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The influence of modifications in imide fragment structure on 5-HT_{1A} and 5-HT_7 receptor affinity and in vivo pharmacological properties of some new 1-(*m*-trifluoromethylphenyl)piperazines

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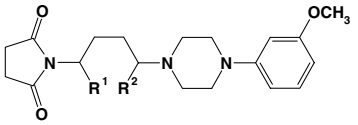
Abstract—New, flexible (**7**, **9**, **11** and **13**) and rigid (**8**, **10**, **12** and **14**) imides with a 1-(*m*-trifluorophenyl)piperazine fragment and a tetramethylene or a 1e,4e-cyclohexylene spacer, respectively, showed very high affinity (K_i = 0.3–34 nM) and agonistic in vivo activity for 5-HT_{1A} receptors. Flexible new compounds and the previously described **5** also bound to 5-HT_7 receptors (K_i = 21–134 nM). Selected glutarimide derivatives, that is, the most potent postsynaptic 5-HT_{1A} receptor agonist rigid compound **8** and its flexible analogue **7**, as well as the previously described full agonist—rigid compound **6** and the partial agonist—its flexible counterpart **5** exhibited moderate affinity for α_1-adrenoceptors (K_i = 85–268 nM), but were practically devoid of any affinity for dopamine D_2 sites. Those glutarimides demonstrated anxiolytic- (**5** and **7**) and antidepressant-like (**5**, **6** and **8**) activity in the four-plate and the swim tests in mice, respectively; at the same time, however, they inhibited the locomotor activity of mice. The antidepressant-like effect of **8** was significantly stronger than that induced by imipramine used as a reference antidepressant.

1. Introduction

Structure–intrinsic activity relationship studies into a group of 1-imido/amido substituted 4-(4-arylpiperazin-1-yl)butane or cyclohexane derivatives have indicated that these compounds show high affinity but diversified functional activity for 5-HT_{1A} receptors. However, the same 5-HT_{1A} receptor intrinsic activity is observed for the majority of investigated pairs that is, flexible butane and rigid cyclohexane counterparts.[1–4] The rigid molecules with restricted conformational freedom possess a defined 3D structure and may thus serve as tools for investigating ligand–5-HT_{1A} receptor interactions.[5]

Additionally, it has been found that compounds with a tetramethylene spacer reveal high 5-HT_7 receptor affinity, while their constrained analogue practically do not bind to these sites. Indeed, compound MM 77 (**1**) and its rigid analogue MP 349 (**2**) (Chart 1), either having an *o*-OCH_3-phenyl fragment, show features of 5-HT_{1A} receptor antagonists, and **1** (but not **2**) has significant affinity for 5-HT_7 sites.[2]

Interestingly, both these ligands demonstrate activity characteristic of anxiolytics.[6] It has also been observed



1 MM 77 $R^1 = R^2 = H$
2 MP 349 $R^1, R^2 = -(CH_2)_2-$

Chart 1.

Keywords: Arylpiperazines; 5-HT_{1A} and 5-HT_7 receptors ligands; Structure–intrinsic activity relationships studies; 5-HT_{1A} receptor agonists; Anxiolytic- and antidepressant-like activity.
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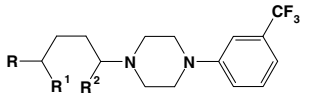
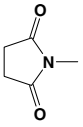
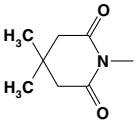
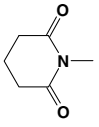
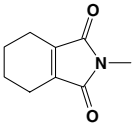
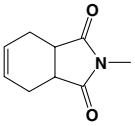
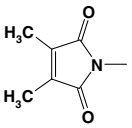
that other ligands with an *o*-OCH_3 group in the aryl moiety and with a cyclic amide system in the opposite terminal show a tendency to block postsynaptic 5-HT_{1A} receptors.[4] The replacement of the *o*-OCH_3 substituent in structures **1** and **2** with an *m*-CF_3 group results in a change in 5-HT_{1A} receptor activity; in fact, these modified ligands **3** and **4** are classified as partial agonists of postsynaptic 5-HT_{1A} receptors[2] (Table 1). Furthermore, it has been shown that the *m*-CF_3 arylpiperazines **5** and **6** containing a 3,3-dimethyl-glutarimide fragment (present in gepirone, a 5-HT_{1A} receptor agonist with antianxiety properties) in place of a succinimide moiety behave like a 5-HT_{1A} receptor partial and a full agonist, respectively.[3] It is noteworthy that there are a limited number of data on the role of the *m*-CF_3 group in the functional activity of arylpiperazine 5-HT_{1A} receptor ligands. In fact, only López-Rodriquez et al.[7] found two such *m*-CF_3 derivatives with agonistic activity toward postsynaptic 5-HT_{1A} receptors.

To carry on investigations with this group of compounds, we synthesized a new series of derivatives whose imide moiety was connected to an *m*-trifluoromethylsubstituted 1-phenylpiperazine fragment via a tetramethylene (**7**, **9**, **11**, **13**) or a conformationally defined 1e, 4e-cyclohexylene (**8**, **10**, **12**, **14**) spacer in order to determine the influence of imide fragment modifications on the affinity and functional 5-HT_{1A} receptor activity. For all new compounds (**7**–**14**), as well as for the previously described **5** and **6**[3] 5-HT_7 receptor and α_1-adrenoceptor affinity was also determined. Moreover, for new derivatives **7**–**14** D_2 dopamine receptor affinity was estimated. 5-HT_{1A} pre- and postsynaptic

Table 1. Structure and affinity data on serotonin (5-HT_{1A} and 5-HT_7), α_1-adrenergic and dopaminergic D_2 receptors of investigated compounds



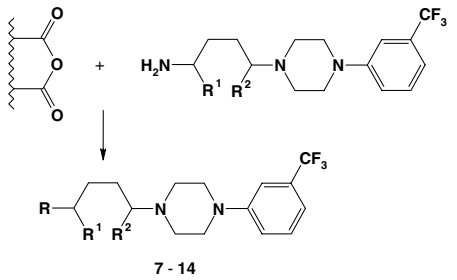
Compound	R	R^1	R^2	K_i (nM) ± SEM			
				5-HT_{1A}	5-HT_7	α_1	D_2
3[a]		H	H	21 ± 1	128 ± 6	483 ± 36	>30000
4[b]		–$(CH_2)_2$–		4 ± 0.5	1820 ± 38	505 ± 62	>25000
5		H	H	4 ± 1[b]	46 ± 3	127 ± 14	2340[b]
6		–$(CH_2)_2$–		9 ± 1[b]	221 ± 17	268 ± 12	>10000[b]
7		H	H	3 ± 1	21 ± 2	101 ± 9	>5000[c]
8		–$(CH_2)_2$–		2.6 ± 0.3	1200 ± 70	85 ± 6	>5000[c]
9		H	H	5.9 ± 0.5	134 ± 11	30 ± 4	>5000[c]
10		–$(CH_2)_2$–		0.3 ± 0.1	>5000	4 ± 1	2640[c]
11		H	H	34 ± 9	31 ± 4	73 ± 7	2720[c]
12		–$(CH_2)_2$–		1.5 ± 0.3	335 ± 22	43 ± 3	4680[c]
13		H	H	2.2 ± 0.6	107 ± 8	48 ± 4	4370[c]
14		–$(CH_2)_2$–		1.3 ± 0.2	>5000	24 ± 2	3600[c]

[a] Data from Ref. 2.
[b] Data from Ref. 3.
[c] The binding experiments to D_2 receptors were carried out at two compound concentrations (each run in triplicate) and ligand affinity was expressed as estimated K_i values.

receptor functional activity was evaluated for **7**–**14** in in vivo models. Compounds **7** and **8**, as well as the previously investigated by us gepirone analogue **5** and **6**, were additionally tested in animal models used for evaluating potential anxiolytic and antidepressant activity.

2. Chemistry

The structures of the compounds under study are shown in Table 1, and their syntheses are illustrated in Scheme 1.



Scheme 1.

The flexible target compounds **7**, **9**, **11** and **13** and the constrained derivatives **10** and **14** were synthesized from 4-[1-(*m*-trifluoromethylphenyl)piperazin-4-yl]-butyl-or 4-[1-(*m*-trifluoromethylphenyl)piperazin-4-yl]cyclohexylamine and the appropriate anhydrides by heating in xylene. In the case of compounds **8** and **12**, intermediate non-cyclic aminoacids were obtained, which were then cyclized in an acetic anhydride according to the procedure described previously.[3] The structures of the newly synthesized compounds were confirmed by ^{1}H NMR spectra and an elemental analysis. In the ^{1}H NMR spectra of the rigid compounds **8**, **10**, **12** and **14**, the observed coupling constants in the cyclohexane ring were consistent with those previously assigned by us to the 1e,4e-diequatorial chair conformation of 1-(2-methoxyphenyl)-4-[4-(2-phthal-imido)cyclohexyl]piperazine[1] and 1-(2-methoxy-phenyl)-4-[4-(2-succinimido)cyclohexyl] piperazine.[8]

3. Pharmacology

The compounds were tested in competition binding experiments for serotonin 5-HT$_{1A}$ and 5-HT$_7$, α_1-adrenergic and dopamine D$_2$ receptors. Affinity data are collected in Table 1.

The functional activity of the investigated compounds at pre- and postsynaptic 5-HT$_{1A}$ receptors was tested in several commonly used in vivo models. It was previously demonstrated that the hypothermia induced by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin] in mice was connected with activation of presynaptic 5-HT$_{1A}$ receptors[9,10] and was abolished by 5-HT$_{1A}$ receptor antagonists such as, for example, WAY 100635 (*N*-{2-[4-(*o*-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2- pyridinyl)-cyclohexanecarboxamide)[11] or MP 3022 (4-[3-(benzotriazol-1-yl)propyl]-1-(*o*-methoxyphenyl)piperazine).[12] Hence the hypothermia produced by the compounds tested in mice (and reduced by WAY 100635) was regarded as a measure of presynaptic 5-HT$_{1A}$ receptor agonistic activity. To determine the postsynaptic 5-HT$_{1A}$ receptor agonistic effect of the tested 5-HT$_{1A}$ ligands, their ability to induce lower lip retraction (LLR) in rats was assessed. The 8-OH-DPAT-induced LLR in rats depended on stimulation of postsynaptic 5-HT$_{1A}$ receptors;[13,14] moreover, it was shown that the latter symptom was sensitive to 5-HT$_{1A}$ receptor antagonists.[8,11,15]

Potential anxiolytic and antidepressant activity was tested in the four-plate[16] and the forced swim tests[17] in mice, respectively.

4. Results and discussion

Like the previously described **3**–**6**,[2,3] all the newly synthesized compounds (**7**–**14**) were characterized by a very high 5-HT$_{1A}$ receptor affinity (K_i = 0.3–34 nM). Therefore it seems that structural changes in both the imide pharmacophore and the spacer constitution are not of importance for the formation of ligand–5-HT$_{1A}$ receptor complexes in the investigated series of compounds. Regarding 5-HT$_7$ receptors, new flexible derivatives **7**, **9**, **11** and **13**, as well as the previously described **5**,[3] but not their constrained analogue, showed distinct affinity; all the same, **9** and **13** were at least 4–5 times less active. The above results confirm our earlier observations that introduction of rigidity into the spacer of imide/amide arylopiperazine derivatives leads to a significant decrease or a complete loss of 5-HT$_7$ receptor affinity. Compounds **5**–**14** exhibited a high or moderate α_1-adrenoceptor affinity (K_i = 4–268 nM)—higher than that described earlier for pair **3/4** though. Like pair **3/4**, compounds **5**–**14** did not bind to D$_2$ receptors.

At a successive stage of our study, we investigated the newly synthesized receptor ligands in in vivo models used for evaluation of pre- and postsynaptic 5-HT$_{1A}$ receptor functional activity. As has been mentioned in the Introduction, in our previous study, the parent compounds **3/4** (with fragment V) and **5/6** (with fragment VI) were characterized by diverse 5-HT$_{1A}$ receptor functional activity; we described **3/4** as partial agonists of postsynaptic 5-HT$_{1A}$ receptors, **5** as an agonist of pre- and a partial agonist of postsynaptic 5-HT$_{1A}$ receptors, and **6** as a full agonist of 5-HT$_{1A}$ sites.[2,3] The investigations described in this paper were conducted mainly in order to determine whether structural changes in the imide termini affected 5-HT$_{1A}$ intrinsic activity. The obtained results indicate that of the new tested derivatives, pairs: **9/10** and **11/12** (both with the bicyclic imide fragment) and the restricted **14** (with two methyl substituents in imide moiety) show features of agonists of presynaptic 5-HT$_{1A}$ receptors and of partial agonists of postsynaptic ones. Indeed, like the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, **9**–**12** and **14** evoked a decrease in mouse body temperature (Table 2), which was sensi-

Table 2. The effect of the tested compounds on the body temperature in mice

Treatment	Dose (mg/kg)	Δ*t* ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	—	0.1 ± 0.1	0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
7	5	−0.5 ± 0.2	−0.4 ± 0.1	−0.4 ± 0.2	−0.1 ± 0.2
	10	−1.3 ± 0.3[b]	−1.1 ± 0.3[b]	−1.0 ± 0.2[b]	−0.9 ± 0.1[b]
8	2.5	−0.7 ± 0.1[a]	−0.5 ± 0.2	−0.5 ± 0.1	−0.3 ± 0.1
	5	−1.6 ± 0.2[b]	−1.5 ± 0.1[b]	−1.5 ± 0.2[b]	−1.3 ± 0.2[b]
Vehicle	—	−0.1 ± 0.1	−0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
9	10	0.2 ± 0.1	0.0 ± 0.2	0.0 ± .0.2	0.0 ± 0.2
	20	−1.8 ± 0.2[b]	−1.3 ± 0.3[b]	−1.3 ± 0.2[b]	−1.1 ± 0.2[b]
10	2.5	−0.4 ± 0.1	−0.5 ± 0.1	−0.4 ± 0.1	0.1 ± 0.1
	5	−1.2 ± 0.2[b]	−1.4 ± 0.2[b]	−0.7 ± 0.1[a]	−0.3 ± 0.2
Vehicle	—	−0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.2	−0.1 ± 0.2
11	10	−0.3 ± 0.2	−0.2 ± 0.2	−0.9 ± 0.3[a]	−0.6 ± 0.2
	20	−1.5 ± 0.3[b]	−1.3 ± 0.3[b]	−1.6 ± 0.3[b]	−1.5 ± 0.2[b]
12	5	−0.7 ± 0.2[a]	−0.4 ± 0.1	−0.4 ± 0.1	−0.1 ± 0.2
	10	−1.7 ± 0.3[b]	−1.3 ± 0.3[b]	−1.1 ± 0.2[b]	−1.0 ± 0.2[b]
Vehicle	—	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
13	5	−0.6 ± 0.1[a]	−0.4 ± 0.1	−0.2 ± 0.1	0.0 ± 0.1
	10	−0.1 ± 0.1	−0.2 ± 0.1	−0.4 ± 0.1	−0.2 ± 0.2
14	5	−0.2 ± 0.1	−0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	10	−1.0 ± 0.2[b]	−0.7 ± 0.2[a]	−0.5 ± 0.1	−0.5 ± 0.1
WAY 100635	0.1	−0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	−0.1 ± 0.1

The tested compounds were administered 30 min before the test. Absolute initial mean body temperatures were within the range of 36.2 ± 0.6 °C; $n = 6$–8 mice per group.

[a] $P < 0.05$.

[b] $P < 0.01$ versus respective vehicle group.

tive to the 5-HT_{1A} receptor antagonist WAY 100635 (Table 3).

Moreover, they produced LLR in rats (Table 4); at the same time, however, the 8-OH-DPAT-induced LLR in rats was reduced by **9**–**12** and **14** (Table 4). Unexpectedly, compound **13** (a flexible analogue of **14**) practically did not change the body temperature of mice (Table 2), but—like WAY 100635—reduced the hypothermia induced by 8-OH-DPAT in a statistically significant manner (data not shown). In the LLR test, **13** behaved like a partial 5-HT_{1A} agonist (Table 4).

The pair **7/8** (with imide moiety I) can be classified as agonists of postsynaptic 5-HT_{1A} receptors; like 8-OH-DPAT, both these compounds induced a marked LLR (a maximal possible effect) in rats, and did affect the LLR evoked by 8-OH-DPAT (Table 4). It is noteworthy that rigid compound **8** produced that symptom at a very low range of doses (0.1–1 mg/kg). Postsynaptic 5-HT_{1A} receptor agonistic activity of the previously investigated analogs (**5/6**) in the LLR test was observed after administration of doses higher than those of compounds **7/8**; moreover, the intensity of symptoms induced by pair **5/6** did not reach the maximal possible effect.[3] In the used model of hypothermia (for the evaluation of presynaptic 5-HT_{1A} activity), derivatives **7** and **8** decreased the body temperature of mice (Table 2), which was not affected by WAY 100635 (Table 3). Therefore a contribution of 5-HT_{1A} receptors to this effect should be excluded.

On the basis of the results of in vivo functional studies presented in this paper, it may be concluded that the glutarimide moiety (I), which is connected to 1-(*m*-CF_3-phenyl)piperazine by a tetramethylene and in particular a 1e,4e-cyclohexylene spacer, is beneficial for postsynaptic 5-HT_{1A} receptor agonistic activity. Such a modification yields compounds **7/8**, potent and very active in vivo agonists of postsynaptic 5-HT_{1A} receptors. Moreover, it seems that the presence of an additional ring (II or III) or substituents (IV) in the imide fragment causes a decrease in postsynaptic 5-HT_{1A} receptor intrinsic activity; the pairs of compounds **9/10**, **11/12** and **13/14** behave like partial agonists of 5-HT_{1A} receptors (Table 5). The functional profile of the investigated 5-HT_{1A} receptor ligands suggests that some of them may show anxiolytic- and/or antidepressant-like activity, since it has been well established that activation of 5-HT_{1A} receptors leads to disclosure of such activity.

Although the relative contribution of pre- and postsynaptic 5-HT_{1A} receptors to the anxiolytic- and antidepressant-like effects of 5-HT_{1A} agonists has not been explicitly determined,[18–20] on the basis of our earlier study we concluded that the anxiolytic- and antidepressant-like effects of azapirones (i.e. ipsapirone and gepi-

Table 3. The effect of WAY 100635 on the hypothermia induced by the tested compounds in mice

Treatment and dose (mg/kg)	$\Delta t \pm$ SEM (°C)	
	30 min	60 min
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **7** (10)	−1.3 ± 0.2[b]	−1.2 ± 0.3[b]
WAY 100635 (0.1) + **7** (10)	−1.0 ± 0.2[b]	−0.8 ± 0.3[a]
Vehicle + vehicle	0.1 ± 0.1	0.0 ± 0.1
Vehicle + **8** (5)	−1.6 ± 0.2[b]	−1.5 ± 0.1[b]
WAY 100635 (0.1) + **8** (5)	−1.2 ± 0.1[b]	−1.2 ± 0.1[b]
Vehicle + vehicle	0.0 ± 0.1	−0.1 ± 0.1
Vehicle + **9** (20)	−1.4 ± 0.2[b]	−1.4 ± 0.1
WAY 100635 (0.1) + **9** (20)	−0.6 ± 0.2[A,a]	−0.2 ± 0.2[B]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **10** (5)	−1.2 ± 0.2[b]	−1.4 ± 0.2[b]
WAY 100635 (0.1) + **10** (5)	0.2 ± 0.1[B]	0.1 ± 0.2[B]
Vehicle + vehicle	0.1 ± 0.1	0.0 ± 0.1
Vehicle + **11** (20)	−1.3 ± 0.2[b]	−1.6 ± 0.2[b]
WAY 100635 (0.1) + **11** (20)	−1.2 ± 0.2[b]	−0.6 ± 0.2[B]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **12** (10)	−1.4 ± 0.1[b]	−1.1 ± 0.2[b]
WAY 100635 (0.1) + **12** (10)	−0.5 ± 0.1[B]	−0.3 ± 0.1[B]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **14** (10)	−1.5 ± 0.2[b]	−1.2 ± 0.2[b]
WAY 100635 (0.1) + **14** (10)	−0.8 ± 0.3[A]	−0.5 ± 0.2[A]
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT (5)	−1.0 ± 0.1[b]	−0.2 ± 0.1
WAY 100635 (0.1) + 8-OH-DPAT (5)	−0.1 ± 0.1[B]	0.2 ± 0.1

WAY 100635 w as administered 15 min before the compounds studied. Body temperature was recorded 30 and 60 min after injection of the tested compounds. Absolute initial mean body temperatures were within the range of 36.2 ± 0.5 °C; n = 7–8 mice per group.
[A] $P < 0.05$.
[B] $P < 0.01$ versus respective vehicle + tested compound group.
[a] $P < 0.05$.
[b] $P < 0.01$ versus respective vehicle + vehicle group.

Table 4. Induction of lower lip retraction (LLR) by the investigated compounds (A) and their effect on the 8-OH-DPAT-induced LLR (B) in rats

Treatment	Dose (mg/kg)	Mean ± SEM LLR score	
		A	B
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
7	2.5	1.6 ± 0.1	2.2 ± 0.2
	5	2.6 ± 0.2[b]	NT
	10	2.8 ± 0.3[b]	NT
8	0.1	1.3 ± 0.2[b]	2.6 ± 0.2
	0.3	2.4 ± 0.3[b]	NT
	1	3.0 ± 0.0[b]	NT
Vehicle	—	0.1 ± 0.1	2.9 ± 0.1
9	2.5	1.9 ± 0.3[b]	2.3 ± 0.3
	5	2.2 ± 0.2[b]	1.5 ± 0.0[b]
	10	2.2 ± 0.2[b]	1.1 ± 0.2[b]
10	5	1.8 ± 0.3[b]	2.5 ± 0.3
	10	2.0 ± 0.2[b]	1.9 ± 0.3[b]
	20	2.0 ± 0.2[b]	1.8 ± 0.2[b]
Vehicle	—	0.1 ± 0.1	2.9 ± 0.1
11	10	1.4 ± 0.2[b]	1.9 ± 0.3[b]
	20	1.8 ± 0.1[b]	0.5 ± 0.2[b]
12	10	0.7 ± 0.1[a]	2.1 ± 0.3
	20	1.3 ± 0.3[b]	1.9 ± 0.2[b]
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
13	10	1.3 ± 0.1[b]	1.4 ± 0.1[b]
	20	1.3 ± 0.2[b]	0.2 ± 0.1[b]
14	10	1.4 ± 0.1[b]	1.8 ± 0.2[b]
	20	1.5 ± 0.0[b]	1.1 ± 0.2[b]
WAY 100635	0.1	0.0 ± 0.0	0.2 ± 0.2[b]

The investigated compounds were administered 15 min before the test (A), or 45 min before 8-OH-DPAT (1 mg/kg); n = 6 rats per group. NT, not tested.
[a] $P < 0.05$.
[b] $P < 0.01$ versus vehicle group (A) or versus vehicle + 8-OH-DPAT group (B).

Table 5. Functional in vivo 5-HT_{1A} receptor activity of the investigated compounds

Compound	5-HT_{1A} activity	
	Presynaptic	Postsynaptic
7	—	Agonist
8	—	Agonist
9	Agonist	Partial agonist
10	Agonist	Partial agonist
11	Agonist	Partial agonist
12	Agonist	Partial agonist
13	Antagonist	Partial agonist
14	Agonist	Partial agonist

rone) stem from stimulation of 5-HT_{1A} receptors localized postsynaptically.[21,22] Taking into account the functional activity of new derivatives, compounds **7/8**, with the strongest postsynaptic 5-HT_{1A} receptor agonistic activity were selected for further in vivo preclinical studies as potential anxiolytics and/or antidepressants. Additionally, we tested the pattern compounds **5/6**, which in our previous study were described as a partial agonist and an agonist of postsynaptic 5-HT_{1A} receptors, respectively.[3] Using the four-plate test in mice (an animal model of anxiety based on spontaneous responses),[16] we showed that **5** and **7** (but not **6** and **8**) induced anxiolytic-like effects (Table 6). In that test, compound **5** induced a strong anti-punishment effect comparable—in terms of its potency and active dose (5 mg/kg)—with the effect evoked by diazepam, used as a reference anxiolytic drug (Table 6). Potential antidepressant activity of **5**–**8** was tested in the forced swim test in mice.[17] It was demonstrated that **5**, **6** and **8** (but not **7**) exerted an effect characteristic of antidepressants (Table 7).

That effect seemed to be specific, since the tested compounds used in doses evoking antidepressant-like activity did not stimulate the locomotor activity of mice (Table 8). It is noteworthy that the anti-immobility effect of **6** was comparable to that induced by imipramine, whereas **8** (0.3 and 1 mg/kg) shortened immobility time in a significantly more potent manner than did imipramine (10–20 mg/kg) (Table 7). A question arises whether the anxiolytic- and/or antidepressant-like effects of **5** and

Table 6. The effect of 5–8 and diazepam in the four-plate test in mice

Treatment	Dose mg/kg	Number of punished crossings mean ± SEM
Vehicle	—	4.3 ± 0.4
5	2.5	5.3 ± 0.5
	5	6.9 ± 0.7[b]
	10	7.6 ± 0.9[b]
		$F(3,32) = 4.787$
		$P < 0.01$
Vehicle	—	3.8 ± 0.3
6	1.25	4.4 ± 0.3
	2.5	4.6 ± 0.4
	5	4.6 ± 0.4
		$F(3,32) = 1.112$
		Ns
Vehicle	—	3.7 ± 0.3
7	2.5	4.3 ± 0.5
	5	5.8 ± 0.5[a]
	10	5.2 ± 0.6
		$F(3,32) = 3.449$
		$P < 0.005$
Vehicle	—	3.6 ± 0.4
8	0.3	3.9 ± 0.4
	1	3.9 ± 0.3
	5	4.2 ± 0.3
		$F(3,32) = 0.539$
		Ns
Vehicle*	—	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]
		$F(3,36) = 9.514$
		$P < 0.001$

The investigated compounds were administered 30 min before the test; $n = 9$–10 mice per group.
[a] $P < 0.05$.
[b] $P < 0.01$ versus vehicle (Dunnett's test), ns: not significant.
* Data from Ref. 23.

Table 7. The effect of 5–8 and imipramine in the forced swim test in mice

Treatment	Dose mg/kg	Immobility time (s) mean ± SEM
Vehicle	—	169.2 ± 11.0
5	5	157.7 ± 10.7
	10	112.1 ± 13.5[b]
		$F(3,32) = 4.892$
		$P < 0.01$
Vehicle	—	161.9 ± 11.6
6	1.25	150.4 ± 9.6
	2.5	98.2 ± 13.7[b]
	5	113.3 ± 11.7[a]
		$F(3,32) = 6.748$
		$P < 0.01$
Vehicle	—	161.2 ± 7.1
7	5	183.9 ± 11.4
	10	196.7 ± 5.6[a]
		$F(2,24) = 4.534$
		$P < 0.05$
Vehicle	—	167.8 ± 7.2
8	0.1	128.3 ± 9.3
	0.3	70.1 ± 11.6[b]
	1	77.7 ± 16.1[b]
		$F(3,32) = 15.782$
		$P < 0.0001$
Vehicle	—	167.1 ± 6.7
Imipramine	10	149.1 ± 10.7
	20	107.8 ± 12.4[b]
		$F(2,27) = 8.760$
		$P < 0.01$

The investigated compounds and imipramine were administered 30 min before the test; $n = 9$–10 mice per group.
[a] $P < 0.05$.
[b] $P < 0.01$ versus vehicle (Dunnett's test).

7 depend exclusively on a 5-HT_{1A} receptor mechanism, since these compounds bind to 5-HT_7 receptors. Recently the data obtained in our laboratory have shown that blockade of 5-HT_7 receptors produces antianxiety- and antidepressant-like effects.[24,25] It is noteworthy that **1**, an antagonist of postsynaptic 5-HT_{1A}[12] and 5-HT_7 (Tokarski K., unpublished data) receptors, induces an anxiolytic-like effect.[6,26,27]

Until now we have not had information about functional 5-HT_7 receptor activity of **5** and **7**, but participation of these sites in psychotropic-like properties of these 5-HT_{1A}/5-HT_7 ligands cannot be excluded. It has also been shown that compounds **5**–**8** reduce the locomotor activity of mice at all the doses producing statistically significant effect in the four-plate test or the forced swim test. Diazepam shows a weak sedative effect in mice at a dose twice as high as this inducing a minimal anxiolytic-like effect, whereas imipramine at a dose active in the forced swim test slightly reduces the locomotor activity of mice. Strong inhibition of the locomotor activity of mice, observed after administration of compound **7**, may be the cause of immobility time prolongation induced by this compound in the swim test. The motor activity of mice seems to be partly regulated by postsynaptic noradrenaline receptors in the brain, thus it cannot be excluded that the sedation of mice induced by the tested compounds **5**–**8** is connected with an inhibition of the noradrenaline system function. The latter compounds are ligands of α_1-adrenoceptors, hence it cannot be excluded that—like in the case of prazosin, an α_1-adrenoceptor blocker[28]—they may reduce the spontaneous locomotor activity of mice due to blockade of postsynaptic α_1-receptors.

Summing up, it has been found that the newly synthesized flexible and rigid imides containing the 1-(*m*-CF_3-phenyl)piperazine fragment (**7**–**14**) are potent ligands of 5-HT_{1A} receptors, and in general—like the previously tested **5**–**6**—show features of agonists of these sites in in vivo studies (hypothermia and LLR models). Therefore, it seems that the *m*-CF_3 substituent in phenylpiperazine, but not structure of the imide fragment, is pivotal for 5-HT_{1A} receptor agonism. Flexible (but not rigid) imides also bind to 5-HT_7 receptors. The selected glutarimides **5**–**8** have properties characteristic of anxiolytics and/or antidepressants, indeed, they increased number of punished crossings in the four-plate test and shortened immobility time in the forced swim

Table 8. The effect of **5**–**8** and imipramine on the locomotor activity of mice

Treatment	Dose mg/kg	Locomotor activity: number of crossings during	
		6 min	30 min
Vehicle	—	393.0 ± 27.12	1054.9 ± 35.8
5	5	121.7 ± 18.0[b]	730.8 ± 88.0[b]
	10	23.0 ± 6.9[b]	265.1 ± 42.6[b]
		$F(2,24) = 99.312$	$F(2,24) = 43.577$
		$P < 0.0001$	$P < 0.0001$
Vehicle	—	406.0 ± 27.3	1092.1 ± 33.1
6	0.3	212.0 ± 33.9[b]	630.1 ± 99.9[b]
	1	211.7 ± 35.6[b]	535.3 ± 80.0[b]
		$F(2,24) = 11.925$	$F(2,24) = 15.213$
		$P < 0.001$	$P < 0.0001$
Vehicle	—	405.4 ± 43.1	948.6 ± 53.9
7	5	217.1 ± 30.0[b]	526.2 ± 76.9[b]
	10	16.3 ± 3.4[b]	179.3 ± 23.3[b]
		$F(2,24) = 41.06$	$F(2,24) = 47.535$
		$P < 0.0001$	$P < 0.0001$
Vehicle	—	405.4 ± 43.1	948.6 ± 53.9
8	0.3	208.2 ± 20.4[b]	537.7 ± 43.3[b]
	1	121.6 ± 19.7[b]	374.3 ± 52.2[b]
		$F(2,24) = 23.872$	$F(2,24) = 34.999$
		$P < 0.0001$	$P < 0.0001$
Vehicle	—	340.6 ± 34.1	892.7 ± 76.6
Imipramine	10	338.0 ± 36.4	876.8 ± 67.0
	20	314.9 ± 24.1	625.3 ± 53.6[a]
		$F(2,27) = 0.1945$	$F(2,27) = 4.930$
		ns	$P < 0.05$

The investigated compounds and imipramine were administered 30 min before the test. $n = 9$–10 mice per group.
[a] $P < 0.05$.
[b] $P < 0.01$ versus vehicle (Dunnett's test).

test in mice. Compounds **5** and **8** exhibit the most potent anxiolytic- and antidepressant-like activity, respectively, but at the same time they also induce a sedative effect.

5. Experimental

5.1. Chemistry

Melting points (mp) were determined with a Boetius apparatus and are uncorrected. ^{1}H NMR spectra were taken with a Varian EM-360L (60 MHz) or a Varian Mercury-VX (300 MHz) spectrophotometer in $CDCl_3$ solutions with TMS as an internal standard. The spectral data of new compounds refer to their free bases. Chemical shifts were expressed in δ (ppm) and the coupling constants J in hertz (Hz). All compounds were routinely checked by TLC using Merck silica gel 60 F_{254} plates (detection at 254 nm). Column chromatography separations were carried out on Merck Kieselgel 60. Elemental analyses were found within ±0.4% of the theoretical values.

The starting 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine and 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl)cyclohexylamine were synthesized by published procedures.[2] The preparation of compounds **5** and **6** had been previously published.[3]

5.1.1. General procedure for the preparation of Compounds 7, 9–11, 13 and 14. Equimolar amounts (2 mmol) of 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine or 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl)cyclohexylamine and proper anhydride were refluxed in xylene (20 mL) for 5 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography. For pharmacological assays free bases were converted into the hydrochloride salts in acetone solutions by the treatment with excess of Et_2O saturated with gaseous HCl.

5.1.1.1. 1-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]butyl}piperidine-2,6-dione (7). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine and glutaric anhydride in 43% yield as a pale yellow oil, $R_f = 0.42$ (SiO_2, $CHCl_3/CH_3OH = 19/1$); ^{1}H NMR (60 MHz) δ 7.5–6.8 (m, 4H, Ar-H), 4.0–3.6 (m, 2H, CH_2–imide), 3.4–3.0 (m, 4H, piperazine $2CH_2$), 2.8–2.2 (cluster, 10H), 2.2–1.2 (cluster, 6H). **7**·2HCl: colourless crystals, mp 191–193 °C. Anal. ($C_{20}H_{26}N_3O_2F_3$·2HCl) C, H, N.

5.1.1.2. 2-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]butyl}-2,3,4,5,6,7-hexahydro-1H-isoindole-1,3-dione (9). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine and 3,4,5,6-tetrahydrophthalic anhydride in 84% yield as a pale yellow oil, $R_f = 0.60$ (SiO_2, $CHCl_3/CH_3OH = 19/1$); ^{1}H NMR (60 MHz) δ 7.6–6.9 (m, 4H, Ar–H), 3.7–3.4 (m, 2H, –CH_2–imide), 3.4–3.1 (m, 4H, piperazine $2CH_2$), 2.7–2.1 (cluster, 10H), 2.1–1.4 (cluster, 8H). **9**·2HCl: colourless crystals, mp 186–188 °C. Anal. ($C_{23}H_{28}N_3O_2F_3$·2HCl) C, H, N.

5.1.1.3. *trans*-2-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}-2,3,4,5,6,7-hexahydro-1H-isoindole-1,3-dione (10). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]cyclohexylamine and 3,4,5,6-tetrahydrophthalic anhydride in 61% yield as colourless crystals, mp 165–167 °C, $R_f = 0.38$ (SiO_2, $CHCl_3/CH_3OH = 49/1$); ^{1}H NMR (300 MHz) δ 7.33 (t, $J = 7.9$ Hz, 1H, aryl H-5), 7.13–7.01 (m, 3H, aryl H-2, H-4 and H-6), 3.88 (tt, $J = 12.3$, 4.0 Hz, 1H, cyclohexane axial H-1), 3.34–3.20 (m, 4H, piperazine $2CH_2$), 2.84–2.68 (m, 4H, piperazine $2CH_2$), 2.58–2.42 (m, 1H, cyclohexane axial H-4), 2.36–2.25 (m, 4H, $2CH_2$ in tetrahydroisoindole-1,3-dione), 2.25–2.08 (m, 2H, cyclohexane axial H's), 2.09–1.96 (m, 2H, cyclohexane equatorial H's), 1.80–1.68 (m, 6H, $2CH_2$ in tetrahydroisoindole-1,3-dione and cyclohexane equatorial H's), 1.50–1.32 (m, 2H, cyclohexane axial H's). **10**·2HCl: colourless crystals, mp 248–250 °C. Anal. ($C_{25}H_{30}N_3O_2F_3$·2HCl) C, H, N.

5.1.1.4. 2-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]butyl}-cis-2,3,3a,4,7,7a-hexahydro-1H-isoindole-1,3-dione (11). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine and *cis*-1,2,3,6-tetrahydrophthalic anhydride in 65% yield as a pale yellow oil, $R_f = 0.45$ (SiO_2, $CHCl_3/CH_3OH = 19/1$); ^{1}H NMR (60 MHz) δ 7.6–6.9 (m, 4H, Ar–H); 6.1–5.8 (m, 2H, –CH=CH–

in tetrahydroisoindole-1,3-dione), 3.8–3.5 (m, 2H, –CH_2–imide), 3.5–2.9 (m, 6H, piperazine 2CH_2 and –$(CH_2)_3$–CH_2–piperazine), 2.9–1.9 (cluster, 10H), 1.9–1.2 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **11**·2HCl: colourless crystals, mp 166–168 °C. Anal. ($C_{23}H_{28}N_3O_2F_3$·2HCl) C, H, N.

5.1.1.5. 3,4-Dimethyl-1-{4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butyl}-3-pyrroline-2,5-dione (13). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]butylamine and 2,3-dimethylmaleic anhydride in 100% yield as a colourless oil, R_f = 0.57 (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^{1}H NMR (60 MHz) δ 7.6–6.9 (m, 4H, Ar–H), 3.8–3.4 (m, 2H, –CH_2–imide), 3.4–3.1 (m, 4H, piperazine 2CH_2), 2.8–2.2 (m, 6H, piperazine 2CH_2 and-$(CH_2)_3$–CH_2–piperazine), 2.0 (s, 6H, 2CH_3), 1.8–1.4 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **13**·2HCl: colourless crystals, mp 177–178 °C. Anal. ($C_{21}H_{26}N_3O_2F_3$·2HCl) C, H, N.

5.1.1.6. *trans*-3,4-Dimethyl-1-{4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}-3-pyrroline-2,5-dione (14). The compound was prepared by the general procedure from 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]cyclohexylamine and 2,3-dimethylmaleic anhydride in 55% yield as colourless crystals: mp 171–173 °C, R_f = 0.55 (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^{1}H NMR (300 MHz) δ 7.34 (t, J = 7.9 Hz, 1H, aryl H-5), 7.15–7.00 (m, 3H, aryl H-2, H-4 and H-6), 3.89 (tt, J = 12.3, 4.0 Hz, 1H, cyclohexane axial H-1), 3.42–3.14 (m, 4H, piperazine 2CH_2), 2.90–2.66 (m, 4H, piperazine 2CH_2), 2.60–2.42 (m, 1H, cyclohexane axial H-4), 2.26–1.98 (m, 4H, cyclohexane), 1.93 (s, 6H, 2CH_3), 1.82–1.68 (m, 2H, cyclohexane equatorial H's), 1.54–1.32 (m, 2H, cyclohexane axial H's). **14**·0.5HCl: colourless crystals, mp 277–279 °C. Anal. ($C_{23}H_{28}N_3O_2F_3$·0.5HCl) C, H, N.

5.1.2. General Procedure for the Preparation of Compounds 8 and 12. Equimolar amounts (2 mmol) of 4-[4-(*m*-trifluoromethylphenyl)piperazin-1-yl]cyclohexylamine and glutaric or *cis*-1,2,3,6-tetrahydrophthalic anhydride were refluxed in xylene (20 mL) for 5 h. The resulting precipitate of non-cyclic amidoacid was filtered off and then was heated in acetic anhydride (20 mL) in the presence of anhydrous sodium acetate (30% excess) for 5 h. After cooling the reaction mixture was poured into ice-water, neutralized with 10% NaOH and extracted with $CHCl_3$ (3 × 30 mL). The combined extracts were dried (K_2CO_3) and evaporated to give the oily residue, which was purified by column chromatography. For pharmacological assays free bases were converted into the hydrochloride salts in acetone solutions by the treatment with excess of Et_2O saturated with gaseous HCl.

5.1.2.1. *trans*-1-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}piperidine-2,6-dione (8). The compound was prepared by the general procedure in 25% yield as colourless crystals, mp 170–172 °C, R_f = 0.24 (SiO_2, $CHCl_3$/CH_3OH = 19/1);^{1}H NMR (300 MHz) δ 7.33 (t, J = 7.9 Hz, 1H, aryl H-5), 7.14–7.00 (m, 3H, aryl H-2, H-4 and H-6), 4.54 (tt, J = 12.2, 3.8 Hz, 1H, cyclohexane axial H-1), 3.34–3.18 (m, 4H, piperazine 2CH_2), 2.84–2.70 (m, 4H, piperazine 2CH_2), 2.62 (t, J = 6.5 Hz, 4H, 2CH_2CO in piperidine-2,6-dione), 2.58–2.46 (m, 1H, cyclohexane axial H-4), 2.46–2.28 (m, 2H, cyclohexane axial H's), 2.10–1.96 (m, 2H, cyclohexane equatorial H's), 1.96–1.84 (m, 2H, –CH_2–CH_2–CH_2– in piperidine-2,6-dione), 1.70–1.58 (m, 2H, cyclohexane equatorial H's), 1.50–1.32 (m, 2H, cyclohexane axial H's). **8**·HCl·0.25H_2O: colourless crystals, mp 244–246 °C. Anal. ($C_{22}H_{28}N_3O_2F_3$·HCl·0.25H_2O) C, H, N.

5.1.2.2. *trans*-2-{4-[4-(*m*-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}-cis-2,3,3a,4,7,7a-hexahydro-1H-isoindole-1,3-dione (12). The compound was prepared by the general procedure in 69% yield as colourless crystals, mp 151–153 °C, R_f = 0.31 (SiO_2, $CHCl_3$/CH_3OH = 49/1); ^{1}H NMR (300 MHz) δ 7.33 (t, J = 7.9 Hz, 1H, aryl H-5), 7.12–7.00 (m, 3H, aryl H-2, H-4 and H-6), 5.93–5.82 (m, 2H, –CH=CH– in tetrahydroisoindole-1,3-dione), 3.93 (tt, J = 12.4, 3.9 Hz, 1H, cyclohexane axial H-1), 3.32–3.14 (m, 4H, piperazine 2CH_2), 3.05–2.94 (m, 2H, CH–CH in tetrahydroisoindole-1,3-dione), 2.82–2.66 (m, 4H, piperazine 2CH_2), 2.64–2.38 (m, 3H, methylene 2H's in tetrahydroisoindole-1,3-dione and cyclohexane axial H-4), 2.34–2.12 (m, 4H, methylene 2H's in tetrahydroisoindole-1,3-dione and cyclohexane axial 2H's), 2.08–1.92 (m, 2H, cyclohexane equatorial 2H's), 1.69–1.55 (m, 2H, cyclohexane equatorial H's), 1.48–1.28 (m, 2H, cyclohexane axial H's). **12**·HCl: colourless crystals, mp 248–250 °C. Anal. ($C_{25}H_{30}N_3O_2F_3$·HCl) C, H, N.

5.2. In vitro radioligand binding assays

For all the assays inhibition constants (K_i) were determined from at least three separate experiments in which 7–9 drug concentrations, run in triplicate, were used. The binding reaction was terminated by rapid filtration through Whatman GF/B filters followed by three 4-mL washes with ice-cold incubation buffer.

The radioactivity retained on the filters was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 ml scintillation fluid (Akwascynt, BioCare). Binding isotherms of the tested compounds were analyzed by non-linear regression (Prism, GrafPad Software Inc., San Diego), using the Cheng-Prusoff equation[29] to calculate K_i values.

5.2.1. Serotonin 5-HT$_{1A}$, dopamine D$_2$ and α_1-adrenergic binding assays. Radioligand studies with native 5-HT$_{1A}$, D$_2$ and α_1-adrenergic receptors were conducted according to the methods previously described by us,[2,30,31] Briefly: 5-HT$_{1A}$ assays used rat hippocampal membranes, [^{3}H]-8-OH-DPAT (106 Ci/mmol, NEN Chemicals) and 5-HT, for nonspecific binding; dopamine D$_2$ assays used rat striatal membranes, [^{3}H]-spiperone (15.0 Ci/mmol, Perkin-Elmer) and butaclamol, for non-specific binding; α_1 assays used rat cortical membranes, [^{3}H]-Prazosin (25.0 Ci/mmol, Amersham) and phentolamine, for non-specific binding.

5.2.2. Expression of the gene for the human 5-HT$_{7(b)}$ receptor. The full length human 5-HTR$_{7(b)}$ cDNA cloned into mammalian expression vector pcDNA3.1(+) was purchased from UMR cDNA Resource Center (www.cdna.org). The receptor cDNA was stably transfected into human embryonic kidney cells (HEK293, ATCC) with use of Lipofectamine 2000 (Invitrogen). A clone yielding high expression level of 5-HTR$_{7(b)}$ was selected during preliminary experiments including Western blot analysis, [^{3}H]-CT saturation binding studies as well as cAMP accumulation assays.

5.2.3. Cell culture and preparation of cell membranes. HEK293 cells with stable expression of 5-HTR$_{7(b)}$ were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's Modifier Eagle's Medium (Gibco BRL) containing 5% dialysed foetal bovine serum (Gibco BRL) and 500 μg/mL G418 sulfate (Sigma–Aldrich). For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate-buffered saline (PBS) and pelleted by centrifugation (1000*g*) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparations pellets were stored at −80 °C. Membranes were prepared from frozen cell pellets by homogenization (Polytron, setting 5 for 15 s) and centrifugation (50,000*g*, 15 min., 4 °C) in 20 volumes of Tris–HCl buffer (50 mM, pH 7.4) containing EDTA (0.1 mM). The pellets were then re-suspended in buffer, incubated 20 min. in 37 °C, and once again centrifuged as described above. The membranes were stored in aliquots at −80 °C until use for binding assays. The protein concentration was determined with bicinchoninic acid protein assay kit (Pierce, USA).

5.2.4. 5-HT$_7$ Receptor binding assay. Binding assays on membranes from HEK 293 cells stably expressing human 5-HT$_{7(b)}$ receptor were performed according to procedure described by Thomas et al.[32] Briefly, the membranes (10 μg protein per tube) were incubated in 50 mM Tris–HCl buffer (pH 7.4) containing 4 mM $MgCl_2$, 0.1 mM pargyline and 0.1% ascorbic acid, in the presence of 7–9 concentrations of test drug and 0.5 nM [^{3}H]-5-CT (93.0 Ci/mmol, Amersham). Nonspecific binding was defined in the presence of 10 μM of 5-HT. After a 1-h incubation at 37 °C, the assay samples were rapidly filtered through Whatman GF/B filters and subsequently washed with ice-cold 50 mM Tris buffer (pH 7.4) using a Brandel harvester.

5.3. 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 February to May), between 9 a.m. and 2 p.m. All the experimental procedures were approved by the local Bioethics Commission 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) was dissolved in saline, N-{2-[4-(o-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexanecarboxamide (trihydrochloride, WAY 100635, synthesized byBoksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) and imipramine (hydrochloride, Polfa-Starogard, Poland) were dissolved in distilled water. Diazepam (Polfa-Poznań, Poland) and the investigated compounds were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc), diazepam, imipramine and the tested compounds were given intraperitoneally (ip) in a volume of 2 ml/kg (rats) or 10 ml/kg (mice). Each experimental group consisted of six to ten animals, and all the animals were used only once. The obtained data were analyzed by one-way analysis of variance followed by Dunnett's test (when only one drug was given) or by Newman–Keuls test (when two drugs were administered).

5.3.1. Body temperature in mice. Effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90 and 120 min after their administration. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **7–12** and **14** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the compounds or 8-OH-DPAT and rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. In another experiment, effect of **13** (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 the basal body temperature, as measured at the beginning of the experiment.

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

5.3.3. Four-plate test in mice. The experiment was carried out according to the method of Aron et al.[16] The box was made of an opaque plastic and was rectangular (25 cm × 18 cm × 16 cm) in shape. The floor was covered with four rectangular metal plates (11 cm × 8 cm), separated by a 4 mm gap. The plates were connected to a source of continuous current which enabled a 120 V dif-

ference of potential between two adjacent plates for 0.5 s when the experimenter pressed the switch. Individual mice were placed gently onto 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 for the following 3 s. The episodes of punished crossing were counted for 60 s.

5.3.4. Forced swim test in mice. The experiment was carried out according to the method of Porsolt et al.[17] 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 keeps its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

5.3.5. Locomotor activity in mice. The spontaneous locomotor activity of mice was recorded by Opto-M3 multichannel activity monitor (MultiDevice Software v. 1.30, Columbus Instruments). The mice were placed individually in plastic cages, and the number of crossing for each channel (ambulation) was counted twice: during the first 6 min, that is, at the time equal to the observation period in the forced swimming test, and during 30-min experimental sessions.

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