}N_4O_2$: C, 71.74; H, 7.22; N, 13.39. Found: C, 71.53; H, 7.53; N, 13.41.

*7.1.3.5. 5-Cyclopropyl-5-phenyl-3-{3-[4-(2-methoxyphenyl)piperazin-1-y]propyl}–imidazolidine-2,4-dione (**16**).* Following the general procedure **16** was obtained from **9** (EtOH). Yield: 49%; m.p. 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60–7.32 (m, 5H), 7.02–6.82 (m, 4H), 6.01 (s, 1H), 3.87–3.83 (s, 3H), 3.62–3.56 (t, $J = 7$ Hz, 2H) 3.11–3.02 (m, 4H), 2.66–2.58 (m, 4H), 2.47–2.39 (m, 2H), 1.91–1.80 (t, $J = 7$ Hz, 2H), 1.75–1.64 (m, 1H), 0.76–0.29 (m, 4H). Anal. calcd for $C_{26}H_{32}N_4O_3$: C, 69.62; H, 7.19; N, 12.49. Found: C, 69.98; H, 7.34; N, 12.67.

*7.1.3.6. 5-Cyclopropyl-5-phenyl-3-{3-[4-(3-chlorophenyl)piperazin-1-yl]propyl}-imidazolidine-2,4-dione (**17**).* Following the general procedure **17** was obtained from **9** (EtOH). Yield: 31%; m.p. 122–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61–7.32 (m, 5H), 7.20–7.11 (m, 1H), 6.87–6.72 (m, 3H), 6.05 (s, 1H), 3.64–3.57 (m, 2H) 3.29–3.17 (m, 4H), 2.74–2.46 (m, 6H), 1.99–1.88 (m, 2H), 1.73–1.65 (m, 1H), 0.75–0.31 (m, 4H). Anal. calcd for $C_{25}H_{29}N_4O_2Cl$: C, 66.29; H, 6.45; N, 12.37. Found: C, 66.13; H, 6.19; N, 12.28.

*7.1.3.7. 1-[3-(4-Phenylpiperazin-1-yl)propyl]-2′,3′-dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**18**).* Following the general procedure **18** was obtained from **10** (EtOH). Yield: 59%; m.p. 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.10 (m, 6H), 6.92–6.83 (m,,3H), 6.25 (s, 1H), 3.64–3.59 (t, $J = 7.2$ Hz, 2H), 3.30–3.22 (m, 1H), 3.18–3.15 (t, $J = 5$ Hz, 4H), 3.08–2.99 (m, 1H), 2.75–2.67 (m, 1H), 2.59–2.57 (t, $J = 5$ Hz, 4H), 2.46–2.41 (t, $J = 7.2$ Hz, 2H), 2.28–2.18 (m, 1H), 1.93–1.83 (q, $J = 7.15$ Hz, 2H). Anal. calcd for $C_{24}H_{28}N_4O_2$: C, 71.26; H, 6.98; N, 13.85. Found: C, 70.94; H, 6.86; N, 13.87.

*7.1.3.8. 1-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl}-2′,3′-dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**19**).* Following the general procedure **19** was obtained from **10** (EtOH). Yield: 72%; m.p. 160–161 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32–7.06 (m, 5H), 7.01–6.80 (m, 3H), 5.91 (s, 1H), 3.85 (s, 3H), 3.65–3.60 (t, J = 7 Hz, 2H), 3.28–3.20 (m, 1H), 3.06 (br.s, 4H), 3.02–2.99 (m, 1H), 2.76–2.67 (m, 1H), 2.63 (br.s, 4H), 2.48–2.43 (t, J = 7 Hz, 2H), 2.29–2.22 (m, 1H), 1.94–1.84 (q, J = 7 Hz, 2H). Anal. calcd for $C_{25}H_{30}N_4O_3$: C, 69.10; H, 6.96; N, 12.89. Found: C, 69.20; H, 6.89; N, 12.84.

*7.1.3.9. 1-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-2′,3′-dihydrospiro[imidazolidine-4,1′-indene]-2,5-dione (**20**).* Following the general procedure **20** was obtained from **10** (EtOH). Yield: 53%; m.p. 138–139 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.14 (m, 5H), 6.90–6.78 (m, 3H), 5.72 (s, 1H), 3.69–3.64 (t, J = 7.2 Hz, 2H), 3.38–3.35 (t, J = 5 Hz, 4H), 3.31–3.20 (m, 1H), 3.13–3.04 (m, 1H) 2.80–2.71 (m, 1H), 2.63–2.59 (t, J = 5 Hz, 4H), 2.51–2.46 (t, J = 7.2 Hz, 2H), 2.33–2.23 (m, 1H), 1.97–1.87 (q, J = 7.10 Hz, 2H). Anal. calcd for $C_{24}H_{27}N_4O_2Cl$: C, 65.67; H, 6.20; N, 12.76. Found: C, 65.66; H, 6.22; N, 12.78.

*7.1.3.10. 1-[3-(4-Phenylpiperazin-1-yl)propyl]-3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione (**21**).* Following the general procedure **21** was obtained from **11** (EtOH). Yield: 56%; m.p. 142–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28–7.04 (m, 6H), 6.94–6.82 (m, 3H), 5.68 (s, 1H), 3.68–3.63 (t, J = 7.2 Hz, 2H), 3.21–3.18 (t, J = 5 Hz, 4H), 2.90–2.84 (m, 2H), 2.62–2.59 (t, J = 5 Hz, 4H), 2.49–2.44 (t, J = 7.2 Hz, 2H), 2.39–2.24 (m, 2H), 2.02–1.77 (m, 4H). Anal. calcd for $C_{25}H_{30}N_4O_2$: C, 71.75; H, 7.22; N, 13.39. Found: C, 71.68; H, 7.22; N, 13.35.

*7.1.3.11. 1-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl}-3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione (**22**).* Following the general procedure **22** was obtained from **11** (EtOH). Yield: 53%; m.p. 205–209 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.12 (m, 3H), 7.05–6.83 (m, 5H), 6.08 (s, 1H), 3.85 (s, 3H), 3.61–3.57 (t, J = 7.2 Hz, 2H), 3.08 (br.s, 4H), 2.92–2.79 (m, 2H), 2.65 (br.s, 4H), 2.48–2.43 (t, J = 7.2 Hz, 2H), 2.32–2.21 (m, 2H), 2.01–1.83 (m, 4H). Anal. calcd for $C_{26}H_{32}N_4O_3$: C, 69.62; H, 7.19; N, 12.49. Found: C, 69.28; H, 7.15; N, 12.27.

*7.1.3.12. 1-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-3′,4′-dihydro-2′H-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione (**23**).* Following the general procedure **23** was obtained from **11** (EtOH). Yield: 52%; m.p. 158–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.03 (m, 5H), 6.91–6.76 (m, 3H) 5.72 (br.s, 1H), 3.63–3.58 (t, J = 7.2 Hz, 2H), 3.20–3.16 (t, J = 4.9 Hz, 4H), 2.90–2.84 (m, 2H), 2.58–2.55 (t, J = 4.9 Hz, 4H), 2.45–2.41 (t, J = 7.2 Hz, 2H), 2.35–2.23 (m, 2H), 2.02–1.75 (m, 4H). Anal. calcd for $C_{25}H_{29}N_4O_2Cl$: C, 66.29; H, 6.45; N, 12.37. Found: C, 66.16; H, 6.40; N, 12.36.

7.1.4. General procedure for the preparation of hydrochlorides

Free bases were dissolved in an excess of concentrated HCl upon heating and were cooled down. Afterwards anhydrous ethanol was added until the salt began to separate. Then hydrochloride salts were recrystallized from anhydrous ethanol.

7.2. In vitro experiments

7.2.1. 5-HT$_{1A}$ receptor binding experiments

[^{3}H]-8-Hydroxy-2-(di-n-propylamino)-tetralin ([^{3}H]-8-OH-DPAT, spec. act. 106 Ci/mM, NEN Chemicals) was used for labeling 5-HT$_{1A}$ receptors. The membrane preparation and the assay procedure were carried out according to the previously published procedure [14] with slight modifications. Briefly, rat cerebral cortex tissues were homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) using Ultra-Turrax® T 25, and were then centrifuged at 30,000*g* for 10 min. The supernatant fraction was discarded, and the pellet was resuspended in the same volume of the Tris–HCl buffer, afterwards it was centrifuged as above. Prior to the third centrifugation, the samples were incubated at 37 °C for 10 min. The final pellet was resuspended in the Tris–HCl buffer containing 10 mM pargyline, 4 mM $CaCl_2$ and a 0.1% ascorbic acid. The final incubation mixture consisted of 1 mL of the tissue suspension (9 mg of wet weight), 100 μl of 10 μM serotonin (for unspecific binding), 100 μl of [^{3}H]-8-OH-DPAT and 100 μl of the analyzed compounds. The sample was incubated at 37 °C for 15 min. The incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters. The filters were then washed 3 times with 5 ml of an ice-cold buffer (50 mM Tris–HCl, pH 7.7) using a Brandel cell harvester. The final [^{3}H]-8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-4} M. Then the filters were placed in scintillation vials with a scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All the assays were carried out in duplicate. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, version 3.0 – San Diego, CA, USA).

7.2.2. 5-HT$_{2A}$ receptor binding experiments

[^{3}H]-Ketanserin (spec. act. 60 Ci/mM, NEN Chemicals) was used for labeling 5-HT$_{2A}$ receptors. The assay was performed according to the method described previously by Leysen et al. [15] Rat cerebral cortex tissues were homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) and centrifuged at 30,000*g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer, preincubated at 37 °C for 10 min and centrifuged for 20 min as above. The final pellet was resuspended in 50 volumes of the same buffer. The final incubation mixture consisted of 1 mL of the tissue suspension, 100 μl of 1 μM mianserin (displacer), 100 μl of [^{3}H]-ketanserin and 100 μl of the analyzed compounds. The sample was incubated at 37 °C for 20 min, followed by a rapid vacuum filtration through Whatman GF/B glass filters, and was then washed three times with 5 ml of an ice-cold Tris–HCl buffer. The final [^{3}H]-ketanserin concentration was 0.6 nM, and the concentrations of the analyzed compounds changed from 10^{-10} to 10^{-4} M. Then the filters were placed in a scintillation vials with scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All the assays were carried out in duplicate. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, version 3.0 – San Diego, CA, USA).

7.2.3. D$_2$ receptor binding experiments

The assay was conducted according to the method described by Ossowska et al. [16]. Rat striatum tissues were thawed in 50 volumes of an ice-cold 50 mM potassium phosphate buffer (pH 7.4) homogenized and centrifuged at 20,000*g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer, and was centrifuged again for 20 min as described above.

Assay tubes contained a membrane suspension (3 mg of wet weight), [^{3}H]-spiperone (15 Ci/mM, NEN) at a concentration of 1 nM, (+)butaclamol (10 μM) or the analyzed compound (from 10^{-10} to 10^{-4} M) at a final volume of 0.5 ml. The sample was incubated at 37 °C for 30 min. The incubation was terminated by rapid filtration over glass filters (Whatman GF/B) using Brandel's manifold. The filters were then washed twice with 5 ml of an ice-cold buffer and were placed in scintillation vials with a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All the assays were carried out in duplicate.

Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, version 3.0 – San Diego, CA, USA).

7.3. In vivo experiments

The experiments were performed on male Wistar rats (290–310 g) or male Albino Swiss mice (24–28 g). The animals were kept at a room temperature of 20 ± 1 °C and had free access to food (standard laboratory pellets) and tap water before the experiment. All the investigations were conducted in the light phase on a natural day–night cycle (from May to September), between 9 a.m. and 2 p.m. All the experimental procedures were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (hydrobromide; 8-OH-DPAT, Tocris, Cookson Ltd., UK) and (±)-2,5-dimethoxy-4-iodoamphetamine (hydrochloride; (±)-DOI, Sigma–Aldrich, Inc., USA) were dissolved in saline; *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (trihydrochloride, WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) and imipramine (hydrochloride; Polfa-Starogard, Poland) were dissolved in distilled water. The investigated compounds were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (*sc*), (±)-DOI, imipramine and the tested compounds were given intraperitoneally (*ip*) at a volume of 2 ml/kg (rats) or 10 ml/kg (mice). Each experimental group consisted of 6–10 animal per dose, and each animal was used only once.

7.3.1. Body temperature in mice

The effects of the tested compounds given alone on rectal body temperature (measured with an Ellab thermometer) were recorded at 30, 60, 90, and 120 min after their administration to mice. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **13**, **14**, **16**, **19**, **22** and 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the former compounds and 8-OH-DPAT, and rectal body temperature was recorded at 30 and 60 min after injection of the tested compounds. In another experiment, the effect of **18**, **20** and **23** (which did not change mouse body temperature) on the 8-OH-DPAT (5 mg/kg)-induced hypothermia was assessed. The tested compounds were administered 45 min before 8-OH-DPAT, and rectal body temperature was measured 15, 30, 45, and 60 min after 8-OH-DPAT injection. The results were expressed as a change in body temperature (Δt) with respect to basal body temperature, as measured at the beginning of the experiment.

7.3.2. Lower lip retraction (LLR) in rats

LLR was assessed according to the method described by Berendsen et al. [19] The rats were individually placed in cages (30 cm × 25 cm × 25 cm) and were scored three times after administration (at 15, 30 and 45 min) of the tested compounds (**13**, **14**, **15**, **16**, **17**, **19**, **20**, **22**) and 8-OH-DPAT as follows: 0 = lower incisors not visible; 0.5 = partly visible; 1 = completely visible. The total maximum score was 3 for each rat. In a separate experiment, the effect of the tested compounds and WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds and WAY 100635 were administered at 45 min and 15 min before 8-OH-DPAT, respectively, and the animals were scored at 15, 30 and 45 min after 8-OH-DPAT administration.

7.3.3. Head twitch response in mice

To habituate mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter) × 20 cm (height) glass cage lined with sawdust, at 30 min before the treatment. Head twitches in mice were induced by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the head twitches were counted throughout 20 min [21]. The tested compounds (**18**, **20**, **21**, **23**) were administered 60 min before (±)-DOI.

7.3.4. Four-plate test in mice

The box was made of opaque plastic and was rectangular (25 cm × 18 cm × 16 cm) in shape. Its floor was covered with four rectangular metal plates (11 cm × 8 cm), separated by a 4mm gap. The plates were connected to the source of a continuous current, which enabled a 120 V potential difference between two adjacent plates for 0.5 s when the experimenter pressed the switch. Individual mice were gently placed on the plate and were allowed to explore for 15 s. Afterwards, each time a mouse passed from one plate to another, the experimenter electrified the whole floor, which evoked a visible flight reaction of the animal. If the animal continued running, it received no new shocks during the following 3 s. The episodes of punished crossing were counted for 60 s [24].

7.3.5. Forced swim test in mice

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

7.3.6. Locomotor activity in mice

The locomotor activity of mice was recorded in photoresistor actometers (24 cm in diameter), illuminated by two light beams which were connected to a counter for the recording of light beam interruptions. The mice were individually placed in the actometers, and the number of crossings of the light beams was assessed twice: during the first 6 min, and during 30-min experimental sessions.

7.4. Statistics

The obtained data were analyzed by a one-way analysis of variance, followed by Dunnett's test (when only one drug was given), or by the Newman–Keuls test (when two drugs were administered). ID50 values were calculated by the method of Litchfield and Wilcoxon.

7.5. Molecular modeling

Docking studies of compound **22** were carried out using a homology, rhodopsin-based model of rat 5-HT_{1A} serotonin receptor described previously [40]. To explore the conformational space of the binding site, a ligand was docked to the population of 100 models which differed significantly in their side-chain conformations, while the polypeptide backbone differed only insignificantly from the original template.

A molecular model of compound **22** was built with CORINA (www2.chemie.uni-erlangen.de/software/corina), followed by geometry optimization using a PM5 quantum semiempirical method with the COnductorlike Screening MOdel (COSMO) approach, to simulate a water environment (MOPAC 2002, implemented in the CAChe Worksystem Pro 6.1; www.cachesoftware.com). The +1 formal charge was located on a protonated nitrogen atom of the piperazine moiety.

Docking was carried out using FlexX (www.biosolveit.de), with default parameters. FlexX rapidly and exhaustively samples the conformational space of a ligand by means of an incremental

construction algorithm that builds the ligand in the site [44]. An interaction constraint (the FlexX-Pharm module of FlexX) was applied on the hydrogen bond between Asp3.32 and the protonated nitrogens of the ligands during docking, since that interaction was considered crucial for all the monoamine neurotransmitter receptors [35]. The obtained ligand–receptor complexes were scored using five scoring functions: F_score, D_score, G_score, Chem_score, and PMF_score, with a subsequent consensus scoring as implemented in the CScore module of SYBYL 7.1. Only complexes with the highest "5" CScore value were considered, and the ranking of compounds was based on the PMF_score, since that scoring function was reported to provide the best enrichment factors in virtual screening experiments [45].

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References

[1] N.M. Barnes, T. Sharp, Neuropharmacology 38 (1999) 1083–1152.
[2] S.J. Oh, H.J. Ha, D.Y. Chi, H.K. Lee, Curr. Med. Chem. 8 (2001) 999–1034.
[3] P. Blier, N.M. Ward, Biol. Psychiatry 53 (2003) 193–203.
[4] C.P. Muller, R.J. Carey, J.P. Huston, M.A. De Souza Silva, Prog. Neurobiol. 81 (2007) 133–178.
[5] M.L. Lopez-Rodriguez, D. Ayala, B. Benhamu, M.J. Morcillo, A. Viso, Curr. Med. Chem. 9 (2002) 443–469.
[6] G. Caliendo, V. Santagada, E. Perissutti, F. Fiorino, Curr. Med. Chem. 12 (2005) 1721–1753.
[7] S. Jordan, R. Chen, V. Koprivica, R. Hamiltona, R.E. Whitehead, K. Tottori, T. Kikuchi, Eur. J. Pharmacol. 517 (2005) 165–173.
[8] Z.A. Hughes, K.R. Starr, C.J. Langmead, M. Hill, G.D. Bartoszyk, J.J. Hagen, D.N. Middlemiss, L.A. Dawson, Eur. J. Pharmacol. 510 (2005) 49–57.
[9] A.D. Feiger, J.F. Heiser, R.K. Shrivastava, K.J. Weiss, W.T. Smith, J.M. Sitsen, M. Gibertini, J. Clin. Psychiatry 64 (2003) 243–249.
[10] Y. Iwasaki, T. Shiojima, M. Kinoshita, K. Ikeda, J. Neurol. Sci. 160 (1998) 92–96.
[11] H. Byrtus, M. Pawłowski, A. Czopek, A.J. Bojarski, B. Duszyńska, G. Nowak, A. Kłodzińska, E. Tatarczyńska, A. Wesołowska, E. Chojnacka-Wójcik, Eur. J. Med. Chem. 40 (2005) 820–829.
[12] H. Byrtus, M. Pawłowski, B. Duszyńska, A. Wesołowska, E. Chojnacka-Wójcik, A.J. Bojarski, Pol. J. Pharmacol. 53 (2001) 395–401.
[13] L.H. Goodson, J.L. Honigberg, J.L. Lehman, W.H. Burton, J. Org. Chem. 25 (1960) 1920–1924.
[14] D.N. Middlemiss, J.R. Fozard, Eur. J. Pharmacol. 90 (1983) 151–153.
[15] J.E. Leysen, C.J. Niemegers, J.M. Van Neuten, P.M. Laduron, Mol. Pharmacol. 21 (1982) 301–314.
[16] G. Ossowska, G. Nowak, R. Kata, B. Klenk-Majewska, Z. Danilczuk, I. Żebrowska-Łupina, J. Neural Transm. 108 (2001) 311–314.
[17] G.M. Goodwin, R.J. De Souza, A. Green, Neuropharmacology 24 (1985) 1187–1194.
[18] E.A. Forster, I.A. Cliffe, D.J. Bill, G.M. Dover, D. Jones, Y. Reilly, A. Fletcher, Eur. J. Pharmacol. 281 (1995) 81–88.
[19] H.H.G. Berendsen, F. Jenck, C.L.E. Broekkamp, Pharmacol., Biochem. Behav. 33 (1989) 821–827.
[20] H.H.G. Berendsen, C.L.E. Broekkamp, A.M.L. van Delft, Behav. Neural Biol. 55 (1991) 214–226.
[21] N.A. Darmani, B.R. Martin, U. Pandey, R.A. Glennon, Pharmacol., Biochem. Behav. 36 (1990) 901–906.
[22] D.L. Willins, H.Y. Meltzer, J. Pharmacol. Exp. Ther. 282 (1997) 699–706.
[23] R.D. Porsolt, A. Bertin, M. Jalfre, Arch. Int. Pharmacodyn 229 (1977) 327–336.
[24] C. Aron, P. Simon, C. Larousse, J.R. Boissier, Neuropharmacology 10 (1971) 459–469.
[25] J.F.W. Deakin, J. Psychopharmacol. 7 (1993) 283–289.
[26] R. Schreiber, J. De Vry, Prog. Neuropsychopharmacol. Biol. Psychiatry 17 (1993) 87–104.
[27] J. De Vry, Psychopharmacology 121 (1995) 1–26.
[28] G. Griebel, D.C. Blanchard, M.C. Rettori, B. Guardiola-Lemaitre, R.J. Blanchard, Pharmacol., Biochem. Behav. 54 (1996) 509–516.
[29] A. Dereń-Wesołek, E. Tatarczyńska, E. Chojnacka-Wójcik, J. Psychopharmacol. 12 (1998) 380–384.
[30] A. Wesołowska, M.H. Paluchowska, K. Gołembiowska, E. Chojnacka-Wójcik, J. Pharm. Pharmacol. 55 (2003) 533–543.
[31] M.J. Millan, Prog. Neurobiol. 70 (2003) 83–244.
[32] C.D. Stader, M.R. Candelore, W.S. Hill, R.A. Dixon, I.S. Sigal, J. Biol. Chem. 264 (1989) 16470–16477.
[33] C.D. Strader, I.S. Sigal, R.A. Dixon, FASEB J. 3 (1989) 1825–1832.
[34] Y.B. Ho, A. Karschin, T. Branchek, N. Davidson, H.A. Lester, FEBS Lett. 312 (1992) 259–262.
[35] C.D. Strader, I.S. Sigal, M.R. Candelore, E. Rands, W.S. Hill, R.A. Dixon, J. Biol. Chem. 263 (1988) 10267–10271.
[36] G. Liapakis, J.A. Ballesteros, S. Parachristou, W.C. Chan, X. Chen, J.A. Javitch, J. Biol. Chem. 275 (2000) 37779–37788.
[37] M.S. Choudhary, S. Craigo, B.L. Roth, Mol. Pharmacol. 43 (1993) 755–761.
[38] V. Cherezov, D.M. Rosenbaum, M.A. Hanson, S.G. Rasmussen, F.S. Thian, T.S. Kobilka, H.J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka, R.C. Stevens, Science 318 (2007) 1258–1265.
[39] M. Kołaczkowski, M. Nowak, M. Pawłowskie, A.J. Bojarski, J. Med. Chem. 49 (2006) 6732–6741.
[40] M. Nowak, M. Kołaczkowski, M. Pawłowski, A.J. Bojarski, J. Med. Chem. 49 (2006) 205–214.
[41] M.L. Lopez-Rodriguez, M.J. Morcillo, E. Fernandez, M.L. Rosado, L. Pardo, K. Schaper, J. Med. Chem. 44 (2001) 198–207.
[42] M.L. Lopez-Rodriguez, B. Vicente, X. Deupi, S. Barrondo, M. Olivella, M.J. Morcillo, Mol. Pharmacol. 62 (2002) 15–21.
[43] M.L. Lopez-Rodriguez, M. Morcillo, E. Fernandez, B. Benhamu, I. Tejada, D. Ayala, A. Viso, M. Olivella, L. Pardo, M. Delgado, J. Manzanares, J.A. Fuentes, J. Med. Chem. 48 (2005) 2548–2558.
[44] M. Rarey, B. Kramer, T. Lengauer, G. Klebe, J. Mol. Biol. 261 (1996) 470–489.
[45] A. Evers, T. Klabunde, J. Med. Chem. 48 (2005) 1088–1097.