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Synthesis and Pharmacological Evaluation of New Arylpiperazines. 3-{4-[4-(3-chlorophenyl)-1-piperazinyl]butyl}-quinazolidin-4-one — A Dual Serotonin 5-HT_{1A}/5-HT_{2A} Receptor Ligand with an Anxiolytic-Like Activity

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Abstract—On the basis of systematic studies on the structure–activity relationships in arylpiperazine group of serotonin ligands, 12 new derivatives containing quinazolidin-4(3H)-one (**1**–**4**), 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**5**–**8**) or 1-phenyl-1,2-dihydropyridazine-3,6-dione (**9**–**12**) fragments were synthesized. The majority of the tested compounds (**2**, **4**, **7**, **8** and **10**–**12**) showed a high affinity for 5-HT_{1A} receptors (K_i = 11–54 nM) and two (**1**, **2**) were found active at 5-HT_{2A} sites (16 and 68 nM, respectively). All the new 5-HT_{1A} ligands tested in vivo revealed an antagonistic activity at postsynaptic 5-HT_{1A} receptors, and three of them behaved as agonists at presynaptic ones. Additionally, both the *meta*-chlorophenylpiperazine derivatives containing quinazolidin-4-one fragment showed features of 5-HT_{2A} receptor antagonists. The dual 5-HT_{1A}/5-HT_{2A} receptor ligand (**2**) was further tested for its potential psychotropic activity. It showed a distinct anxiolytic-like activity in a conflict drinking test in rats and the observed effect was more potent in terms of the active dose, than that produced by diazepam (used as a reference drug).

Introduction

Arylpiperazine is a core fragment of many bioactive compounds which exhibit a variety of pharmacological effects. It has been shown that their action can be mediated by different subpopulations of serotonin (5-hydroxytryptamine, 5-HT), dopamine and adrenergic receptors. Numerous studies have indicated that even minor modifications in the chemical structure of arylpiperazine derivatives strongly affect the affinity and selectivity for the above-mentioned targets (for a review see Glennon et al.[1]). Therefore designing compounds with a desired receptor profile is extremely difficult. Nevertheless, a potential multireceptor activity of arylpiperazines implicates their frequent use as a source of new agents with different therapeutic properties.

The most thoroughly studied group of arylpiperazine derivatives, called long chain arylpiperazines (LCAPs), can be found as serotonin receptor ligands, especially 5-HT_{1A} and 5-HT_{2A} ones. Their general chemical structure consists of an alkyl chain (2–4 methylene units) attached to the N4 atom of the piperazine moiety, and a terminal amide or an imide fragment. The significance of the respective parts of LCAPs for 5-HT_{1A} affinity, intrinsic activity and selectivity has been the subject of many structure–activity relationship studies.[2] Although the influence of the aryl group (typically a

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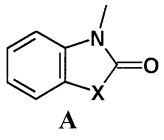
substituted phenyl or heteroaromatic moiety), as well as the length of the alkyl chain are rather well established, the function of the terminal fragment is still unclear. A great number of such fragments tested (even those without the amide group) suggest that different forces are engaged in stabilizing the ligand–receptor complex in this region. If we assume that an arylpiperazine fragment serves as an anchoring point, the highly flexible alkyl chain allows a terminal fragment of LCAPs to interact with different sites of a binding pocket.[2]

During our systematic structure–affinity and structure–intrinsic activity studies within the LCAP group of 5-HT_{1A} ligands different termini were used.[3–6] The main structural features explored included changes in the relative position of the amide group with regard to the aromatic ring, varied ring sizes (five- or six-membered) and introduction of an additional carbonyl group and/or the oxygen atom (Scheme 1). Although the studied compounds exhibited diversified 5-HT_{1A} affinities and pharmacological profiles one generalization could be observed. Ligands with a nitrogen atom attached directly to the benzene ring (type A)—even those showing a high 5-HT_{1A} affinity—were not active in vivo.[4] On the contrary, compounds of type B were usually highly potent in vivo 5-HT_{1A} receptor ligands.[6]

In order to further extend diversity of terminal amides B we present the synthesis, 5-HT_{1A} and 5-HT_{2A} receptor in vitro and in vivo studies of a new model arylpiperazines connected by three or four methylene group spacer with quinazolidin-4-one (**1**–**4**), 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**5**–**8**) or 1-phenyl-1,2-dihydropyridazine-3,6-dione (**8**–**12**) moieties. On the basis of the obtained results, the most promising compound **2** was then tested in several animal models of anxiety and depression.

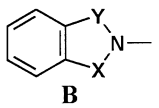
Chemistry

Final compounds **1**–**12** were obtained in a similar way (Scheme 2) as their recently described phenylpiperazine and pyrimidylpiperazine analogues.[7] In short, the starting quinazolidin-4(*3*H)-one, 2-phenyl-2,3-dihydrophtalazine-1,4-dione and 1-phenyl-1,2-dihydropyridazine-3,6-dione were prepared from antranilic acid,[8] ftalic anhydride[9] and maleic anhydride,[10,11] respectively, according to the published procedures.



A

X = CH_2–CH_2, CH=CH, CH_2–O, O–CH_2, CH_2, O



B

X	Y
CH_2–CH_2	C=O
CH_2–C=O	CH_2
CH=CH	C=O
O–C=O	C=O

Scheme 1.

Subsequent alkylation with 1-bromo-3-chloropropane or 1,4-dibromobutane in the presence of K_2CO_3 in acetonitrile led to the formation of halogen intermediates and the symmetrically disubstituted by-products.[7] Target compounds were obtained upon condensation of halogen derivatives with the respective 1-arylpiperazine. The structure of free bases **1**–**12** was confirmed by ^1H NMR spectra, and by an elemental analysis after conversion to hydrochloride salts. Physicochemical data of **1**–**12** are collected in Table 1.

Pharmacology

All the compounds were evaluated for their affinity at 5-HT_{1A} and 5-HT_{2A} receptors; additionally, the affinity of derivative **2** for dopamine D_2 sites was also determined. The most in vitro potent ligands were tested in vivo to assign their agonistic/antagonistic properties towards 5-HT_{1A} and/or 5-HT_{2A} receptors.

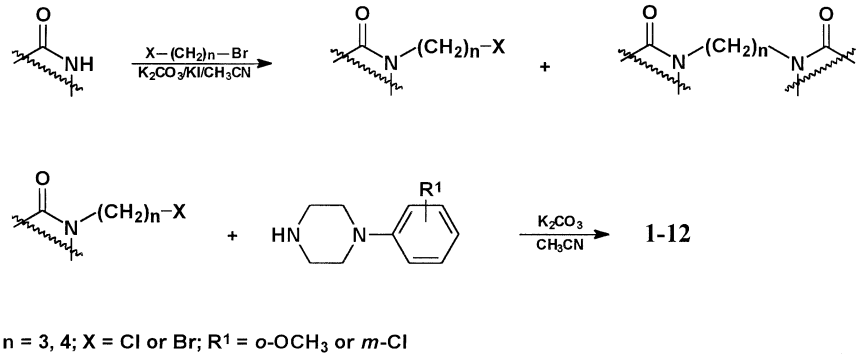
Several models were proposed for measuring the responses evoked by activation of pre- and postsynaptic 5-HT_{1A} receptors in vivo.[12,13] Administration of 8-hydroxy-2-(di-*n*-propyloamino)tetraline (8-OH-DPAT)—a full 5-HT_{1A} agonist—evoked a hypothermic effect in mice (a model representative of activity at presynaptic 5-HT_{1A} receptors)[14,15] and lower lip retraction (LLR) in rats,[16] as well as a behavioral syndrome that is a flat body posture (FBP) and forepaw treading (FT) in reserpinized rats (models reflecting 5-HT_{1A} postsynaptic activity).[17] The antagonistic 5-HT_{1A} activity of the tested compounds was assessed on the basis of the blockade of the above-mentioned 8-OH-DPAT-induced in vivo effects. Their ability to mimic the 8-OH-DPAT-induced action was regarded as a 5-HT_{1A} agonistic activity. Since hypothermia is a nonspecific in vivo effect, we checked if it was abolished by the highly selective 5-HT_{1A} antagonist WAY 100635.[18]

The ability of the tested compounds to antagonize head twitches in mice, observed after administration of (±)-DOI [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane]—a 5-HT_{2A} receptor agonist, was used to evaluate 5-HT_{2A} receptor antagonistic properties.[19]

The potential anxiolytic activity of compound **2** was measured in two classic models in rats: the conflict drinking (Vogel)[20] and the elevated plus-maze[21] tests. The forced swimming test in mice was a behavioral paradigm selected to determine potential antidepressant properties.[22] In order to measure the effect of compound **2** administration on the exploratory activity in rats, the open field test was also performed.[23]

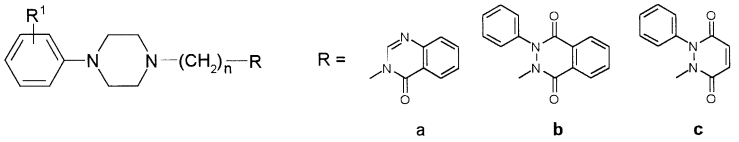
Results and Discussion

The affinity of the tested compounds for 5-HT_{1A} receptors varied from 11 nM (**12**) to 415 nM (**5**), whereas for 5-HT_{2A} receptors it ranged from 16 nM to 2100 for **1** and **11**, respectively (Table 1). Generally, *ortho*-methoxyphenylpiperazine derivatives were always more



Scheme 2. Synthesis of the investigated compounds **1**–**12**.

Table 1. Structure, physical data and 5-HT_{1A} and 5-HT_{2A} binding affinities of the compounds **1**–**12**



Compound	R^1	*n*	R	Yield (%)[a]	Mp (°C)	Molecular formula[b]	$K_i \pm$SEM (nM)	
							5-HT_{1A}	5-HT_{2A}
1	*m*-Cl	3	a	57	209–211	$C_{21}H_{23}N_4OCl \cdot HCl \cdot 0.5H_2O$	235±13	16±3
2	*m*-Cl	4	a	67	215–218	$C_{22}H_{25}N_4OCl \cdot HCl$	50±9	68±10
3	*o*-OCH_3	3	a	53	199–202	$C_{22}H_{26}N_4O_2 \cdot HCl \cdot H_2O$	100±3	460±4
4	*o*-OCH_3	4	a	59	184–187	$C_{23}H_{28}N_4O_2 \cdot HCl$	36±1	566±6
5	*m*-Cl	3	b	59	223–226	$C_{27}H_{27}N_4O_2Cl \cdot HCl \cdot 0.5H_2O$	415±13	660±17
6	*m*-Cl	4	b	61	209–212	$C_{28}H_{29}N_4O_2Cl \cdot HCl$	400±20	580±29
7	*o*-OCH_3	3	b	74	222–225	$C_{28}H_{30}N_4O_3 \cdot HCl \cdot H_2O$	30±1	300±20
8	*o*-OCH_3	4	b	66	188–191	$C_{29}H_{32}N_4O_3 \cdot 2 \cdot HCl$	43±9	375±20
9	*m*-Cl	3	c	79	207–210	$C_{23}H_{25}N_4O_2Cl \cdot HCl \cdot 0.5H_2O$	206±9	270±16
10	*m*-Cl	4	c	68	179–182	$C_{24}H_{27}N_4O_2Cl \cdot HCl \cdot H_2O$	50±8	1830±26
11	*o*-OCH_3	3	c	74	192–195	$C_{24}H_{28}N_4O_3 \cdot HCl \cdot H_2O$	54±8	2120±28
12	*o*-OCH_3	4	c	64	184–187	$C_{25}H_{30}N_4O_3 \cdot HCl \cdot 0.5H_2O$	11±2	1450±24

[a]Yield of a free base.
[b]Anal. C, H, N.

active at 5-HT_{1A} receptors than the respective *meta*-chloro analogues. Moreover, compounds with a 4-membered alkyl chain spacer (even numbers) were more potent 5-HT_{1A} ligands than were those containing 3 methylene groups. Thus the in vitro results were in line with the general trends concerning affinities within the arylpiperazine group of ligands. However, a closer examination of the obtained 5-HT_{1A} binding data reveals that the influence of alkyl chain length, as well as the arylpiperazine used depend on the terminal fragment. In the case of series **a** and **c**, elongation of the spacer caused app. 4-fold enhancement of 5-HT_{1A} affinity, but in series **b** it was without effect. On the other hand, an increase in 5-HT_{1A} affinity, connected with the replacement of *m*-Cl by *o*-OCH_3 in the phenyl ring, was the most significant for series **b** (10-fold) and less important for series **c** and **a** (4- and 2-fold, respectively).

Regarding 5-HT_{2A} receptors, only two relatively potent compounds were found (**1** and **2**, $K_i = 16$ and 68 nM, respectively), either containing a *meta*-chlorophenylpiperazine and a quinazolidin-4-one (R = **a**) fragments. Other compounds displayed a low affinity for 5-HT_{2A} receptors. Derivatives **10**–**12** were the least potent 5-HT_{2A} ligands ($K_i = 1450$–2130 nM), but at the same time showing a high affinity for 5-HT_{1A} receptors, hence the highest 5-$HT_{2A/1A}$ selectivity ($S_{2A/1A} > 35$).

The most in vitro active compounds ($K_i < 70$ nM) were further tested in several in vivo models to determine their functional profile at 5-HT_{1A} (**2**, **4**, **7**, **8**, **10**–**12**) and 5-HT_{2A} (**1**, **2**) receptors. All of the 5-HT_{1A} ligands tested given alone produced (like 8-OH-DPAT) a dose-dependent decrease in body temperature in mice; the maximum hypothermic effect was observed 30 min after injection (Table 2). The hypothermia evoked by **2** (1.25 mg/kg), **7** (5mg/kg), **10** (10 mg/kg), or the reference 8-OH-DPAT (5 mg/kg) was abolished by WAY100635 (0.1 mg/kg), hence those compounds were classified as agonists of presynaptic 5-HT_{1A} receptors. Since WAY100635 did not affect the decrease in body temperature in mice induced by **4**, **8** (10 mg/kg), **11** (5 mg/kg) and **12** (2.5 mg/kg), it seems that 5-HT_{1A} receptors are not involved in these hypothermias (Table 3).

All the investigated compounds given alone in doses up to 10 or 20 mg/kg, evoked neither LLR in rats nor flat body posture (FBP) or forepaw treading (FT) in reser-

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

Treatment	Dose (mg/kg)	$\Delta t \pm$SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	—	0.0±0.1	0.1±0.1	0.0±0.1	0.1±0.1
2	1.25	−1.3±0.3[b]	−0.5±0.2	−0.1±0.1	0.1±0.1
	2.5	−2.3±0.1[b]	−1.4±0.1[b]	−1.0±0.1[b]	−0.5±0.1
Vehicle	—	0.0±0.1	0.1±0.1	0.0±0.1	0.1±0.1
4	5	−0.9±0.2[a]	0.0±0.2	0.3±0.2	0.6±0.1[a]
	10	−2.0±0.2[b]	−1.4±0.2[b]	−0.7±0.2	−0.1±0.1
Vehicle	—	−0.2±0.1	−0.1±0.1	−0.1±0.1	0.1±0.1
7	5	−0.9±0.1[b]	−0.6±0.1[b]	−0.7±0.1[b]	−0.4±0.1[b]
	10	−1.3±0.3[b]	−1.1±0.3[b]	−0.8±0.2[b]	−0.2±0.2
Vehicle	—	−0.2±0.1	−0.1±0.1	−0.1±0.1	0.1±0.1
8	5	−0.6±0.3	−0.8±0.1[b]	−0.2±0.1	−0.1±0.1
	10	−1.5[b]±0.2	−1.3[b]±0.2	−0.9[b]±0.2	−0.4±0.1
Vehicle	—	−0.1±0.2	−0.2±0.1	0.01±0.1	−0.1±0.1
10	5	−1.1±0.2[b]	−0.7±0.2[a]	−0.6±0.2[a]	−0.5±0.2
	10	−1.5±0.2[b]	−0.9±0.2	−0.9±0.2[b]	−0.7±0.2
Vehicle	—	−0.1±0.2	−0.2±0.1	0.01±0.1	−0.1±0.1
11	5	−1.5±0.2[b]	−0.7±0.2[a]	−0.4±0.2	−0.1±0.2
	10	−2.5±0.3[b]	−1.7±0.3[b]	−0.9±0.2[b]	−0.4±0.2
Vehicle	—	−0.1±0.1	−0.1±0.1	−0.1±0.2	−0.1±0.1
12	2.5	−1.8±0.2[b]	−1.4±0.1[b]	−0.8±0.1[b]	−0.6±0.1
	5	−2.3±0.2[b]	−2.4±0.2[b]	−1.5±0.2[b]	−1.1±0.2[b]
WAY 100635	0.1	0.2±0.1	0.2±0.1	0.1±0.1	0.2±0.1

The investigated compounds (ip) and WAY 100635 (sc) were administered 30 min before the test. The absolute mean body temperatures were within a range of 36.8±0.3 °C.
[a]$p < 0.05$ versus vehicle.
[b]$p < 0.01$ versus vehicle.

pinized rats (data not shown); therefore it seems that they have no agonistic activity at postsynaptic 5-HT_{1A} receptors.

The LLR induced by 8-OH-DPAT (1 mg/kg, the maximum possible score being 93%) was dose-dependently antagonized by compounds **2**, **4** (1.25–10 mg/kg), **7**, **10** and **11** (10–20 mg/kg) and **12** (5–10 mg/kg), whereas the effect of **8** (10–20 mg/kg) was weak irrespective of the dose used (Table 4). Both the symptoms of the 8-OH-DPAT-induced behavioral syndrome in reserpinized rats (5 mg/kg, the maximum behavioral scores for FBP and FT being 12.0–14.3 and 12.7–14.4, respectively) were attenuated by **4** (5–20 mg/kg), **11** (10–20 mg/kg) and **12** (5–10 mg/kg). Compounds **8** (10–20 mg/kg), **2** (10 mg/kg) and **7** (20 mg/kg) reduced the FT, but failed to inhibit the FBP, whereas **10** (10–20 mg/kg) did not affect any component of the 5-HT syndrome (Table 4). It is noteworthy that only derivative **4** in the higher dose used (20 mg/kg) produced an almost complete blockade of the FBP and FT.

In those models, all the tested ligands—like the reference compound WAY 100635—showed features characteristic of postsynaptic 5-HT_{1A} receptor antagonists. Moreover, it should be stressed that only quinazolinone derivatives **2** and **4** with a tetramethylene spacer demonstrated a high potency, since they inhibited the effects of 8-OH-DPAT already in a dose of 1.25 mg/kg, however still higher than in the case of WAY 100635.

Compounds **1** and **2** behaved like 5-HT_{2A} receptor antagonists, since either of them—like the reference compound ketanserin- dose-dependently antagonized head twitches in mice, observed after (±)-DOI (2.5 mg/kg) administration. ED_{50} values were 6.2 (4.4–8.7) mg/kg, 9.2 (6.1–13.8) mg/kg and 0.14 (0.07–0.20) mg/kg for **1**, **2** and ketanserin, respectively.

On the basis of the results of a functional study, and considering the premises described below, compound **2** —an agonist of presynaptic and an antagonist of postsynaptic 5-HT_{1A} receptors with a 5-HT_{2A} receptor antagonistic activity—was selected for further in vivo preclinical studies as a potential psychotropic agent.

It is well established that 5-HT_{1A} ligands possess anxiolytic and antidepressive properties,[24,25] but their underlying mechanism of action is still unclear.[26] To date, there are a lot of evidences indicating that partial agonists (such as the well-known drug buspirone), as well as full agonists (flesinoxan) show intense activity in a number of models of anxiety and depression.[27–29] Moreover, it has been demonstrated that 5-HT_{1A} antagonists (WAY 100635, WAY100135 and MM77) produce anxiolytic-like effects in some animal studies,[30,31] however a number of data to the contrary have also been reported.[32,33]

Furthermore, not only 5-HT_{1A}, but also 5-HT_{2A} receptors have been proposed to be involved in the regulation of anxiety and depression.[34] Studies with nonselective

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

Treatment dose (mg/kg)	$\Delta t \pm$SEM (°C)	
	30 min	60 min
Vehicle + vehicle	0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **2** (1.25)	−1.3 ± 0.3[b]	−0.5 ± 0.2
WAY 100635 (0.1) + **2** (1.25)	−0.5 ± 0.2[c]	−0.2 ± 0.1
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **4** (10)	−2.1 ± 0.2[b]	−1.5 ± 0.2[b]
WAY 100635 (0.1) + **4** (10)	−3.2 ± 0.2[b,c]	−2.5 ± 0.3[b]
Vehicle + vehicle	−0.2 ± 0.1	0.2 ± 0.1
Vehicle + **7** (5)	−0.9 ± 0.2[a]	−0.7 ± 0.1[a]
WAY 100635 (0.1) + **7** (10)	−0.2 ± 0.1[c]	−0.1 ± 0.1[c]
Vehicle + vehicle	0.0 ± 0.1	−0.1 ± 0.1
Vehicle + **8** (10)	−1.7 ± 0.2[b]	−1.0 ± 0.1[b]
WAY 100635 (0.1) + **8** (10)	−1.4 ± 0.1[b]	−0.5 ± 0.1
Vehicle + vehicle	−0.2 ± 0.1	−0.2 ± 0.1
Vehicle + **10** (10)	−1.7 ± 0.2[b]	−0.8 ± 0.2[b]
WAY 100635 (0.1) + **10** (10)	−0.3 ± 0.1[c]	−0.1 ± 0.1[c]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **11** (5)	−2.0 ± 0.1[b]	−0.8 ± 0.2[b]
WAY 100635 (0.1) + **11** (5)	−1.4 ± 0.2[b]	−0.4 ± 0.1
Vehicle + vehicle	−0.1 ± 0.1	0.1 ± 0.1
Vehicle + **12** (2.5)	−1.8 ± 0.2[b]	−1.4 ± 0.1[b]
WAY 100635 (0.1) + **12** (2.5)	−1.5 ± 0.2[b]	−1.0 ± 0.1[b]
Vehicle	0.1 ± 0.1	0.1 ± 0.1
8-OH-DPAT (5)	−1.0 ± 0.1[b]	−0.7 ± 0.2
WAY100635 (0.1) + 8-OH-DPAT (5)	−0.1 ± 0.1[c]	0.1 ± 0.1[c]

WAY 100635 was administered (sc) 15 min before investigated compounds. The absolute mean body temperatures were within a range of 36.7 ± 0.4 °C.
[a] $p < 0.05$ versus vehicle + vehicle.
[b] $p < 0.01$ versus vehicle + vehicle.
[c] $p < 0.01$ versus respective vehicle + compound.

serotonin drugs (e.g., ritanserin,[35] adantanserin,[36] nefazodon[37]) have shown that their therapeutic effects can also be related with the antagonistic activities at 5-HT_{2A} receptors. Regarding compounds with such a 'mixed activity', much attention has been given to drugs combining 5-HT_{1A} agonistic/antagonistic and 5-$HT_{2A/2C}$ antagonistic properties, as it is believed that they may increase the therapeutic response of psychotropic drugs. Such was the case with adatanserin, flibanserin[38] and a number of other 5-HT_{1A} and 5-HT_{2A} receptor ligands which showed robust activity in preclinical models of anxiety and/or depression and are now awaiting clinical validation.[28] In addition, as follows from a number of preclinical and clinical investigations, ligands combining D_2/5-HT_{2A} antagonism with 5-HT_{1A} receptor activity for example, ziprasidone[39] may have potential antipsychotic properties.

Thus, as a first step, the radioligand binding profile of compound **2** was completed by the determination of its affinity for dopamine D_2 receptors. Since the affinity found ($K_i = 430 \pm 10$ nM) was low, further in vivo examination in that direction was unfounded.

The successive phase of our studies with compound **2** focused on its evaluation as a potential anxiolytic and/or antidepressant agent.

In the conflict drinking test in rats, compound **2** (0.31–2.5 mg/kg) dose-dependently increased the number of punished licks (Table 5), but used in a higher dose (5 mg/kg) it induced sedation and other behavioral disturbances (e.g., abduction, weak tremor), so that dose was not tested. It seems that the observed effect was specifically anxiolytic, since when compound **2** was given in doses evoking an anticonflict activity, it affected neither the shock threshold nor the non-punished water consumption. It should be noted here that the anticonflict effect of **2** was even more potent in terms of active dose than that produced by diazepam, used as a reference drug (Table 5), and comparable to that of the partial 5-HT_{1A} receptor agonists buspirone and MM199,[40] or of mixed 5-HT_{1A}/5-HT_{2A} ligands, for example, adatanserin.[41]

On the other hand, compound **2** (0.31–1.25 mg/kg) was practically inactive in the plus-maze test in rats, in which diazepam (2.5–5 mg/kg) showed a marked anxiolytic-like activity. However there also exist contradictory data about the effects of 5-HT_{1A} partial agonists (e.g., buspirone) in this test, since anxiolytic-like,[42] lack of effect[43] or even anxiogenic effects[44–46] were described.

The results of our experiments also showed that **2** (2.5–10 mg/kg) did not change immobility time in the Porsolt test in mice, while the typical antidepressant imipramine (30 mg/kg, a 70% decrease in immobility time, $P < 0.01$), used as a reference drug, showed distinct activity in that model. However, such a result could be expected, since the 5-HT_{1A} receptor partial agonists buspirone and ipsapirone after systemic administration did not reduce immobility, either; [40,47–49] they exhibited antidepressant-like activity in animals pretreated with an inhibitor of drug metabolism.[47]

Compound **2** administered in a dose of 1.25 mg/kg did not change exploratory locomotor activity in the open field test in rats, but in doses of 2.5 and 5 mg/kg it potently and dose-dependently attenuated general exploratory behavior (time spent walking, ambulation and rearing + peeping; Table 6). Although the latter dose produced almost complete reduction of rats activity, it should be noted that it was 16-fold higher than that evoking a minimal, but statistically significant, anticonflict effect. In comparison, diazepam administered in a dose of 2.5 mg/kg practically did not affect the exploratory activity of rats, but its dose of 5 mg/kg significantly reduced that exploration in that test (Table 6).

Conclusions

Here we reported the preparation, structure–activity studies and discussion of pharmacological results of a series of 12 new model arylpiperazines containing quinazolidin-4-one, 2-phenyl-2,3-dihydrophthalazine-1,4-dione or 1-phenyl-1,2-dihydropyridazine-3,6-dione moieties. The differences in 5-HT_{1A} binding constants were explained on the basis of the known effects of the length of an alkyl linker and the substituents used (*m*-Cl or

Table 4. The effect of the tested compounds on the 8-OH-DPAT-induced lower lip retraction (LLR) in rats (A) and on the 8-OH-DPAT-induced behavioral syndrome in reserpinized rats (B)

Compound	Dose mg/kg	Behavioral score, mean±SEM		
		A: LLR	B: Flat body posture	Forepaw treading
Vehicle	—	2.8±0.1	14.3±0.3	12.7±0.3
2	1.25	1.6±0.2[b]	NT	NT
	2.5	1.3±0.3[b]	NT	NT
	5	1.3±0.2[b]	13.8±0.8	11.8±0.7
	10	0.8±0.2[b]	13.7±0.9	8.7±0.6[b]
Vehicle	—	2.8±0.1	14.3±0.3	12.7±0.3
4	1.25	1.7±0.1[b]	NT	NT
	2.5	1.2±0.1[b]	NT	NT
	5	1.0±0.2[b]	8.2±1.1[b]	8.7±0.7[b]
	10	0.5±0.2[b]	3.5±1.2[b]	8.3±0.7[b]
	20	NT	0.8±0.7[b]	2.0±0.7[b]
Vehicle	—	2.8±0.1	14.3±0.3	13.2±0.7
7	10	2.0±0.2[b]	14.2±0.5	10.8±0.8
	20	1.5±0.4[b]	14.2±0.4	10.3±0.8[a]
Vehicle	—	2.8±0.1	13.8±0.8	13.2±0.7
8	10	1.9±0.2[b]	13.7±0.5	8.5±0.7[b]
	20	2.1±0.2[a]	13.3±0.7	9.3±0.7[b]
Vehicle	—	2.8±0.1	12.0±1.0	13.2±0.7
10	10	1.2±0.2[b]	10.2±0.6	10.3±0.4
	20	0.8±0.3[b]	11.7±0.8	9.5±0.9
Vehicle	—	2.8±0.1	12.8±1.1	14.4±0.2
11	10	1.4±0.2[b]	9.0±0.8[a]	8.7±1.6
	20	1.5±0.3[b]	9.2±1.1[a]	5.0±1.5[b]
Vehicle	—	2.8±0.2	12.0±1.0	13.2±0.7
12	5	2.1±0.1[a]	9.0±1.1	6.7±1.3[b]
	10	0.4±0.1[b]	6.0±0.4[b]	2.0±0.4[b]
WAY 100635	0.1	0.3±0.2[b]	0.7±0.4[b]	1.3±0.8[b]

(A) The tested compounds (ip) and WAY 100635 (sc) were administered 45 min, before 8-OH-DPAT (1 mg/kg, sc); (B) reserpine (1 mg/kg, sc), the tested compounds (ip) and WAY 100635 (sc) were administered 18 h, 60 min and 30 min, respectively, before 8-OH-DPAT (5 mg/kg, *sc*); $n=6$ rats per group. NT — not tested.
[a]$p<0.05$ versus vehicle.
[b]$p<0.01$ versus vehicle.

Table 5. The effect of compound 2 and diazepam in the conflict drinking test (A) and plus-maze test (B) in rats

Treatment	Dose mg/kg	A	B	
		Number of shocks accepted/5 min	% Of time in open arms	% Of open arms entries
Vehicle	—	7.6±0.9	10.1±2.3	41.1±5.6
2	0.08	14.7±3.1	NT	NT
	0.16	18.3±4.7	NT	NT
	0.31	33.6±6.3[b]	16.6±6.8	28.5±8.0
Vehicle	—	9.6±1.8		
	0.625	33.5±6.5[b]	33.5±15.8	37.3±13.8
	1.25	34.4±3.8[b]	25.4±15.6	30.6±15.6
	2.5	39.3±6.4[b]	NT	NT
Vehicle	—	9.3±2.1	10.9±1.1	38.5±3.1
Diazepam	2.5	29.7±3.5[b]	47.2±5.3[a]	73.8±4.2[a]
	5.0	38.3±6.9[b]	70.4±10.9[b]	76.2±8.8[b]

Compound **2** and diazepam were administered 30 and 60 min, respectively before the tests.
[a]$p<0.05$ versus vehicle.
[b]$p<0.01$ versus vehicle.

o-OCH_3) on a phenylpiperazine fragment. All the compounds tested in functional in vivo models (**2**, **4**, **7**, **8**, **10**–**12**) behaved like antagonists of postsynaptic 5-HT_{1A} receptors and moreover **2**, **7** and **10** may be regarded as agonists of presynaptic sites. Since the quinazolidin-4-one derivative **2** also showed features of a 5-HT_{2A} receptor antagonist, it was further examined in in vivo tests as a potential psychotropic agent. This dual 5-HT_{1A}/5-HT_{2A} ligand displayed distinct anxiolytic-like activity in the Vogel test, but was inactive in the plus-maze model, nor did it exert antidepressant-like properties in the forced swimming test in mice. Although

Table 6. The effect of compound **2** and diazepam on the exploratory activity in the open field test in rats

Treatment	Dose mg/kg	Exploratory activity		
		Time of walking (s)	Ambulation	Peeping + rearing
Vehicle	—	37.7±4.7	14.7±3.1	13.3±1.9
2	1.25	39.2±7.2	13.7±3.1	13.2±3.3
	2.5	23.0±7.1	6.0±1.7[a]	4.3±1.9[a]
	5.0	2.3±1.0[b]	0.5±0.2[b]	0.3±0.2[b]
Vehicle	—	37.0±4.0	17.0±2.3	11.2±2.4
diazepam	2.5	27.0±4.1	12.0±1.9	7.8±2.1
	5.0	17.8±4.6[b]	5.0±1.3[b]	4.4±2.0[a]

Compound **2** and diazepam were administered 30 and 60 min, respectively before the test.
[a]$p < 0.05$ versus vehicle.
[b]$p < 0.01$ versus vehicle.

Compound **2** revealed anxiolytic-like properties already at low doses (from 0.31 mg/kg), the sedative effect exerted by its dose of 5 mg/kg, excluded that ligand from being regarded as a potential drug. New derivatives of quinazolidin-4-one are currently being developed.

Experimental

Chemistry

Elemental analyses were performed on a Perkin–Elmer 2400 analyzer located in Regional Laboratory of Jagiellonian University. ^{1}H NMR spectra were recorded in deuterochloroform with a Tesla 487C (80 MHz) spectrometer and tetramethylsilane (TMS) as an internal standard; chemical shifts are reported in ppm (δ); coupling constants were taken from the expanded spectrum. Melting points were determined in a Boetius apparatus and are uncorrected.

For biological experiments, free bases **1**–**12** were converted into hydrochloride salts, and their molecular formulas and molecular weights were established on the basis of an elemental analysis.

General procedure for the preparation of compounds 1–12

The mixture of the corresponding chloro derivative (0.01 mol), arylpiperazine (0.01 mol), powdered K_2CO_3 (4.14 g, 0.03 mol) and catalytic amount of KI in 30 mL of acetonitrile was stirred for 48 h at 50–60 °C (Scheme 2). Then the inorganic precipitate was filtered off, the filtrate was evaporated, and the residue was purified using silica gel chromatography with chloroform/methanol = 9:1 (**3**, **10**, **12**) or crystallized from the appropriate solvent.

General procedure for the preparation of hydrochlorides

Free bases **1**–**12** were converted into hydrochlorides by dissolving the corresponding base in acetone (10 mL/g) and treating with ethanol saturated with HCl. The precipitate was filtered off and washed with acetone. Some of the hydrochlorides were additionally purified by crystallization.

3-{3-[4-(3-Chlorophenyl)-1-piperazinyl]propyl}-quinazolidin-4-one (1). Base **1** was obtained in 57% yield, mp 59–61 °C (acetone–H_2O 5:1); ^{1}H NMR: δ 1.94–2.18 (m, 2H, $CH_2CH_2CH_2$), 2.36–2.63 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.09–3.23 (m, 4H, $(CH_2)_2NAr$), 4.11 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 8.13 (s, 1H, $CH{=}N$), 6.75–8.37 (m, $8H_{Arom}$); MS: *m*/*z* (I%); M 382 (21), 209 (16), 187 (100); hydrochloride mp 209–211 °C (2-propanol). Anal. calcd for $C_{21}H_{23}N_4OCl{\cdot}HCl{\cdot}0.5H_2O$ (428.36): C, 58.88; H, 5.88; N, 13.08. Found: C, 59.01; H, 5.94; N, 12.89.

3-{4-[4-(3-Chlorophenyl)-1-piperazinyl]butyl}-quinazolidin-4-one (2). Base **2** was obtained in 67% yield, mp 87–89 °C (acetone); ^{1}H NMR: δ 1.58–2.04 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.34–2.63 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.11–3.25 (m, 4H, $(CH_2)_2NAr$), 4.05 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 8.04 (s, 1H, $CH{=}N$), 6.72–8.37 (m, $8H_{Arom}$); MS: *m*/*z* (I%); M 396 (33), 209 (100); hydrochloride mp 215–218 °C (2-propanol–acetone 1:1). Anal. calcd for $C_{22}H_{25}N_4OCl{\cdot}HCl$ (433.38): C, 60.97; H, 6.05; N, 12.93. Found: C, 60.72; H, 5.89; N, 13.01.

3-{3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl}-quinazolidin-4-one (3). Base **3** was obtained as an oil in 53% yield; ^{1}H NMR: δ 1.94–2.19 (m, 2H, $CH_2CH_2CH_2$), 2.35–2.62 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.04–3.24 (m, 4H, $(CH_2)_2NAr$), 3.86 (s, 3H, CH_3O), 4.12 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 8.17 (s, 1H, $CH{=}N$), 6.78–8.37 (m, $8H_{Arom}$); MS: *m*/*z* (I%); M 378 (100), 205 (31), 187 (94); hydrochloride mp 199–202 °C (acetone–ethanol 10:1). Anal. calcd for $C_{22}H_{26}N_4O_2{\cdot}HCl{\cdot}H_2O$ (432.95): C, 61.03; H, 6.75; N, 12.94. Found: C, 60.89; H, 6.82; N, 12.92.

3-{4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl}-quinazolidin-4-one (4). Base **4** was obtained in 59% yield, mp 59–62 °C (acetone); ^{1}H NMR: δ 1.62–2.03 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.37–2.70 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.03–3.18 (m, 4H, $(CH_2)_2NAr$), 3.86 (s, 3H, CH_3O), 4.05 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 8.05 (s, 1H, $CH{=}N$), 6.88–8.37 (m, $8H_{Arom}$); MS: *m*/*z* (I%); M 392 (92), 205 (100); hydrochloride mp 184–187 °C (2-propanol–acetone 1:1). Anal. calcd for $C_{23}H_{28}N_4O_2{\cdot}HCl$ (428.96): C, 64.40; H, 6.81; N, 13.06. Found: C, 64.13; H, 6.68; N, 13.29.

3-{3-[4-(3-Chlorophenyl)-1-piperazinyl]propyl}-2-phenyl-2,3-dihydrophtalazine-1,4-dione (5). Base **5** was obtained in 59% yield, mp 52–54 °C (methanol); ^{1}H NMR: δ 2.00–2.28 (m, 2H, $CH_2CH_2CH_2$), 2.50–2.71 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.12–3.31 (m, 4H, $(CH_2)_2NAr$), 4.44 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.78–8.55 (m, 13H_{Arom}); MS: *m*/*z* (I%); M 474 (7), 279 (12), 209 (100); hydrochloride mp 223–226 °C (acetone–ethanol 10:1). Anal. calcd for $C_{27}H_{27}N_4O_2Cl{\cdot}HCl{\cdot}0.5H_2O$ (520.46): C, 62.31; H, 5.62; N, 10.76. Found: C, 62.41; H, 5.58; N, 10.58.

3-{4-[4-(3-Chlorophenyl)-1-piperazinyl]butyl}-2-phenyl-2,3-dihydrophtalazine-1,4-dione (6). Base **6** was obtained in 61% yield, mp 78–80 °C (acetone); ^{1}H NMR: δ 1.75–2.00 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.41–2.67 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.14–3.26 (m, 4H, $(CH_2)_2NAr$), 4.40 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.79–8.47 (m, 13H_{Arom}); MS: *m*/*z* (I%); M 488 (6), 209 (100); hydrochloride mp 209–212 °C (1-butanol). Anal. calcd for $C_{28}H_{29}N_4O_2Cl{\cdot}HCl$ (528.48): C, 64.00; H, 5.75; N, 10.66. Found: C, 63.81; H, 5.82; N, 10.48.

3-{3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl}-2-phenyl-2,3-dihydrophtalazine-1,4-dione (7). Base **7** was obtained in 74% yield, mp 116–118 °C (acetone); ^{1}H NMR: δ 2.00–2.26 (m, 2H, $CH_2CH_2CH_2$), 2.57–2.76 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.10–3.26 (m, 4H, $(CH_2)_2NAr$), 3.87 (s, 3H, CH_3O), 4.44 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.94–8.55 (m, 13H_{Arom}); MS: *m*/*z* (I%); M 470 (4), 279 (5), 205 (100); hydrochloride mp 222–225 °C (acetone–ethanol 10:1). Anal. calcd for $C_{28}H_{30}N_4O_3{\cdot}HCl{\cdot}H_2O$ (525.05): C, 64.05; H, 6.34; N, 10.67. Found: C, 64.30; H, 6.22; N, 10.71.

3-{4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl}-2-phenyl-2,3-dihydrophtalazine-1,4-dione (8). Base **8** was obtained in 66% yield, mp 91–93 °C (acetone); ^{1}H NMR: δ 1.72–1.99 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.41–2.72 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.12–3.22 (m, 4H, $(CH_2)_2NAr$), 3.86 (s, 3H, CH_3O), 4.40 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.90–8.44 (m, 13H_{Arom}); MS: *m*/*z* (I%); M 484 (11), 205 (100); hydrochloride mp 188–191 °C (2-propanol–acetone 1:1). Anal. calcd for $C_{29}H_{32}N_4O_3{\cdot}2HCl$ (557.52): C, 62.48; H, 6.15; N, 10.05. Found: C, 62.76; H, 6.23; N, 10.03.

2-{3-[4-(3-Chlorophenyl)-1-piperazinyl]propyl}-1-phenyl-1,2-dihydropyridazine-3,6-dione (9). Base **9** was obtained in 79% yield, mp 92–94 °C (acetone–methanol 1:1); ^{1}H NMR: δ 1.87–2.09 (m, 2H, $CH_2CH_2CH_2$), 2.47–2.69 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.12–3.28 (m, 4H, $(CH_2)_2NAr$), 4.25 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.81–7.73 (m, 11H_{Arom}); MS: *m*/*z* (I%); M 424 (11), 229 (5), 209 (100); hydrochloride mp 207–210 °C (acetone–ethanol 10:1). Anal. calcd for $C_{23}H_{25}N_4O_2Cl{\cdot}HCl{\cdot}0.5H_2O$ (470.40): C, 58.73; H, 5.79; N, 11.91. Found: C, 58.95; H, 5.89; N, 11.69.

2-{4-[4-(3-Chlorophenyl)-1-piperazinyl]butyl}-1-phenyl-1,2-dihydropyridazine-3,6-dione (10). Base **10** was obtained as an oil in 68% yield; ^{1}H NMR: δ 1.62–1.86 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.34–2.66 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.12–3.27 (m, 4H, $(CH_2)_2NAr$), 4.20 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.84–7.73 (m, 11H_{Arom}); MS: *m*/*z* (I%); M 438 (13), 209 (100); hydrochloride mp 179–182 °C (acetone–ethanol 4:1). Anal. calcd for $C_{24}H_{27}N_4O_2Cl{\cdot}HCl{\cdot}H_2O$ (493.43): C, 58.42; H, 6.13; N, 11.35. Found: C, 58.31; H, 6.21; N, 11.09.

2-{3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl}-1-phenyl-1,2-dihydropyridazine-3,6-dione (11). Base **11** was obtained in 74% yield, mp 57–60 °C (acetone); ^{1}H NMR: δ 1.91–2.12 (m, 2H, $CH_2CH_2CH_2$), 2.50–2.75 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.03–3.19 (m, 4H, $(CH_2)_2NAr$), 3.86 (s, 3H, CH_3O), 4.25 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.91–7.73 (m, 11H_{Arom}); MS: *m*/*z* (I%); M 420 (63), 229 (−), 205 (100); hydrochloride mp 192–195 °C (acetone–ethanol 10:1). Anal. calcd for $C_{24}H_{28}N_4O_3{\cdot}HCl{\cdot}H_2O$ (474.99): C, 60.69; H, 6.58; N, 11.80. Found: C, 60.60; H, 6.59; N, 11.83.

2-{4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl}-1-phenyl-1,2-dihydropyridazine-3,6-dione (12). Base **12** was obtained as an oil in 64% yield; ^{1}H NMR: δ 1.66–1.94 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.38–2.75 (m, 6H, $CH_2N(CH_2)_2$ and $CH_2N(CH_2)_2$), 3.04–3.21 (m, 4H, $(CH_2)_2NAr$), 3.85 (s, 3H, CH_3O), 4.20 (t, 2H, $CH_2NC{=}O$, $J = 6.6$ Hz), 6.91–7.74 (m, 11H_{Arom}); MS: *m*/*z* (I%); M 434 (12), 205 (100); hydrochloride mp 184–187 °C (2-propanol–acetone 1:1). Anal. calcd for $C_{25}H_{30}N_4O_3{\cdot}HCl{\cdot}0.5H_2O$ (480.00): C, 62.56; H, 6.72; N, 11.67. Found: C, 62.79; H, 6.56; N, 11.66.

In vitro studies — receptor binding

5-HT_{1A} and 5-HT_{2A} receptor binding assays. Radioligand binding experiments were conducted in rat hippocampus for 5-HT_{1A} receptors, and in the cortex for 5-HT_{2A} receptors according to the published procedures.[50] The radioligands used were [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham) and [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A} and 5-HT_{2A} receptors, respectively.

D_2 dopaminergic receptor binding assay. Competition binding studies were performed in rat striatal membranes prepared according to the previously published procedure.[51] An assay was carried out in a 96-well filter plate (containing glass fiber type C, Millipore), presoaked with 100 μL of ice-cold 50 mM potassium phosphate buffer (pH 7.4) and filtered using a Millipore Vacuum Manifold prior to sample addition. 150 μL aliquots of striatal membrane preparations, 50 μL of the radioligand ([^{3}H]-spiperone, 15.70 Ci/mmol, NEN Chemicals), and either 50 μL of the buffer (for total binding assay) or 50 μL of (±)-butaclamol (5 μM) to determine the unspecific binding, or 50 μL of the compounds to be tested, were added to each well. Additionally, to prevent [^{3}H]-spiperone binding to 5-HT_{2A} receptors, ketanserin (50 nM) was included in the assay buffer. After incubation at 37 °C for 30 min, binding reaction was terminated by vacuum filtration and washed 3 times with 200 μL of buffer. Radioactivity was determined by liquid scintillation counting in a Beckman LS 6500 apparatus.

K_i values were determined from at least three competition binding experiments in which 10 drug concentrations, run in triplicate, were used. The Cheng and Prusoff equation was used for K_i calculations.[52]

In vivo studies

The experiments were carried out on male Wistar rats (250–300 g) or male Albino-Swiss mice (25–30 g). The animals were kept at a room temperature of $20\pm1\,^{\circ}C$ and were housed under standard laboratory conditions with free access to food and tap water before the experiment. All experiments were conducted in the light phase, on a natural light–dark cycle (from May to December), between 9 am and 2 pm. All the experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. (±)-DOI (Research Biochemical, Inc.), 8-OH-DPAT (Research Biochemical, Inc), reserpine (Ciba, ampules) and WAY 100635 (synthesized by Dr. J. Boksa, Institute of Pharmacology, PAS, Kraków) were dissolved in saline. Diazepam (Polfa, SA) and the investigated salts of the tested compounds were used in the form of freshly prepared suspensions in a 1% Tween 80. 8-OH-DPAT, reserpine and WAY 100635 were injected subcutaneously (sc), diazepam and the tested compounds intraperitoneally (ip) in a volume of 2 mL/kg (rats) or 10 mL/kg (mice). Each group consisted of 6–9 animals. The obtained data were analyzed by Dunnett's test.

The effect on the body temperature in mice

Rectal body temperature (measured with an Ellab thermometer) was recorded in mice 30, 60, 90 and 120 min after injection of the tested compounds. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by **2**, **4**, **7**, **8** and **10**–**12** or 8-OH-DPAT was investigated. Rectal body temperature was measured 30 and 60 min after injection of the drugs tested. WAY 100635 was given 15 min before the tested compounds.

Lower lip retraction in rats

LLR was assessed according to the method described by Berendsen et al.[16] The rats were placed individually in cages (30×25×25 cm) and were scored three times (at 15, 30 and 45 min after the tested compounds or 8-OH-DPAT) as follows: 0 = lower incisors invisible, 0.5 = partly visible, 1 = clearly visible. The summed up maximum scores amounted to 3 for each rat. The effect of the tested compounds and WAY 100635 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.

Behavioral syndrome in reserpinized rats

The rats were placed individually 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. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The maximum score, summed up over five observation periods, amounted to 15 for each symptom/animal.[17] The effect of the tested compounds and WAY 100635 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 administration. Reserpine (1 mg/kg) was given 18 h before the test.

Head twitches in mice

In order to habituate the mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter)×20 cm (height) glass cage, lined with sawdust, 30 min prior to treatment. Head twitches in mice were induced by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the number of head twitches was counted for 30 min.[19] The tested compounds or saline were administered 60 min before (±)-DOI.

Conflict drinking test (Vogel test)

A modification of the method of Vogel et al.[20] was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a Plexiglass box (27×27×50 cm), equipped with a grid floor of stainless steel bars and with a drinking bottle containing tap water. After the initial adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for a further 10-min adaptation period during which they had free access to the drinking bottle. Afterwards, they were allowed a 30 min free-drinking session in their home cage. After another 24-h period of water deprivation, the rats (those that drank water the day before) were placed again in the test chamber and allowed to drink for 30 s. Immediately afterwards, their drinking attempts were punished with an electric shock (0.5 mA). Impulses were released every 2 s (timed from the moment when a preceding shock was delivered) in 1-s periods, between the grid floor and the spout of the drinking bottle. The number of shocks accepted throughout a 5-min experimental session was counted by an experimenter who observed a behavioral reaction (e.g., body jerks) of rats to an electric shock. The tested compounds were administered 60 min before the test.

Shock threshold and free-drinking tests

To control the possibility of drug-induced changes in the perception of a stimulus or in the thirst drive, which might have contributed to the activity in the conflict drinking test, stimulus threshold measurements and a free-drinking experiment were also carried out. In both those cases, the rats were treated before the experiment in the same manner as described in the conflict drinking test, including two 24-h water deprivation periods separated by 30 min of water availability. In the shock

threshold test, the rats were placed individually in the box and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise at 15 s shock-free intervals by increasing manually the current (0.1, 0.2, 0.3, 0.4 and 0.5 mA); the shock lasted for 1 s and was delivered through the grid-floor until a rat showed an avoidance reaction (jump, or jerk) to the electric stimulus.

In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of the water (mL) consumed during 5 min was recorded for each rat. The tested compound was administered 60 min before tests.

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.[21] Each rat was placed in the centre of the plus-maze, facing one of the enclosed arms immediately after a 5-min adaptation in a wooden box ($60\times60\times35$ cm). During a 5-min test period, two experimenters who were sitting in the same room approximately 1 m from the end of one of the open arms recorded the number of entries into the closed or the open arm. An entry with all four feet put into one arm was defined as an arm entry. At the end of each trial the maze was wiped clean. The tested compounds were administered 60 min before test.

Open field test

The studies were carried out on rats according to the slightly modified method of Janssen et al.[23] The open field consisted of a circular arena (1 m in diameter) without walls, which was divided into six symmetrical sectors and illuminated with a 75 W electric bulb hung 75 cm directly above it. The laboratory was dark throughout the experiment. Individual control or drug-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 episodes (looking over the edge of the arena) were recorded for 3 min. The tested compound was administered 60 min before the test.

Forced swimming test

The forced swimming test was carried out on mice according to the method of Porsolt et al.[22] Briefly, the mice were dropped individually into a glass cylinders (25 cm high, 10 cm in diameter) containing 10 cm of water maintained at 23–25 °C, and were left there for 6 min. The duration of immobility was recorded during the last 4 of the 6 min testing period.

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