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Short communication

Synthesis and 5-HT_{1A}, 5-HT_{2A} receptor activity of new β-tetralonohydantoins

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Abstract

A series of new 3-[4-(4-arylpiperazinyl)-butyl]-β-tetralonohydantoins (**8a**–**13a**) were synthesized. The compounds exhibited high affinity for 5-HT_{1A} receptors (K_i = 6 to 55 nM) combined with moderate-to-high 5-HT_{2A} receptor affinities (K_i = 45 to 213 nM). The results of in vivo studies indicated that of the compounds tested, 3-[4-(4-phenylpiperazinyl)-butyl-β-tetralonohydantoin (**8a**) showed features of full (pre- and postsynaptic) 5-HT_{1A} receptor agonists, whereas compounds **9a**–**13a** behaved like antagonists of postsynaptic 5-HT_{1A} receptors; additionally, compound **13a** produced an effect characteristic of presynaptic 5-HT_{1A} receptor agonists. Moreover, compounds **8a** and **10a**–**13a** exhibited properties of 5-HT_{2A} receptor antagonists. Due to the most interesting 5-HT_{1A}/5-HT_{2A} functional profile compounds **8a** and **13a** were further tested for their potential psychotropic activity. In fact, compound **8a** (but not **13a**) showed diazepam—like anxiolytic activity and behaved like a weak antidepressant.

Keywords: 1-arylpiperazine derivatives; β-tetralonohydantoins; $5HT_{1A}$, $5HT_{2A}$, D_1/D_2 receptor affinity; 5-HT_{1A}/5-HT_{2A} functional activity; Anxiolytics/antidepressants

1. Introduction

The neurotransmitter serotonin (5-HT) modulates the activity of the central nervous system and peripheral tissues through a number of receptor subtypes. Due to a wide range of physiological and pathophysiological systems in which 5-HT is known to play an important role, close attention has been focused on the identification of agents which act selectively at each of these receptor subtypes [1]. Among them, 5-HT_{1A} receptor partial agonists and 5-HT_{2A} receptor antagonists are particular interest, since clinical studies indicate that these drugs are effective in treating mood disorders [2,3].

Consequently, it has been suggested that compounds interact simultaneously with 5-HT_{1A} and 5-HT_{2A} receptors may have a more advantageous therapeutic profile. Such has been the case with adatanserin and flibanserin (both 5-HT_{1A} receptor agonist and 5-HT_{2A} receptor antagonist) which show potent activity in number models of anxiety and depression [3]. Moreover "atypical" antypsychotic drugs such as e.g. olanzapine and ziprazidon are antagonists of 5-HT_{2A} and dopaminergic D_2 receptors and, interestingly enough, ziprazidon is also 5-HT_{1A} receptor agonist [4]. In the past years a great number of 1-arylpiperazines have been synthesized and the class of long chain arylpiperazines (LCAPs) derivatives is considered to be of great importance for studies into ligand 5-HT_{1A} and 5-HT_{2A} receptor interactions [5].

On the other hand, 5,5-disubstituted hydantoins connected at N3 position to arylpiperazines with an alkylen spacer showed a wide spectrum of activity on the central nervous system, mediated by α_1-adrenergic, D_2-dopaminergic and also 5-HT_{1A}, 5-HT_{2A} receptors [6–11]. In contrast, LCAPs con-

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taining the bicyclic fragment based on the hydantoin moiety, showed moderate-to-high affinity for 5-HT_{1A} and α_1 receptor binding sites, but low affinity for D_2 receptors [12].

Of the above discussed LPCAs three selected compounds i.e. 1′,5-cyclopentanospiro-3-[4-(4-phenyl-1-piperazinyl)-butyl]-hydantoin (**1**), its 1′,5-cyclohexanospiro analogue (**2**), and 1′,5-cyclohexanospiro-3-{4-[4-(-2-methoxyphenyl)-1-piperazinyl]-butyl}-hydantoin (**3**) (Fig. 1), synthesized in our laboratory, exhibited high 5-HT_{1A} receptor affinity (K_i = 33, 17 and 0.51 nM, respectively) and moderate 5-HT_{2A} receptor affinity (K_i = 133–293 nM) [6].

Lopez-Rodriguez et al. [12,13] described a series of structurally related compounds-phenylpiperazine derivatives of 1,3-dioxoperhydroimidazo[1,5-a]pyridine and 1,2-dioxoperhydropyrrolo[1,2-c]imidazole-as new, selective 5-HT_{1A} receptor ligands among which compound **4** (Fig. 1) showed the highest 5-HT_{1A} receptor affinity (K_i = 7.2 nM).

In a successive study we presented a series (**14**–**19**) of new arylpiperazinyl derivatives with cyclohexano-1′,5-spirohydantoin modified by a fused aromatic ring in the cyclohexane moiety, connected to unsubstituted, 2-OCH_3, 3-Cl, 2-F, 4-F, 3-CF_3 phenylpiperazine with a propylen spacer. The majority (**14**, **16**–**18**) of those agents displayed high 5-HT_{2A} receptor affinity and low-to-moderate 5-HT_{1A} receptor affinity, only 3-CF_3 derivative (**19**) showed comparable high affinity for both 5-HT_{1A} and 5-HT_{2A} receptors [8].

The described results prompted us to further investigate that group of compounds. Since LCAPs with a four-unit spacer generally display higher affinity for 5-HT_{1A} receptors than do shorter-chain ones [14], we synthesized butyl analogs (**8a**–**13a**) of the previously described compounds (**14**–**19**) an attempt to obtain agents displaying high affinity for both 5-HT_{1A} and 5-HT_{2A} (and D_2) receptor subtypes. As has been mentioned above, such a receptor profile is characteristic of some anxiolytics, antidepressants or "atypical" neuroleptics. To explore the effect of spacer elongation on pharmacological properties, compounds **8a**–**13a** were tested for their 5-HT_{1A}/5-HT_{2A} receptor affinity. Additionally, the D_1/D_2 receptor affinity was examined for selected, mixed 5-HT_{1A}/5-HT_{2A} receptor ligands (**8a** and **13a**). On the basis of binding studies, the functional 5-HT_{1A} receptor profile was determined for all compounds; moreover, 5-HT_{2A} receptor antagonistic activity was assessed for the new 5-HT_{2A} ligands (**8a**, **11a**–**13a**). Compounds **8a** and **13a**, which showed the most interesting 5-HT_{1A}/5-HT_{2A} functional profile, were examined using animal models of anxiety and depression.

2. Chemistry

The compounds were synthesized according to Scheme 1. β-Tetralonohydantoin (7,8-benzo-1,3-diazaspiro- [4,5]-decane-2,4-dione) **6** was prepared from ketone **5** by means of the Bucherer Berg reaction with modifications described by Goodson et al. [15]. Compounds **8**–**13** (free bases) were obtained in a two-step condensation. β-Tetralonohydantoin **6** were alkylated with 1-bromo-4-chlorobutane, in the presence of K_2CO_3 and in acetone medium, at position N3 to

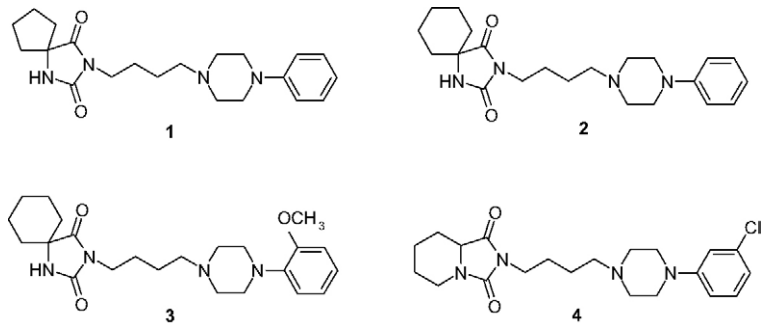
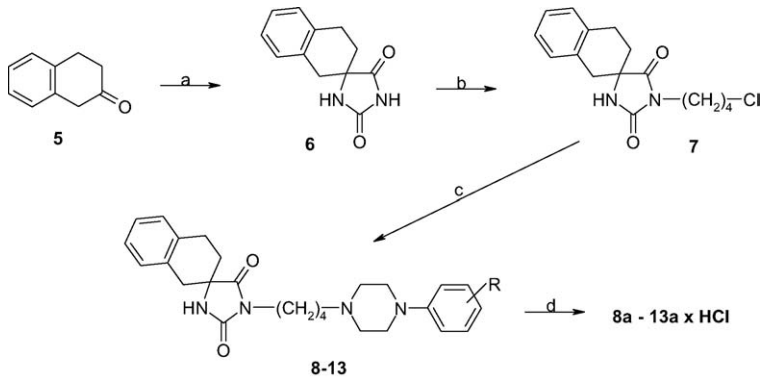


Fig. 1. Chemical structure of selected hydantoin derivatives.



Scheme 1. Synthesis pathways of the investigated compounds. Reagents, reaction conditions: (a) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol; (b) $Br(CH_2)_4Cl$, K_2CO_3, acetone, reflux 8 h; (c) 1-phenylpiperazine derivative, anhydrous ethyl alcohol, reflux 8 h; (d) 36% HCl.

give intermediate product **7**. The subsequent condensations of 4-chlorobutyl derivative **7** with the appropriate arylpiperazine gave **8–13** transformed into water solubled hydrochlorides **8a–13a**.

Compounds **8–13** were recrystallized from a mixture of ethanol and acetone.

The molecular formulas of **8a–13a** were established on the basis of the results of elemental (C, H, N) analyses (data not shown). The structures of hydrochlorides **8a–13a**, as well as their physicochemical data together with m.p.'s of the appropriate bases are presented in Table 1. The structures of the compounds were confirmed by ^{1}HNMR spectra (Table 2).

3. Pharmacological results

3.1. In vitro studies

The results of in vitro binding studies of the newly synthesized compounds **8a–13a**, as well as the data of the previously published analogs **14–19** [8] are shown in Table 3.

The investigated compounds **8a–13a** showed high affinity for 5-HT$_{1A}$ receptors (K_i = 6–55 nM); moreover, they displayed high to moderate affinity for 5-HT$_{2A}$ receptor sites (K_i = 45–213 nM). The most active derivatives at serotonin receptors (**8a**, **13a**) were selected and evaluated in the respect of their affinity for dopaminergic D$_1$ and D$_2$ receptors. Compound **8a** demonstrated very low affinity for D$_1$ and D$_2$ receptors (K_i = 2200 ± 300 and 950 ± 200 nM, respectively), whereas **13a** practically did not bind to those receptors (K_i > 20,000 nM) (data not shown).

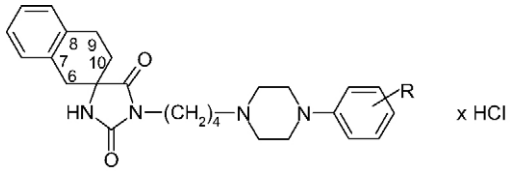
3.2. In vivo studies

For the newly synthesized compounds **8a–13a**, functional 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor activity in vivo was tested.

3.2.1. Lower lip retraction (LLR) in rats

Among the investigated compounds only derivative **8a** (10 and 20 mg/kg) induced LLR in rats, the maximum pos-

Table 1
Physicochemical properties of the hydrochlorides of 3-(4-arylpiperazinylalkyl)-β-tetralonohydantoin derivatives **8a–13a**



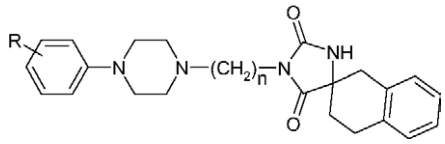
Comp.	R	Yield [a] (%)	M.p. [b] (°C)		Formula [c] M.W.	Molecular weight
			Base	Salt		
8a	H	50	152–153	227–229	$C_{26}H_{32}N_4O_2 \times HCl$	469.01
9a	2-OCH$_3$	42	166–168	234–237	$C_{27}H_{34}N_4O_3 \times HCl$	499.01
10a	3-Cl	52	150–153	210–212	$C_{26}H_{31}N_4O_2Cl \times HCl$	503.45
11a	2-F	48	147–149	229–230	$C_{26}H_{31}N_4O_2F \times HCl$	487.00
12a	4-F	40	172–174	218–220	$C_{26}H_{31}N_4O_2F \times HCl$	487.00
13a	3-CF$_3$	53	147–150	215–217	$C_{27}H_{31}N_4O_2F_3 \times HCl$	537.01

[a] Yield (%) calculated for free bases **8–13**.
[b] All the bases were recrystallized from a mixture of anhydrous ethanol and acetone (1:1).
[c] The values obtained by in an elemental analysis were within 0.4% of theoretical values.

Table 2
^{1}H-NMR spectral data of compounds **8–13**

Compound	δ ppm in CDCl$_3$ (200 MHz)
8	1.56–1.95 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.15–2.27 (m, 1H, C10-*H*), 2.42 (t, *J* = 7.5 Hz, 2H, *CH$_2$*-piperazine), 2.60 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.70 (d, *J* = 16.0 Hz, 1H, C6-*H*), 2.88–3.02 (m, 2H, C9-*H*), 3.20 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 3.35 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.56 (t, *J* = 7.0 Hz, 2H, N3-*CH$_2$*), 5.88 (brs, 1H, N1-*H*), 6.82–7.32 (m, 9H, aromat)
9	1.59–1.91 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.16–2.32 (m, 1H, C10-*H*), 2.44 (t, *J* = 7.5 Hz, 2H, *CH$_2$*-piperazine), 2.65 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.75 (d, *J* = 16.5 Hz, 1H, C6-*H*), 2.90–3.10 (m, 2H, C9-*H*), 3.15 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 3.38 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.56 (t, *J* = 7.5 Hz, 2H, N3-*CH$_2$*), 3.86 (s, 3H, *CH$_3$*), 5.72 (brs, 1H, N1-*H*), 6.84–7.16 (m, 8H, aromat)
10	1.53–1.91 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.16–2.32 (m, 1H, C10-*H*), 2.46 (t, *J* = 7.5 Hz, 2H, *CH$_2$*-piperazine), 2.62 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.74 (d, *J* = 16.0 Hz, 1H, C6-*H*), 2.90–3.10 (m, 2H, C9-*H*), 3.22 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 3.38 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.58 (t, *J* = 7.0 Hz, 2H, N3-*CH$_2$*), 5.77 (brs, 1H, N1-*H*), 6.75–6.80 (m, 3H, aromat), 7.08–7.19 (m, 5H, aromat)
11	1.49–1.92 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.18–2.32 (m, 1H, C10-*H*), 2.42 (t, *J* = 7.5 Hz, 2H, *CH$_2$*-piperazine), 2.60 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.75 (d, *J* = 16.0 Hz, 1H, C6-*H*), 2.90–3.05 (m, 2H, C9-*H*), 3.12 (t, *J* = 5.0 Hz, 4H, *CH$_2$*- piperazine), 3.35 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.55 (t, *J* = 7.0 Hz, 2H, N3-*CH$_2$*), 5.98 (brs, 1H, N1-*H*), 6.90–7.26 (m, 8H, aromat)
12	1.42–1.94 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.18–2.32 (m, 1H, C10-*H*), 2.42 (t, *J* = 7.0 Hz, 2H, *CH$_2$*-piperazine), 2.58 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.75 (d, *J* = 16 Hz, 1H, C6-*H*), 2.84–3.02 (m, 2H, C9-*H*), 3.10 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 3.35 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.55 (t, *J* = 7.0 Hz, 2H, N3-*CH$_2$*), 5.92 (brs, 1H, N1-*H*), 6.83–7.15 (m, 8H, aromat)
13	1.45–1.90 (m, 5H, CH$_2$*CH$_2$*CH$_2$*CH$_2$, m, C10-*H*), 2.16–2.32 (m, 1H, C10-*H*), 2.45 (t, *J* = 7.5 Hz, 2H, *CH$_2$*-piperazine), 2.60 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 2.74 (d, *J* = 16.0 Hz, 1H, C6-*H*), 2.88–3.10 (m, 2H, C9-*H*), 3.12 (t, *J* = 5.0 Hz, 4H, *CH$_2$*-piperazine), 3.34 (d, *J* = 16.0 Hz, 1H, C6-*H*), 3.58 (t, *J* = 7.0 Hz, 2H, N3-*CH$_2$*), 5.90 (s, 1H, N1-*H*), 6.85–7.18 (m, 8H, aromat)

Table 3
Structure of the investigated compounds and the binding data on 5-HT_{1A} and 5-HT_{2A} receptors



Compound	*n*	R^1	K_i (nM)	
			5-HT_{1A}	5-HT_{2A}
8a	4	H	25 ± 3	45 ± 1
9a	4	2–OCH_3	6 ± 1	213 ± 10
10a	4	3–Cl	15 ± 1	114 ± 6
11a	4	2–F	17 ± 1	75 ± 3
12a	4	4-F	55 ± 1	57 ± 3
13a	4	3-CF_3	43 ± 3	69 ± 1
14*	3	H	70 ± 1	34 ± 2
15*	3	2-OCH_3	92 ± 12	462 ± 10
16*	3	3-Cl	96 ± 1	17 ± 2
17*	3	2-F	408 ± 2	117 ± 5
18*	3	4-F	950 ± 21	14 ± 2
19*	3	3-CF_3	53 ± 1	76 ± 4

* Data from [5].

sible score being 50% and 53% ($P < 0.01$), respectively. The remaining compounds **9a**–**12a** (10–20 mg/kg) and **13a** (1.25–5 mg/kg) given alone showed no activity in that test (data not shown). At the same time, **10a**–**12a** (10–20 mg/kg) and **13a** (1.25–5 mg/kg)-like WAY 100635, used as a standard 5-HT_{1A} receptor antagonist-inhibited the LLR induced by 8-OH-DPAT in rats in a dose-dependent manner; at the highest doses used, that inhibition ranged from 40% (**12a**) to 71% (**13a**). Compounds **8a** and **9a** (10–20 mg/kg) practically did not affect the 8-OH-DPAT-induced LLR (Table 4).

3.2.2. Behavioral syndrome in reserpinized rats

None of the investigated compounds (**8a**–**13a**) in doses up to 20 mg/kg, given alone, evoked changes in the behavior of reserpine-pretreated rats (data not shown). Among the tested compounds, derivatives **9a** (2.5–5 mg/kg) and **11a** (10–20 mg/kg)-like WAY 100635-reduced dose-dependently the 8-OH-DPAT-induced flat body posture (FBP) and forepaw treading (FT); in that respect, compound **9a** was more effective, as after the highest dose used it reduced FBP and FT by 58% and 77%, respectively. Derivatives **12a** and **13a** (10–20 mg/kg) attenuated FT but failed to inhibit the FBP produced by 8-OH-DPAT in rats. Compounds **8a** and **10a** (10–20 mg/kg) practically did not affect either symptom induced by 8-OH-DPAT in reserpinized rats (Table 4).

3.2.3. Body temperature in mice

All the investigated compounds **8a**–**13a** (2.5–20 mg/kg) decreased rectal body temperature in mice in a dose-dependent manner. The maximum hypothermic effect was observed at 30 min after their administration and lasted up to 60 min (**8a**, **13a**) or longer (**9a**–**12a**) (Table 5). WAY 100635 (0.1 mg/kg) reduced the hypothermia induced by **8a** (5 mg/kg), **10a** (10 mg/kg) and **13a** (10 mg/kg, statistically non-significant)

Table 4
Effect of the investigated compounds on the 8-OH-DPAT-induced LLR in rats (A) and their effect on the 8-OH-DPAT-induced behavioral syndrome in reserpine-pretreated rats (B)

Compounds	Dose (mg/kg)	Mean ± S.E.M. LLR score A	Mean ± S.E.M. behavioral score B	
			FBP	FT
Vehicle	–	2.6 ± 0.2	13.8 ± 0.8	12.8 ± 1.2
8a	10	2.3 ± 0.2	13.2 ± 0.7	13.7 ± 0.6
	20	2.1 ± 0.2	11.5 ± 0.3	10.3 ± 1.1
Vehicle	–	2.8 ± 0.2	12.3 ± 0.5	12.0 ± 0. 6
9a	2.5	NT	8.8 ± 2.8	4.3 ± 1.2 [a]
	5	NT	5.2 ± 1.4 [a]	2.8 ± 0.8 [a]
	10	2.3 ± 0.3	NT	
	20	2.5 ± 0.1	NT	
Vehicle	–	2.7 ± 0.2	13.8 ± 0.8	12.8 ± 1.2
10a	10	1.4 ± 0.3 [a]	14.2 ± 0.6	11.2 ± 1.2
	20	1.1 ± 0.2 [a]	12.5 ± 0.5	8.7 ± 1.6
Vehicle	–	2.8 ± 0.1	14.5 ± 0.3	12.8 ± 0.5
11a	10	2.1 ± 0.3	11.7 ± 0.9 [b]	9.5 ± 0.6 [a]
	20	1.3 ± 0.4 [a]	10.3 ± 0.6 [a]	6.7 ± 0.6 [a]
Vehicle	–	2.8 ± 0.1	14.0 ± 0.4	12.3 ± 0.5
12a	10	2.4 ± 0.3	13.7 ± 0.3	8.7 ± 0.6 [a]
	20	1.8 ± 0.2 [a]	14.1 ± 0.3	9.3 ± 0.5 [a]
Vehicle	–	2.8 ± 0.1	14.0 ± 0.3	12.6 ± 0.9
13a	1.25	2.0 ± 0.2 [a]	NT	
	2.5	1.3 ± 0.1 [a]	NT	
	5	0.8 ± 0.3 [a]	12.8 ± 0.7	7.0 ± 1.0 [a]
	10	NT	12.5 ± 0.9	5.8 ± 1.0 [a]
	20	NT	11.0 ± 1.5	5.3 ± 1.4 [a]
WAY 100635	0.1	0.3 ± 0.2[a]	0.8 ± 0.4[a]	1.2 ± 0.7 [a]

A: The investigated compounds (i.p.) and WAY 100635 (s.c.) were administered 45 and 15 min, respectively, before 8-OH-DPAT (1 mg/kg, s.c.). B: Reserpine (1 mg/kg, s.c.), the investigated compounds (i.p.) and WAY 100635 (s.c.) were administered 18 h, 60 and 30 min, respectively, before 8-OH-DPAT (5 mg/kg, i.p.). Each group consisted of 6 rats. [a] $P < 0.01$ vs. respective vehicle +8-OH-DPAT group (Dunnett's test). NT-not tested.

but not **9a** (10 mg/kg), **11a** (2.5 mg/kg) or **12a** (5 mg/kg), in mice (Table 6). WAY 100635 (0.1 mg/kg), used as a standard 5-HT_{1A} receptor antagonist, did not change body temperature (Table 5), but completely abolished the hypothermic effect of 8-OH-DPAT in mice (Table 6).

3.2.4. Head twitch response in mice

The investigated compounds **8a**, **10a**–**13a** inhibited in a dose-dependent manner the (±)DOI-induced head twitches in mice; the ID_{50} values ranged between 4.7 (**8a**) and 19.0 (**13a**) mg/kg. Their 5-HT_{2A} antagonistic activity was compared to ketanserin (ID_{50} = 0.14 mg/kg), a well-known 5-HT_{2A} receptor antagonist (Table 7).

3.2.5. Elevated plus-maze test in rats

The total number of entries (open + closed arm entries) made by control rats during a 5-min test session was about six to eight in the present set of experiments. Thirty-five to 39% of entries were made into the open arms, and 11–12% of the total time (239–270 s) spent in the arms (either type) was spent in the open arms.

Table 5
The effect of the investigated compounds on the body temperature of mice

Compounds	Dose (mg/kg)	Δ*t* ± S.E.M. (°C) [a]			
		30 min	60 min	90 min	120 min
Vehicle	–	–0.1 ± 0.1	–0.1 ± 0.1	–0.1 ± 0.1	0.0 ± 0.1
8a	2.5	–0.5 ± 0.2	–0.3 ± 0.1	–0.3 ± 0.1	0.0 ± 0.1
	5	–2.2 ± 0.3[b]	–0.7 ± 0.1[c]	0.0 ± 0.1	0.2 ± 0.1
	10	–2.7 ± 0.3[b]	–1.8 ± 0.5[b]	–0.8 ± 0.3	–0.5 ± 0.2
Vehicle	–	–0.2 ± 0.1	–0.1 ± 0.1	–0.2 ± 0.1	–0.3 ± 0.1
9a	10	–2.3 ± 0.3[b]	–1.8 ± 0.3[b]	–1.1 ± 0.3[c]	–0.9 ± 0.1[c]
	20	–3.6 ± 0.3[b]	–3.1 ± 0.3[b]	–1.8 ± 0.3[b]	–1.3 ± 0.2[b]
Vehicle	–	–0.2 ± 0.1	0.0 ± 0.1	–0.3 ± 0.1	–0.1 ± 0.1
10a	10	–1.5 ± 0.2[b]	–0.8 ± 0.1[c]	–0.8 ± 0.1[c]	–0.5 ± 0.1
	20	–3.3 ± 0.3[b]	–2.4 ± 0.3[b]	–1.5 ± 0.2[b]	–0.7 ± 0.2[c]
Vehicle	–	0.0 ± 0.1	0.0 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1
11a	2.5	–1.5 ± 0.2[b]	–0.6 ± 0.2	–0.4 ± 0.2	–0.2 ± 0.1
	5	–2.0 ± 0.3[b]	–1.7 ± 0.3[b]	–1.1 ± 0.3[b]	–0.9 ± 0.1[b]
	10	–3.3 ± 0.4[b]	–2.9 ± 0.4[b]	–1.7 ± 0.3[b]	–1.1 ± 0.2[b]
Vehicle	–	0.1 ± 0.1	–0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
12a	2.5	–0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
	5	–1.5 ± 0.2[b]	–0.7 ± 0.1[b]	–0.2 ± 0.2	0.1 ± 0.1
	10	–2.9 ± 0.3[b]	–2.1 ± 0.3[b]	–1.6 ± 0.2[b]	–1.1 ± 0.2[c]
Vehicle	–	–0.1 ± 0.1	–0.1 ± 0.1	–0.1 ± 0.1	0.1 ± 0.1
13a	10	–0.9 ± 0.1[b]	–0.8 ± 0.1[b]	–0.4 ± 0.2	–0.2 ± 0.2
	20	–2.0 ± 0.2[b]	–1.4 ± 0.2[b]	–0.8 ± 0.3	–0.5 ± 0.3
WAY 100635	0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1

The investigated compounds (i.p.) and WAY 100635 (s.c.) were administered 30 min before the test. Each group consisted of seven to eight mice. [a] Absolute mean initial body temperatures were within a range of 36.7 ± 0.3 °C; [b] $P < 0.01$, [c] $P < 0.05$ vs. respective vehicle group (Dunnett's test).

Compound **8a** administered in a dose of 2.5 mg/kg changed the percentage of the open arm entries and the time spent in the open arms; when given in the doses of 5 and 10 mg/kg, it induced an anxiolytic-like effect, having significantly increased the percentage of entries into the open arms (up to 49% and 92%, respectively), and the time spent therein (up to 34% and 96%, respectively) (Table 8). Nevertheless, the active doses of compound **8a** (5 and 10 mg/kg) modified the general activity of rats, and decreased the total number of entries by 32% and 80%, respectively (data not shown). Compound **13a** (10–20 mg/kg) did not change the percentage of the time spent in the open arms and the entries into the open arms (data not shown). Diazepam (2.5 and 5 mg/kg), used as a reference drug, significantly increased the percentage of entries into the open arms (up to 74% and 76%, respectively), as well as the time spent in the open arms (up to 47% and 70%, respectively). Diazepam (5 mg/kg, but not lower) significantly reduced (by 52%) the total number of entries (data not shown).

3.2.6. Forced swimming test in rats

Compound **8a** (2.5 and 5 mg/kg) poorly reduced the immobility time in rats (by 22% and 24%, respectively); its higher dose was without effect. The antiimmobility effect of **8a** was weaker than that of imipramine, used as a reference antidepressant drug; the latter drug at a dose of 40 mg/kg patently (by 47%) inhibited immobility time in rats (Table 9). Compound **13a** (5–20 mg/kg) was practically inactive in that test (data not shown).

Table 6
The effect of WAY 100635 on the hypothermia induced by the investigated compounds in mice

Compounds, dose (mg/kg)	Δ*t* ± S.E.M. (°C) [a]	
	30 min	60 min
Vehicle	0.0 ± 0.1	0.0 ± 0.1
8a (5)	–2.9 ± 0.3 [b]	–2.1 ± 0.2 [b]
WAY 100635 + **8a** (5)	–1.8 ± 0.3 [B]	–0.9 ± 0.2[bA]
Vehicle	–0.2 ± 0.1	–0.2 ± 0.1
9a (10)	–1.4 ± 0.1 [b]	–1.2 ± 0.2 [b]
WAY 100635 + **9a** (10)	–1.6 ± 0.1 [b]	–1.4 ± 0.3 [b]
Vehicle	–0.1 ± 0.1	–0.1 ± 0.2
10a (10)	–2.4 ± 0.2 [b]	–1.3 ± 0.1 [b]
WAY 100635 + **10a** (10)	–1.3 ± 0.2[bA]	–0.8 ± 0.2
Vehicle	–0.1 ± 0.1	–0.1 ± 0.1
11a (2.5)	–1.8 ± 0.2 [b]	–1.0 ± 0.2 [b]
WAY 100635 + **11a** (2.5)	–1.5 ± 0.3 [b]	–0.8 ± 0.2 [c]
Vehicle	0.0 ± 0.1	0.1 ± 0.1
12a (5)	–2.9 ± 0.6 [b]	–1.7 ± 0.4 [b]
WAY 100635 + **12a** (5)	–3.2 ± 0.5 [b]	–1.5 ± 0.3 [b]
Vehicle	–0.1 ± 0.2	–0.2 ± 0.1
13a (10)	–1.2 ± 0.3 [b]	–0.8 ± 0.2 [c]
WAY 100635 + **13a** (10)	–0.7 ± 0.1[bB]	–0.3 ± 0.2
Vehicle	0.1 ± 0.1	0.1 ± 0.1
8-OH-DPAT (5)	–1.0 ± 0.1 [b]	–0.7 ± 0.2
WAY 100635 + 8-OH-DPAT (5)	–0.1 ± 0.1 [A]	0.1 ± 0.1 [A]

The investigated compounds (i.p.) and 8-OH-DPAT (s.c.) were administered 30 and 15 min, respectively, before the test. WAY 100635 (0.1 mg/kg, s.c.) was administered 15 min before the investigated compounds. [a] Absolute mean initial body temperatures were within a range of 36.5 ± 0.5 °C. [b] $P < 0.01$, [c] $P < 0.05$ vs. respective vehicle group; [A] $P < 0.01$, [B] $P < 0.05$ vs. respective vehicle + compound group (Dunnett's test).

Table 7
The effects of the investigated compounds on the (±)-DOI-induced head twitch response in mice

Compounds	ID_{50}[a] (mg/kg, i.p.)
8a	4.7 (2.9–7.5)[b]
10a	8.5 (5.3–13.6)
11a	5.5 (3.4–8.8)
12a	10.0 (6.9–14.5)
13a	19.0 (12.7–28.5)
Ketanserin	0.14 (0.07–0.20)

[a] ID_{50}-the dose inhibiting the head twitches in mice by 50%.
[b] The investigated compounds (i.p.) were administered 60 min before (±)-DOI (2.5 mg/kg, i.p.).

Table 8
The effects of compound **8a** and diazepam in the plus-maze test in rats

Compounds	Dose (mg/kg)	Percent of open arm entries mean ± S.E.M.	Percent of time in open arms mean ± S.E.M.
Vehicle	–	34.9 ± 6.5	11.5 ± 2.7
8a	2.5	43.7 ± 3.4	31.5 ± 11.2
	5	49.0 ± 5.0[a]	34.1 ± 7.0[a]
	10	91.7 ± 8.3[b]	96.1 ± 3.9[b]
Vehicle	–	38.5 ± 3.1	10.9 ± 1.1
Diazepam	2.5	73.8 ± 4.2[a]	47.2 ± 5.3[a]
	5	76.2 ± 8.8[b]	70.4 ± 10.9[b]

Compound **8a** and diazepam were administered i.p. 60 min before the test. Each group consisted of six to eight rats. [a] $P < 0.05$, [b] $P < 0.01$ vs. respective vehicle group (Dunnett's test).

Table 9
The effects of compound **8a** and imipramine in the forced swimming test in rats

Compounds	Dose (mg/kg)	Immobility time (s) mean ± S.E.M.	%
Vehicle	–	235.9 ± 5.7	100
8a	1.25	206.6 ± 10.7	87.6
	2.5	184.0 ± 12.1[a]	78.0
	5	179.9 ± 5.6[a]	76.3
	10	198.7 ± 9.9	84.2
Vehicle	–	215.3 ± 13.1	100
Imipramine	20	176.3 ± 14.9	81.9
	40	113.5 ± 8.8[a]	52.7

Compound **8a** and imipramine were administered i.p. 60 min before the test. Each group consisted of eight rats. [a] $P < 0.01$ vs. respective vehicle group (Dunnett's test).

3.2.7. Open field test in rats

Compound **8a**, used at doses effective in the plus-maze test or forced swimming test (2.5–10 mg/kg), potently and dose-dependently attenuated the exploratory activity (time of walking, ambulation and peeping + rearing) of rats; an almost complete reduction of the rats' activity was observed after administration of 10 mg/kg (Table 10).

Diazepam (2.5–5 mg/kg) attenuated the exploratory activity of rats, whereas imipramine (20–40 mg/kg) did not change the rats' activity in that test.

Table 10
The effect of compound **8a** on the exploratory activity of rats in the open field test

Compound	Dose (mg/kg)	Exploratory activity		
		Time of walking (s) mean ± S.E.M.	Ambulation mean ± S.E.M.	Peeping + rearing mean ± S.E.M.
Vehicle	–	40.0 ± 7.0	16.0 ± 1.8	15.5 ± 1.1
8a	2.5	38.5 ± 9.1	12.7 ± 3.5	7.0 ± 1.5[b]
	5	21.8 ± 3.3[b]	5.7 ± 1.0[b]	2.8 ± 0.7[b]
	10	9.0 ± 1.5[b]	1.8 ± 0.4[b]	0.8 ± 0.4[b]
Vehicle	–	37.0 ± 4.0	17.0 ± 2.3	11.2 ± 2.4
Diazepam	2.5	27.0 ± 4.1[b]	12.0 ± 1.9	7.8 ± 2.1
	5	17.8 ± 4.6[b]	5.0 ± 1.3[b]	4.4 ± 2.0[a]
Vehicle	–	37.3 ± 3.6	15.5 ± 2.2	7.8 ± 1.5
Imipramine	20	33.3 ± 3.3	11.8 ± 3.2	7.5 ± 1.8
	40	32.5 ± 4.6	11.0 ± 1.4	5.8 ± 1.8

Compound **8a**, diazepam and imipramine were given i.p. 60 min before the test. Each group consisted of six rats. [a] $P < 0.05$, [b] $P < 0.01$ vs. vehicle group (Dunnett's test).

4. Discussion

Compounds **8a**–**13a** containing a tetramethylene group alkylen spacer exhibit high 5-HT_{1A} receptor affinity K_i ranging from 6 to 55 nM for 2-OCH_3 (**9a**) and 4-F (**12a**) derivatives, respectively; whereas among the described earlier trimethylene spacer analogs (**14**–**19**), only 3-CF_3 (**19**) was a potent 5-HT_{1A} receptor ligand (K_i = 42 nM) [8] (Table 3). As expected, elongation of the spacer enhanced 5-HT_{1A} receptor affinity, irrespective of the substituent type in the phenyl ring (Fig. 2). A comparison of the pairs of analogues showed

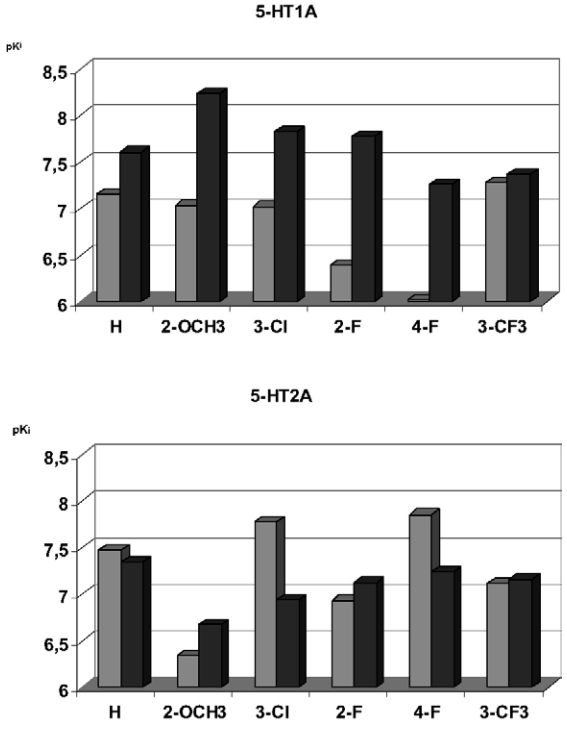


Fig. 2. Relationships between the pK_i values and different spacer fragments (light gray: trimethylene spacer analogs, dark gray: tetramethylene spacer analogs).

that the affinity increased 24-fold for 2-F (**17** vs. **11a**), but only threefold in the case of the unsubstituted phenylpiperazines **14** and **8a**.

Moderate but significant affinities (K_i = 45–213 nM) of compounds **8a**–**13a** were observed for the 5-HT_{2A} receptor and their changes with the respect to the analogues with a shorter spacer [8] were not uniform (Fig. 2). Surprisingly, the elongation of the spacer in the case of the most potent 5-HT_{2A} receptor ligands **16** and **18** (K_i = 17 and 14 nM, respectively) [8] caused a significant decrease in the affinity of the resultant tetramethylene derivatives **10a** (K_i = 114 nM) and **12a** (K_i = 57 nM).

For the selected mixed 5-HT_{1A}/5-HT_{2A} receptor ligands (**8a**, **13a**), affinity for D_1 or D_2 receptors was determined; both compounds practically did not bind to those receptor sites. Summing up, we obtained either compounds with dual 5-HT_{1A}/5-HT_{2A} receptor affinity (**8a**, **11a**–**13a**) or selective 5-HT_{1A} receptor ligands (**9a** and **10a**).

In the successive phase of our investigation we studied newly synthesized receptor ligands in vivo models used for evaluation of 5-HT_{1A} and 5-HT_{2A} functional activity.

To determine postsynaptic 5-HT_{1A} agonistic effects of the tested compounds, their ability to induce a LLR in rats and a behavioral syndrome, i.e. FBP and FT in reserpinized rats, were tested. The LLR and behavioral syndrome in rats induced by 8-OH-DPAT, a well-known 5-HT_{1A} receptor agonist, are dependent on the stimulation of postsynaptic 5-HT_{1A} receptors [16,17]. Moreover, evidence was presented that those symptoms are sensitive to 5-HT_{1A} receptor antagonists e.g. WAY 100635 [18], MP 3022 [19] and WAY 100135 [20]. Hence, the ability of the investigated compounds to inhibit the symptoms produced by 8-OH-DPAT was regarded as a postsynaptic 5-HT_{1A} antagonistic activity. On the other hand, the 8-OH-DPAT-induced hypothermia in mice was shown to be related to the activation of presynaptic 5-HT_{1A} receptors [21], and was abolished by 5-HT_{1A} antagonists [18–20,22]. Thus the decrease in body temperature in mice, induced by the investigated derivatives and reduced by WAY 100635 (a silent 5-HT_{1A} antagonist), was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity. In order to determine the antagonistic activity of the investigated compounds at 5-HT_{2A} receptors, their ability to inhibit the head twitches induced by (±)-DOI, a 5-HT_{2A} receptor agonist, was studied in mice [23].

The results obtained in vivo studies indicate that among the tested 4-(4-arylpiperazinyl)-3-butyl-β-tetralonohydantoines, compound **8a** (with an unsubstituted phenylpiperazine fragment) shows features of a full (pre- and postsynaptic) 5-HT_{1A} receptor agonist. In fact, like 8-OH-DPAT, compound **8a** evoked hypothermia in mice (sensitive to WAY 100635) and LLR in rats. At the same time, it did not change the effects induced by 8-OH-DPAT. The 3-Cl (**10a**) and 3-CF_3 (**13a**) analogs of **8a** exhibited some presynaptic 5-HT_{1A} agonistic activity in the hypothermia model in mice, whereas no such activity was observed in that test for compounds with the 2-OCH_3 (**9a**), 2-F (**11a**) or 4-F (**12a**) substituents, since the hypothermia induced by **9a**, **11a** or **12a** was not changed by WAY 100635. On the other hand, introduction of the substituents to the phenylpiperazine part of **8a** yielded derivatives with postsynaptic 5-HT_{1A} receptor antagonistic activity. Compound **9a**–**13a** reduced the LLR, FBP or FT induced by 8-OH-DPAT in rats; moreover, and when they were given alone, they did not mimic the action of that 5-HT_{1A} agonist in the models used. The observed postsynaptic 5-HT_{1A} receptor antagonistic activity of **9a**–**13a** was weaker than that of WAY 100635; however, in the case of **13a**, it was higher (the LLR test) or comparable (the behavioral syndrome test) to that of WAY 100135 [20] or MP 3022 [19]. With the respect to the 5-HT_{2A} functional activity, compounds **8a**, **10a**, **13a** may be classified as antagonists of these receptors, since-like the reference drug ketanserin-they inhibited in a dose-dependent manner the (±)-DOI-induced head twitches in mice, the latter effect being connected with activation of 5-HT_{2A} receptors [23].

In our earlier study [8] we showed that among the trimethylene spacer analogs of the tested compounds only the 3-CF_3 derivative (an analog of **13a**) showed high 5-HT_{1A}/5-HT_{2A} receptor affinity and a functional profile of a 5-HT_{1A} receptor partial agonist; additionally-like unsubstituted (an analog of **8a**), 3-Cl (an analog of **10a**) or 4-F (an analog of **12a**) derivatives-that compound was a 5-HT_{2A} receptor antagonist.

On the basis of the results of in vivo studies presented in this paper, it may be concluded that elongation of the trimethylene spacer to a tetramethylene one, which connects the arylpiperazine portion to the β-tetralonohydantoin moiety, is beneficial for 5-HT_{1A} receptor activity, since such modification yields **8a**–**13a**, potent and active in vivo 5-HT_{1A} receptor ligands with different 5-HT_{1A} receptor intrinsic activity. Moreover, it seems that the presence of the substituent in the arylpiperazine part of the tested compounds causes a total decrease in their postsynaptic 5-HT_{1A} receptor intrinsic activity in vivo, since substituted **9a**–**13a** are antagonists and the unsubstituted **8a** is an agonist of 5-HT_{1A} receptors. The elongation of the spacer and the presence of a substituent in the phenyl ring, as well as the mode of substitution seem to be insignificant for 5-HT_{2A} receptor activity, since **8a**, **10a**–**13a**-like the majority of the previously described trimethylene analogs [8]-showed features of 5-HT_{2A} receptor antagonists.

A number of preclinical studies suggest a role of 5-HT receptors in particular 5-HT_{1A} and 5-HT_{2A}, in many psychiatric disorders. The development of non-benzodiazepine anxiolytics/antidepressants, e.g. buspirone, gepirone and related compounds which act as 5-HT_{1A} receptor partial agonists, provides further substantial support to an interrelationship between 5-HT and anxiety or/and depression. A pharmacological study with these drugs has shown that their anxiolytic/antidepressant-like action is correlated to the activation of 5-HT_{1A} receptors; however, the precise mechanism of action presynaptic versus postsynaptic is still controversial [24,25]. It has also been suggested that 5-HT_{1A} receptor antagonists may have a beneficial effect on anxiety and

depression; a number of new substances with 5-HT_{1A} antagonistic activity are described as potential anxiolytic/antidepressant drugs [26–30]; but the involvement of 5-HT_{1A} receptors in their anxiolytic/antidepressant-like activity still needs to be corroborated.

The present paper describes also the results of studies with compounds **8a** and **13a** showing the most interesting 5-HT_{1A}/5-HT_{2A} functional profile, **8a** is a potent full 5-HT_{1A} receptor agonist/5-HT_{2A} receptor antagonist and **13a** is the most active postsynaptic 5-HT_{1A} antagonist with weak presynaptic 5-HT_{1A} agonistic activity and 5-HT_{2A} receptor antagonist, in animal models used for detecting potential anxiolytic and antidepressant activity. The obtained results indicate that compound **8a**, but not **13a**, exhibits characteristics of an anxiolytic and antidepressant drug. In fact, it showed anxiolytic-like activity in the plus-maze test in rats, as it increased the percentage of entries into the open arms and the time spent in them. The anxiolytic-like activity of **8a** at a dose of 5 mg/kg was weaker than the effect observed after administration of diazepam in the same dose. A visibly stronger anxiolytic-like effect was observed after administration of **8a** at a dose of 10 mg/kg; however, the obtained results may be untrustworthy since the rats were sedated and the total number of entries was reduced. Compound **8a**, but not **13a**, also behaved like a weak antidepressant, as it slightly reduced the immobility time in rats in the forced swimming test. Its effect could be seen after administration of two medium doses (2.5–5 mg/kg) only, whereas imipramine (used as a reference antidepressant) potently inhibited the immobility of rats. However further studies with **8a** as a potential anxiolytic/antidepressant agent seem unnecessary, since this compound at doses active in the plus-maze test showed a very potent sedative effect in rats, while diazepam only partly reduced the rats' exploratory activity and imipramine did not change it.

The obtained results suggest that the structure of phenylpiperazine linked with a tetramethylene spacer to β-tetralonohydantoin remain a worthy of future research for obtaining new derivatives with potential anxiolytic/antidepressant activity.

5. Experimental protocols

5.1. Chemistry

Melting points are uncorrected. All the compounds used (bases **8–13** and hydrochlorides **8a–13a**) were subjected to a quantitative elemental (C, H, N) analysis by a micro method using the elemental Vario EI III Elementar analyzer (data not shown). For a thin-layer chromatography (TLC), Kieselgel 60 F_{254} plates (Merck) and the solvents: acetone/isopropanol/chloroform (20:10:1) and methanol/25% NH_3 (100:1,5) were used.

^{1}H-NMR Spectra (in $CDCl_3$) were recorded on a Varian Gemini 200 (200 MHz); chemical shifts are expressed in parts per million (δ) downfield of tetramethylsilane (TMS) used as an internal standard.

Materials: appropriate substituted 1-phenylpiperazines, 1-bromo-4-chlorobutane, β-tetralone (**5**) and other chemicals were commercially available from Aldrich or Fluka.

5.1.1. β-Tetralonohydantoin (*6*) [15]

5.1.1.1. 3-(4-Chlorobutyl)-β-tetralonohydantoin (7). A suspension of β-tetralonehydantoin (**6**) (10.08 g, 0.05 mol) and anhydrous K_2CO_3 (20 g, 0.15 mol) in acetone (150 ml) was refluxed under intensive stirring for 20 min. Afterwards, a solution of 1-bromo-4-chlorobutane (9.43 g, 0.055 mol) in acetone (50 ml) was added in one portion, and the reaction was carried on for the following 8 h. The hot reaction mixture was filtered off, the solvent was evaporated and the oily residue was purified by crystallization from a 96° ethanol.

M.p. 130–132 °C, yield 80%, ^{1}H-NMR ($CDCl_3$) δ: 1.56–1.95 (m, 5H, $CH_2CH_2CH_2CH_2$, m, 1H, C10-*H*), 2.12–2.33 (m, 1H, C10-*H*), 2.72 (d, J = 16.0 Hz, 1H, C6-H), 2.75–3.11 (m, 2H, cyclohexan-CH_2), 3.35 (d, J = 16.5 Hz, 1H, C6-*H*), 3.55 (m, 4H, N3CH_2, CH_2Cl), 5.88 (brs, 1H, N1*H*), 7.04–7.21 (m, 4H, arom.).

5.1.1.2.3-[4-(4-Arylpiperazin-1-yl)butyl]-β-tetralonehydantoins 8–13. **General procedure.** A mixture of 3-(4-chlorobutyl)-β-tetralonehydantoin **7** (1.53 g, 0.005 mol), the appropriate 1-phenylpiperazine derivative (0.001 mol) and anhydrous ethanol (50 ml) was refluxed for 17 h (compounds **8**, **9**) or for 20 h (compounds **10–13**). Then the solvent was evaporated and the residue was treated with water (50 ml); the precipitate was filtered off, washed with water and the crude product was purified by recrystallization. Melting points and the percentage of yield are summarized in Table 1.

Preparation of hydrochlorides (**8a–13a**). The bases (**8–13**) were dissolved in an excess (2 ml per 0.001 mol) of conc. HCl upon heating. After cooling, anhydrous ethanol 10 ml was added until salt precipitation was observed. The product was recrystallized from anhydrous ethanol. ^{1}H-NMR data of compounds **8–13** are summarized in Table 2.

5.2. In vitro experiments

The affinity of serotonin 5-HT_{1A}, 5-HT_{2A} and dopaminergic D_1 and D_2 receptors for the newly synthesized compounds was determined by standard competitive displacement assays [31,32]. The following tissue sources and radioligands were used: (a) serotonin 5-HT_{1A} receptors of rat brain hippocampus membranes, [^{3}H]-8-OH-DPAT (215 Ci/mmol, Amersham); (b) serotonin 5-HT_{2A} receptors of rat brain cortex membranes, [^{3}H]-ketanserin (63 Ci/mmol, NEN Chemicals); (c) dopamine D_1 receptors of rat striatal membranes, [^{3}H]-SCH-23390 (75 Ci/mmol, NEN Chemicals); (d) dopamine D_2 receptors of rat striatal membranes, [^{3}H]-spiperone (15.7 Ci/mmol, NEN Chemicals).

K_i values were determined on the basis of at least three competition binding experiments in which the tested compounds were used at concentrations of 10^{-10}–10^{-3} M, run in triplicate.

5.3. In vivo experiments

All the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

The experiments were carried out on male Wistar rats (260–300 g) and male Albino–Swiss mice (25–30 g). The animals were kept at an ambient temperature of 20 ± 1 °C and had free access to food (standard laboratory pellets, LSM) and tap water. All experiments were conducted in the light phase on a natural light-dark cycle (from July to December), between 09:00 and 14:00 h. 8-Hydroxy-2-(di-n-propylamino)-tetralin hydrobromide (8-OH-DPAT, Research Biochemical, Inc.), imipramine hydrochloride (Polfa, Poznań), reserpine (Ciba; ampules), N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635; synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride ((±)-DOI; Research Biochemicals, Inc.) were dissolved in saline. Diazepam (Polfa, Poznań) and the investigated compounds were used in a form of freshly prepared suspensions in a 1% Tween 80. 8-OH-DPAT, reserpine and WAY 100635 were injected subcutaneously (s.c.); (±)-DOI, imipramine, diazepam and the tested compounds were given intraperitoneally (i.p.) in a volume of 2 ml/kg (rats) or 10 ml/kg (mice). Each group consisted of six to nine animals. The obtained data were analyzed by the Dunnett's test.

5.3.1. LLR in rats

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

5.3.2. Behavioral syndrome in reserpinized rats

The rats were individually placed in cages (30 × 25 × 25 cm) 5 min before injection of the tested compounds or 8-OH-DPAT. Observation sessions, lasting 45 s each, began 3 min after drug administration and were repeated every 3 min. FBP and reciprocal FT were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The maximum score, summed up over 5 observation periods, amounted to 15 for each symptom per animal [17]. The effect of the tested compounds on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg) was assessed in a separate experiment. The investigated compounds were administered 60 min before 8-OH-DPAT, and the animals were scored at 3, 6, 9, 12 and 15 min after 8-OH-DPAT treatment. Reserpine (1 mg/kg) was administered 18 h before the test.

5.3.3. Body temperature in mice

The effects of the tested compounds given alone on the rectal body temperature of mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. The effect of WAY 100635 on hypothermia induced by the investigated compounds was tested in a separate experiment. WAY 100635 (0.1 mg/kg) was administered 15 min before the tested compounds and the rectal body temperature was recorded 30 and 60 min after the injection of the investigated compound.

The results are expressed as a change in body temperature (Δt) with respect to basal body temperature, as measured at the beginning of the experiments.

5.3.4. Head twitch response in mice

In order 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 20 min before the treatment head twitches of mice were induced by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the number of head twitches was counted during 20 min [23]. The investigated compounds were administered 60 min before (±)-DOI.

5.3.5. Elevated plus-maze test in rats

The construction and the testing procedure of an elevated plus-maze were based on a method described by Pellow and File [33]. Each rat was placed in the center of the plus-maze, facing one of the enclosed arms immediately after a 5-min adaptation period in a wooden box (60 × 60 × 35 cm). During a 5-min test period, two experimenters, who were sitting in the same room approximately 1 m from the end of one the open arms, recorded the number of entries into the closed or open arms, as well as the time spent in either type of the arms. The entry with all the four feet put into one arm was defined as an arm entry. At the end of each trial, the maze was wiped clean. The investigated compounds **8a**, **13a** and diazepam were administered 60 min before the test.

5.3.6. Forced swimming test in rats

The experiment was carried out using the method of Porsolt et al. [34]. Briefly, the animals were placed individually in plexiglass cylinders (40 cm high; 18 cm in diameter), containing 15 cm of water maintained at 25 °C. After 15 min, the rats were removed to a drying room (30 °C) for 30 min. They were replaced in the cylinder 24 h later, and the total duration of immobility was measured for 5-min test period. The rats were used only once in that test. The time during which the animals were immobile was measured by two experimenters unaware of the treatment that the rats had received. The investigated compounds **8a**, **13a** and imipramine were administered 60 min before the test.

5.3.7. Open field test in rats

The studies were carried out on rats according to the slightly modified method of Janssen et al. [35]. The center of an open arena (1 m diameter), divided into six symmetrical sectors without walls, was illuminated with a 75 W electric bulb hanging directly 75 cm above it. During all the experiments the laboratory room was dark. Individual control or compound-injected animals were placed gently in the center of the arena and were allowed to explore freely. The time of walking, ambulation (the number of sector line crossings) and the number of rearing and peeping (looking under the edge of the arena) episodes were recorded for 3 min. Compound **8a,** diazepam and imipramine were administered 30 min before the test.

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