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ARTICLE *in* ARCHIV DER PHARMAZIE · NOVEMBER 2013

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Full Paper

Synthesis and Pharmacological Evaluation of Novel Tricyclic[2,1-*f*]theophylline Derivatives

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The multireceptor strategy was implemented to obtain potential antipsychotics and/or antidepressants in a series of long-chain arylpiperazines bearing a tricyclic theophylline fragment. Their binding profile toward monoaminergic receptors (α_1, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2, D_3) was determined as well. The selected compounds **7** and **9** were tested in functional *in vivo* models and showed, like atypical antipsychotic drugs, presynaptic 5-HT_{1A} receptor agonistic and postsynaptic 5-HT_{1A}, 5-HT_{2A}, and D_2 receptor antagonistic activity.

Keywords: Depression / Imidazo[2,1-*f*]purine-2,4-diones / Long-chain arylpiperazines / Schizophrenia / Serotonin

Received: July 10, 2013; Revised: August 10, 2013; Accepted: August 15, 2013

DOI 10.1002/ardp.201300257

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Introduction

Over the past decade, atypical antipsychotic drugs (AADs) such as risperidone, olanzapine, clozapine, quetiapine, ziprasidone, and aripiprazole have revolutionized the pharmacologic treatment of schizophrenia and related disorders [1]. The observed aggregation of effects of AADs can be explained as the result of multiple mechanism of their action. AADs interact with a large number of G-protein coupled receptors (GPCRs) including: (i) many serotonergic receptors including 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7; (ii) dopaminergic receptors; (iii) adrenergic and histamine receptors; and (iv) all five muscarinic receptors [2, 3]. From the chemical point of view, one of the explored classes of GPCRs ligands comprises 1-arylpiperazines. Structural modifications of this pharmacophore led to 4-substituted derivatives with a flexible aliphatic chain of different lengths, called long-chain arylpiperazines (LCAPs). Numerous studies have indicated that even minor modifications in the chemical structure of LCAP may strongly affect the affinity and selectivity for 5-HT receptor sites, other monoaminergic receptors, and their central activity *in vivo* [4–6]. Furthermore, the selectivity problem for the D2 and α1 receptors has not been clearly determined due to the high degree of homology between these receptors [6]. LCAPs are usually thought of as 5-HT_{1A} ligands; however, many of these agents bind at 5-HT_2, dopamine, and/or adrenergic receptors as well as at 5-HT_7 receptors [4].

For several years, our attention has been focused on the development of LCAPs with a complex terminal part based on purine moiety to find selective 5-HT_{1A}/5-HT_{2A} receptor ligands. The most potent for serotonin receptors were compounds with double bonds at the annelated ring at the 7,8 position of theophylline. In the majority of the obtained compounds with high or very high 5-HT_{1A} receptor affinity, lack of affinity for 5-HT_{2A} subtype and diversified pharmacological profile were observed [7–9]. In terms of functional intrinsic activity, derivatives of pyrimido[2,1-*f*]purine **I** (Fig. 1) behaved like an agonist of presynaptic and as a partial agonist of postsynaptic 5-HT_{1A} receptors and resembled ipsapirone [8]. In turn, preclinical studies indicated that derivatives of imidazo[2,1-

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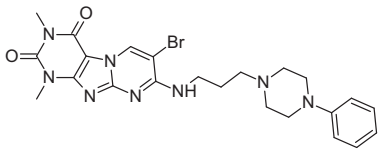
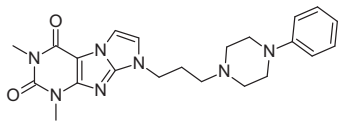


5-HT$_{1A}$ K_i = 11 ± 0.1 nM agonist / partial agonist
5-HT$_{2A}$ K_i = 297 ± 20 nM
D_2 K_i = 414 ± 120 nM



5-HT$_{1A}$ K_i = 28.8 ± 1.3 nM

Figure 1. Chemical structures and binding affinities of previously synthesized LCAPs bearing a pyrimido- or imidazo[2,1-*f*]purine-2,4-dione fragment.

f]purine-2,4-dione **II** showed high *in vitro* activity at 5-HT$_{1A}$ receptors and its intrinsic *in vivo* activity at this receptor subtype was diversified [9].

Continuing our study in a group of tricyclic theophylline derivatives, and in order to extend the studies aimed at verification of the impact of purine moiety as terminal part of LCAPs, and the length of the linker, we designed a series of imidazo[2,1-*f*]purine-2,4-diones and pyrimido[2,1-*f*]purine-2,4,8-triones with the most thoroughly studied arylpiperazines. Herein, we provide a report on their synthesis and an evaluation of selected serotoninergic (5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$, 5-HT$_7$), dopaminergic (D_2, D_3), and adrenergic α_1 receptors, as well as determination of their intrinsic activity. The present work aims at exploration of the multireceptor action capabilities of the designed compounds, which, in turn, can lead to obtain potential antipsychotic- and/or antidepressant-like activity [10].

Results and discussion

Chemistry

The final derivatives of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione **5**–**13** were obtained in a reaction of cyclocondensation of 7-ketonyl derivatives of 8-bromotheophylline **1**–**3** with appropriate arylpiperazinylalkylamine **4** according to the previously reported method [9]. The target compounds **15**–**22** were synthesized by substitution reaction of 1,3-dimethyl-(1*H*,3*H*,9*H*)-7-bromo-pyrimido[2,1-*f*]purine-2,4,8-trione **14** with appropriate arylpiperazinylalkylamine **4** (Scheme 1). For the purpose of pharmacological evaluation, compounds were converted into water-soluble hydrochloride salts. The structure elucidations of the newly synthesized compounds were carried out using different spectroscopic techniques like ^{1}H NMR and LC–MS. Further confirmations of the compounds were carried out by elemental analysis (±0.4%). The elemental analysis data and some physical properties of these compounds are reported in the Experimental part.

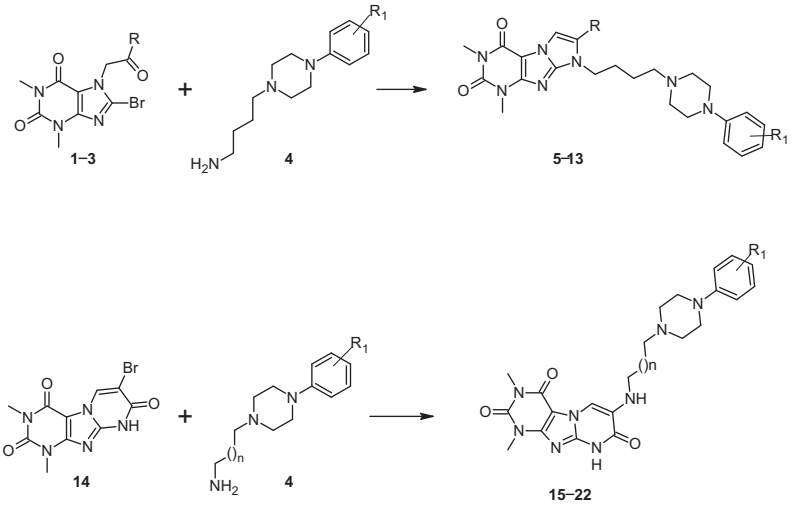
In vitro evaluation

Affinities of the newly synthesized compounds for cloned 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$, 5-HT$_7$, D_2, D_3, α_1 receptors, and serotonin transporter (SERT) were determined by standard competitive displacement assays. All compounds were tested in screening assays in two concentrations: 1 and 0.22 μM (Supporting Information). For selected compounds with percent of total binding up to 20 (%T), the inhibition constants K_i were determined. The affinity data are shown in Table 1.

All tested derivatives of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione are potent 5-HT$_{1A}$ receptor ligands (except **13**) with K_i within the range of 0.5–52.2 nM and demonstrate lack of affinity for 5-HT$_{2A}$ subtype (except **7**). Low or very low affinity for D_2 receptors was demonstrated for 7-subtituted (methyl or phenyl) arylpiperazinylbutyl derivatives of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione. Moreover, compounds **7**, **9**, and **20** are potent 5-HT$_7$ receptor ligands. In case of derivatives of pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione only compound **20** demonstrated affinity for all receptors.

Functional *in vivo* evaluation

The functional activity of selected compounds at 5-HT$_{1A}$, 5-HT$_{2A}$, and D_2 receptors was tested in several commonly used *in vivo* models [11–16]. The decrease in body temperature in mice induced by the investigated compounds and reduced by WAY 100635 was regarded as a measure of their presynaptic 5-HT$_{1A}$ receptor agonistic activity. The ability of the tested compounds to attenuate 8-OH-DPAT-induced LLR was considered as their postsynaptic 5-HT$_{1A}$ receptor antagonist activity. The antagonistic 5-HT$_{2A}$ properties were assessed in the 2,5-dimethoxy-4-iodoamphetamine ((±)DOI)-induced head twitch responses in mice. To determinate D_2 receptor antagonistic effect of the tested compounds, their ability to abolish the apomorphine-induced climbing test in mice was tested. The compounds **7** and **9** administered alone, like 8-OH-DPAT, produced a dose-dependent decrease in the body temperature in mice, and the maximum effects were observed at 30 min after their administration. Hypothermia induced by **7** and **9** was reduced by WAY 100635, a 5-HT$_{1A}$ receptor antagonist; hence, those compounds may be classified as presynaptic 5-HT$_{1A}$ receptor agonists. The results are presented in Tables 2 and 3. In a behavioral experiment used to assess the function at postsynaptic 5-HT$_{1A}$ receptors, compounds **7** and **9** did not



	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	21	22
R	-H	-H	-H	$-CH_3$	$-CH_3$	$-CH_3$	$-C_6H_5$	$-C_6H_5$	$-C_6H_5$	–	–	–	–	–	–	–	–
R_1	-H	2-OMe	3-Cl	-H	2-OMe	3-Cl	-H	2-OMe	3-Cl	-H	2-OMe	3-Cl	2,3-diCl	-H	2-OMe	4-Cl	2,3-diCl
n	–	–	–	–	–	–	–	–	–	1	1	1	1	2	2	2	2

Scheme 1. Synthesis pathways of the investigated compounds.

mimic the effect of 8-OH-DPAT in LLR test in rats. The LLR induced by 8-OH-DPAT was partially reduced by **7** and **9**. The results obtained in the LLR model indicate that **7** and **9** exhibit some postsynaptic 5-HT_{1A} receptor antagonistic activity. The results are presented in Table 4. The intraperitoneal injection of (±)DOI, the 5-HT_{2A} receptor agonist, induces a characteristic behavioral effect consisting of head twitches in mice. Compounds **7** and **9** produced a dose-dependent decrease in the number of (±)DOI-induced head twitches in mice, but significant effects were observed only in the case of the highest doses used, i.e., 20 and 60 mg/kg, respectively. These results indicate that **7** and **9** exhibit some weak 5-HT_{2A} receptor antagonistic activity. The results are presented in Table 5. Apomorphine (3 mg/kg s.c.) evoked climbing behavior, lasting about 100 s. Compound **7** administered in all investigated doses (2.5, 5, 10, and 20 mg/kg) significantly reduced the apomorphine-induced climbing; however, the results did not reach a statistically significant level. Compound **9** reduced the time of climbing induced by apomorphine in a dose-dependent manner. Thus, both compounds studied may be classified as D_2 receptor antagonists. The results are presented in Table 6.

Table 1. The binding affinities of selected compounds

	K_i (nM)			
Comp.	**5-HT_{1A}**	**5-HT_{2A}**	**D_2**	**5-HT_7**
5	4.3	147.8	–	–
6	18.7	>1000	138.6	–
7	0.5	182.8	62.7	124.6
8	52.2	471.4	–	–
9	0.96	>1000	78.2 ± 0.6	35.15
10	45	79.3	–	–
11	47.9	117.1	69	–
12	9	866	91.6	–
13	>1000	907	–	–
16	126.6	>1000	>1000	>1000
20	12.46	62.7	104.2	89.39

K_i are the inhibition constants (nM).

Conclusion

Summarizing, the present study indicates that reduction of the ring size from six to five members at the 7,8-position of theophylline had influence on receptor affinity in comparison with pyrimido[2,1-*f*]purine derivatives. We established, in turn, that imidazo[2,1-*f*]theophylline had the highest impact on affinity in the case of the multireceptor strategy. Moreover, for compounds **7** and **9**, there was no relationship between the type of substituent in the aromatic part of LCAP and the observed 5-HT_{1A} intristic activity. Chemical modification of gepirone showed that 5-HT_{1A} intristic activity was sensitive to the mode of substitution in the aromatic part of arylpiperazine [17]. It was found that ligands with the

Table 2. Effect of the investigated compounds and WAY 100635 on the body temperature in mice

		$\Delta t \pm$ SEM (°C)			
Treatment	**Dose (mg/kg)**	**30 min**	**60 min**	**90 min**	**120 min**
7	5	-0.5 ± 0.2	-0.5 ± 0.1	-0.3 ± 0.1	0.1 ± 0.2
	10	-1.3 ± 0.1[a)]	-1.0 ± 0.2[a)]	-0.9 ± 0.2[a)]	-0.5 ± 0.2[a)]
9	5	-0.6 ± 0.2	-0.4 ± 0.1	-0.1 ± 0.2	-0.1 ± 0.2
	10	-1.5 ± 0.1[a)]	-0.9 ± 0.1[a)]	-0.9 ± 0.1[a)]	-0.4 ± 0.2
WAY 100635	0.3	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. The absolute mean initial body temperatures were within a range of 36.1 ± 0.5°C.
[a)] $p < 0.01$ versus vehicle (one-way ANOVA followed by Bonferroni's *post hoc* test).

Table 3. Effect of WAY 100635 on the hypothermia induced by compounds **7** and **9** or 8-OH-DPAT in mice

	$\Delta t \pm$ SEM (°C)	
Treatment and dose (mg/kg)	**30 min**	**60 min**
Vehicle + vehicle	0.0 ± 0.1	0.1 ± 0.1
Vehicle + **7** (10)	-1.1 ± 0.2[a)]	-0.9 ± 0.2[a)]
WAY 100635 (0.3) + **7** (10)	-0.4 ± 0.2[A]	-0.2 ± 0.2[A]
Vehicle + vehicle	-0.1 ± 0.1	-0.1 ± 0.1
Vehicle + **9** (10)	-1.7 ± 0.1[a)]	-0.9 ± 0.1[a)]
WAY 100635 (0.3) + **9** (10)	-1.1 ± 0.2[a),A]	-0.6 ± 0.1
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1[A]
Vehicle + 8-OH-DPAT (5)	-1.2 ± 0.1[a)]	-0.8 ± 0.2[a)]
WAY 100635 (0.3) + 8-OH-DPAT (5)	-0.1 ± 0.1[B]	0.1 ± 0.1[B]

WAY 100635 was administered (s.c.) 15 min before the investigated compounds (i.p.) or 8-0H-DPAT (s.c.). The absolute mean initial body temperatures were within a range of 36.4 ± 0.5°C.
[a)] $p < 0.01$ versus vehicle + vehicle (one-way ANOVA followed by Newman–Keuls *post hoc* test). [A]$p < 0.05$; [B]$p < 0.01$ versus vehicle + investigated compound (one-way ANOVA followed by Newman–Keuls *post hoc* test).

Table 4. Induction of lower lip retraction (LLR) by the investigated compounds and WAY 100635 (A); their effect on the 8-OH-DPAT-induced LLR (B) in rats

		Mean ± SEM LLR score	
Treatment	**Dose (mg/kg)**	**A**	**B**
Vehicle	–	0.0 ± 0.0	2.8 ± 0.1
7	10	0.2 ± 0.1	1.9 ± 0.2[a)]
	20	0.2 ± 0.1	1.3 ± 0.3[a)]
9	10	0.3 ± 0.2	1.7 ± 0.2[a)]
	20	0.3 ± 0.1	1.8 ± 0.2[a)]
WAY 100635	0.3	0.1 ± 0.1	0.3 ± 0.2[a)]

The investigated compounds (i.p.) and WAY 100635 (s.c.) were administrated 15 min before the test (A) or 45 min before 8-OH-DPAT (1 mg/kg s.c.) (B).
[a)] $p < 0.01$ versus vehicle + 8-OH-DPAT (B) (one-way ANOVA followed by Newman–Keuls *post hoc* test).

Table 5. Effect of compounds **7** and **9** on (±)DOI-induced head-twitches reaction in mice

Treatment	**Dose (mg/kg)**	**Head twitches/20 min (mean ± SEM)**	**ID_{50}**[a)] **(mg/kg; i.p.)**
(±)DOI	2.5	34.8 ± 8.3	9.88
7	5	20.0 ± 6.8	(8.25–11.51)
	10	12.0 ± 3.9	
	20	6.0 ± 1.6[b)]	
		$F(3.18) = 5.047$	
		$p < 0.05$	
(±)DOI	2.5	26.2 ± 3.8	37.32
9	20	20.8 ± 4.7	(wide range)
	40	12.8 ± 2.1	
	60	7.0 ± 2.3[c)]	
		$F(3.24) = 4.937$	
		$p < 0.01$	

The investigated compounds (i.p.) were administrated 60 min before DOI (i.p.).
[a)] ID_{50} – the dose inhibiting head twitches in mice by 50%; confidence limits (95%) given in parentheses (Graph Pad Prism 5 Software).
[b)] $p < 0.01$ versus (±)DOI treated group (one-way ANOVA followed by Bonferroni's *post hoc* test).
[c)] $p < 0.05$.

ortho-OCH_3 group in the aryl moiety and cyclic imide system in the opposite terminal have a tendency to block postsynaptic 5-HT_{1A} receptors, whereas unsubstituted, *meta*-Cl substituted and derivatives with cyclic amide moiety show agonistic or partial agonist properties. The results of our *in vivo* functional study demonstrated that the investigated compounds behaved as presynaptic 5-HT_{1A} receptor agonists and postsynaptic 5-HT_{1A}, 5-HT_{2A}, and D_2 receptor antagonists. The observed functional profile suggest that compounds **7** and **9** modulate D_2, 5-HT_{1A} and 5-HT_{2A} receptors in the same way as ziprasidone, an atypical antipsychotic drug [2] (Table 7). The obtained results imply that compounds **7** and **9** are worthy of future research for their potential antipsychotic- and/or antidepressant-like activity.

Table 6. Effect of compounds **7** and **9** on apomorphine-induced climbing behavior in mice

Treatment	Dose (mg/kg)	Climbing time [s]/2 min (mean ± SEM)	ID_{50} [a] (mg/kg, i.p.)
Apomorphine	3	109.4 ± 3.3	–
7	2.5	55.5 ± 17.5[b]	
	5	30.2 ± 9.3[c]	
	10	40.1 ± 12.6[c]	
	20	35.7 ± 12.2[c]	
		$F(4.59) = 12.197$	
		$p < 0.0001$	
Apomorphine	3	101.7 ± 8.4	10.18 (5.64–14.72)
9	5	64.5 ± 15.7	
	10	55.5 ± 4.7	
	20	42.7 ± 22.7[b]	
		$F(3.23) = 4.332$	
		$p < 0.05$	

The investigated compounds (i.p.) were administrated 60 min and apomorphine (s.c.) 20 min before the test.
[a] ID_{50} – the dose inhibiting climbing behavior in mice by 50%; confidence limits (95%) given in parentheses (Graph Pad Prism 5 Software).
[b] $p < 0.05$.
[c] $p < 0.01$ versus apomorphine treated group (one-way ANOVA followed by Bonferroni's *post hoc* test).

Table 7. Functional *in vivo* activity of compounds **7** and **9**

Receptor		7	9
5-HT_{1A}	Presynaptic	Agonist	Agonist
	Postsynaptic	Antagonist	Antagonist
5-HT_{2A}	Postsynaptic	Antagonist	Antagonist
D_2	Postsynaptic	Antagonist	Antagonist

Experimental

General

Organic solvents (from Sigma–Aldrich and Chempur) were of reagent grade and used without purification. All other reagents were from Sigma–Aldrich and Alfa Aesar. Purity of the synthesized compounds was confirmed by TLC performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany) with the following solvents: dichloromethane/methanol/99.5% acetic acid (60:20:10) (A) and dichloromethane/methanol (90:10) (B). Spots were detected by their absorption under UV light ($\lambda = 254$ nm). ^{1}H NMR spectra were recorded at 300 MHz (Varian BB 200 spectrometer) using tetramethylsilane (TMS) (0.00 ppm) and chloroform-d_1; *J* values are in Hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). The LC/MS system consisted of a Waters Acquity UPLC, coupled to Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All analyses were carried out using an Acquity UPLC BEH C18, 50 mm × 2.1 mm reversed-phase column. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 100 to 700 *m/z*; eight scans were summed up to produce the final spectrum. Elemental analyses were found within ±0.4% of the theoretical values. Melting points (m.p.) were determined with a Büchi apparatus and are uncorrected. Column chromatography separations were carried out on columns with Merck Kieselgel 60 using the solvents: dichloromethane/methanol (90:10) as eluent. The arylpiperazinylalkylamines were obtained using a slightly modified Gabriel method [18]. 7-Ketonyl derivatives of 8-bromotheophylline **1**–**3** were obtained according to the previously described procedures [19]. 1,3-Dimethyl-7-bromo-pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione **14** was obtained during debenzylation of 1,3-dimethyl-7-bromo-9-benzyl-pyrimido [2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione [8] in a 90% sulfuric acid environment.

General procedures for the preparation of derivatives of imidazo[2,1-f]purine-2,4-dione

A mixture of 7-ketonyl derivatives of 8-bromotheophylline **1**–**3** (5 mmol) with double amount of appropriate arylpiperazinylalkylamine (**4**) (10 mmol) was refluxed in 2-methoxyethanol (20 mL) for 6–12 h. The products were isolated from the mixture as free bases according to the method: A or B.

Method A: The reaction mixture was refrigerated (−15°C) for 12 h. The precipitated product was filtered off, washed with a small amount of cold anhydrous ethanol, and recrystallized from anhydrous ethanol.

Method B: The solvent was distilled from the reaction mixture, under reduced pressure, and to the oily residue, 50 mL of acetone was added. The precipitated product was filtered off; the filtrate was evaporated, and the oily residue was dissolved in anhydrous ethanol (20 mL). After cooling, the separated product was recrystallized from ethanol 96%.

*8-[4-(N4-Phenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**5**)*

Method A: yield 62%; m.p. 217–219°C; $R_f = 0.76$ (A); ^{1}H NMR ($CDCl_3$) δ: 2.08–2.09 (m, 4H, $CH_2CH_2CH_2CH_2$), 3.02–3.13 (m, 6H, $CH_2CH_2CH_2CH_2 + (CH_2)_2N$), 3.43 (s, 3H, N3–$CH_3$), 3.58–3.78 (m, 7H, $N(CH_2)_2$ + N1–CH_3), 4.14 (t, $J = 12.05$, 2H, N8CH_2), 6.92–6.98 (m, 4H, arom + C7*H*), 7.27–7.33 (m, 2H, arom), 7.42 (d, $J = 2.3$ Hz, 1H, C6*H*). ESI-MS (*m/z*) 436.6 $(M+H)^+$. Anal. calcd. for $C_{23}H_{29}N_7O_2$: C, 63.43; H, 6.71; N, 22.51. Found: C, 63.33; H, 6.58; N, 22.82.

*8-[4-(N4-2′-Methoxyphenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**6**)*

Method A: yield 81%; m.p. 279–280°C; $R_f = 0.71$ (A); ^{1}H NMR ($CDCl_3$) δ: 2.08–2.09 (m, 4H, $CH_2CH_2CH_2CH_2$), 3.02–3.13 (m, 6H, $CH_2CH_2CH_2CH_2 + (CH_2)_2N$), 3.13–3.20 (m, 4H, $NCH_2)_2$), 3.43 (s, 3H, N3–CH_3), 3.58 (s, 3H, N1–CH_3), 3.89 (s, 3H, OCH_3), 4.19 (t, $J = 12.3$, 2H, N8CH_2), 6.92–6.98 (m, 3H, arom + C7*H*), 7.27–7.33 (m, 2H, arom), 7.42 (d, $J = 2.3$ Hz, 1H, C6*H*); ESI-MS (*m/z*) 446.6 $(M+H)^+$. Anal. calcd. for $C_{24}H_{31}N_7O_3$: C, 61.91; H, 6. 71; N, 21.06. Found: C, 62.08; H, 6.51; N, 20.92.

*8-[4-(N4-3′-Chlorophenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**7**)*

Method A: yield 69%; m.p. 252–255°C; $R_f = 0.52$ (A); ^{1}H NMR ($CDCl_3$) δ: 1.55–1.58 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.94–1.99 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.41–2.46 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.54–2.61 (m, 4H, $(CH_2)_2N$), 3.18–3.3 (m, 4H, $N(CH_2)_2$), 3.43 (s, 3H, N3–CH_3),

3.58 (s, 3H, N1–CH_3), 4.11 (t, $J = 14.1$, 2H, N8CH_2), 6.92–6.98 (m, 3H, arom), 7.12–7.17 (m, 1H, C7*H*) 7.23–7.25 (m, 1H, arom), 7.41 (d, $J = 2.05$ Hz, 1H, C6*H*); ESI-MS (*m/z*) 470.98 $(M+H)^+$. Anal. calcd. for $C_{23}H_{28}N_7O_2Cl$: C, 58.78; H, 6.01; N, 20.86. Found: C, 58.86; H, 6.23; N, 20.67.

*7-Methyl-8-[4-(N4-phenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**8**)*

Method B: yield 87%; m.p. 259–260°C, $R_f = 0.79$ (A); ^{1}H NMR ($CDCl_3$) δ: 2.02–2.17 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.35 (s, 3H, 7CH_3), 2.95–2.99 (m, 2H, $CH_2CH_2CH_2CH_2$), 3.10–3.20 (m, 4H, $(CH_2)_2$N), 3.42 (s, 3H, N3–CH_3), 3.57–3.64 (m, 7H, N1–CH_3 + N$(CH_2)_2$), 4.08 (t, $J = 13.5$, 2H, N8CH_2), 6.91–6.98 (m, 4H, arom + C6*H*), 7.24–7.32 (m, 2H, arom); ESI-MS (*m/z*) 452.98 $(M+H)^+$. Anal. calcd. for $C_{24}H_{31}N_7O_2$: C, 63.38; H, 6.71; N, 22.51. Found: C, 63.58; H, 6.94; N, 22.59.

*7-Methyl-8-[4-(N4-2′-methoxyphenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**9**)*

Method B: yield 69%, m.p. 237–239°C; $R_f = 0.75$ (A); ^{1}H NMR ($CDCl_3$) δ: 2.02–2.06 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.35 (s, 3H, 7CH_3), 2.95–2.99 (m, 2H, $CH_2CH_2CH_2CH_2$), 3.10–3.20 (m, 4H, $(CH_2)_2$N), 3.42 (s, 3H, N3–CH_3), 3.60–3.84 (m, 7H, N1–CH_3 + N$(CH_2)_2$), 3.91 (s, 3H, OCH_3), 4.10 (t, $J = 13.4$, 2H, N8CH_2), 6.91–6.98 (m, 2H, arom), 7.24 (m, 3H, arom + C6*H*); ESI-MS (*m/z*) 480.59 $(M+H)^+$. Anal. calcd. for $C_{25}H_{33}N_7O_3$: C, 62.61; H, 6.94; N, 20.44. Found: C, 62.58; H, 6.84; N, 20.59.

*7-Methyl-8-[4-(N4-3′-chlorophenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**10**)*

Method B: yield 80%; m.p. 138–140°C, $R_f = 0.61$ (A); ^{1}H NMR ($CDCl_3$) δ: 1.64 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.00–2.01 (m, 4H, $(CH_2)_2$N), 2.34 (s, 3H, 7CH_3), 2.95–2.99 (m, 2H, $CH_2CH_2CH_2CH_2$), 3.13 (t, $J = 14.05$, 2H, $CH_2CH_2CH_2CH_2$), 3.42 (s, 3H, N3–CH_3), 3.56–3.64 (m, 7H, N1–CH_3 + N$(CH_2)_2$), 4.08 (t, $J = 13.3$, 2H, N8CH_2), 6.91–6.98 (m, 3H, arom), 7.17–7.20 (m, 2H, arom + C6*H*); ESI-MS (*m/z*) 485.09 $(M+H)^+$. Anal. calcd. for $C_{24}H_{30}N_7O_2Cl$: C, 59.96; H, 6.25; N, 20.26. Found: C, 59.27; H, 6.33; N, 20.20.

*7-Phenyl-8-[4-(N4-phenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**11**)*

Method B: yield 52%; m.p. 160.7–163°C; $R_f = 0.93$ (A); ^{1}H NMR ($CDCl_3$) δ: 1.60–1.65 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.88–1.92 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.91–2.97 (m, 4H, $(CH_2)_2$N), 3.32–3.35 (m, 2H, $CH_2CH_2CH_2CH_2$), 3.44 (s, 3H, N3–CH_3), 3.61–3.74 (m, 7H, N1–CH_3 + N$(CH_2)_2$), 4.15 (t, $J = 13.8$, 2H, N8CH_2), 6.89–6.98 (m, 3H, arom), 7.25–7.56 (m, 8H, arom + C6*H*); ESI-MS (*m/z*) 512.69 $(M+H)^+$. Anal. calcd. for $C_{29}H_{33}N_7O_2$: C, 68.08; H, 6.50; N, 19.16. Found: C, 68.12; H, 6.37; N, 19.22.

*7-Phenyl-8-[4-(N4-2′-methoxyphenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**12**)*

Method B: yield 53%; m.p. 187–189°C; $R_f = 0.97$ (A); ^{1}H NMR ($CDCl_3$) δ: 1.40–1.48 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.81–1.85 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.3 (t, $J = 14.6$, 2H, $CH_2CH_2CH_2CH_2$), 3.03–3.19 (m, 4H, $(CH_2)_2$N), 3.32–3.39 (m, 4H, N$(CH_2)_2$), 3.45 (s, 3H, N3–CH_3), 3.63 (m, 3H, N1–CH_3), 3.85 (s, 3H, OCH_3), 4.14 (t, $J = 14.8$, 2H, N8CH_2), 6.83–6.99 (m, 4H, arom + C6*H*), 7.26–7.52 (m, 5H, arom); ESI-MS (*m/z*) 542.66 $(M+H)^+$. Anal. calcd. for $C_{20}H_{35}N_7O_3$: C, 66.52; H, 6.51; N, 18.10. Found: C, 66.24; H, 6.71; N, 18.17.

*7-Phenyl-8-[4-(N4-3′-chlorophenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**13**)*

Method B: yield 53%; m.p. 201–203°C; $R_f = 0.83$ (A); ^{1}H NMR ($CDCl_3$) δ: 1.65–1.88 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.89–1.92 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.91–2.97 (m, 4H, $(CH_2)_2$N), 3.32 (t, $J = 13.3$, 2H, $CH_2CH_2CH_2CH_2$), 3.45 (s, 3H, N3–CH_3), 3.61 (s, 3H, N1–CH_3), 3.48–3.75 (m, 4H, N$(CH_2)_2$), 4.15 (t, $J = 14.3$, 2H, N8CH_2), 6.90–6.93 (m, 3H, arom), 7.00–7.56 (m, 7H, arom + C6*H*); ESI-MS (*m/z*) 582.56 $(M+H)^+$. Anal. calcd. for $C_{29}H_{32}N_7O_2Cl$: C, 63.79; H, 5.91; N, 17.95. Found: C, 63.94; H, 6.06; N, 18.02.

*1,3-Dimethyl-7-bromo-pyrimido[2,1-f]purine-2,4,8-(1H,3H,9H)-trione (**14**)*

To the stirred solution of 90% H_2SO_4 (25 mL) 1,3-dimethyl-7-bromo-9-benzyl-pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione (0.01 mol) was added in portions. The obtained mixture was allowed to react overnight at room temperature. Then water was added carefully. The precipitate was filtered off. The solution was diluted by water and the product was filtered off.

Yield 80%; m.p. >300°C; ^{1}H NMR (DMSO-d_6) 3.23 (s, 3H, N3-CH_3), 3.41 (s, 3H, N1-CH_3), 8.42 (d, $J = 8.1$, C6*H*); ESI-MS (*m/z*) 327.14 $(M+H)^+$. Anal. calcd. for $C_{10}H_8BrN_5O_3$: C, 36.83; H, 2.47; N, 21.48. Found: C, 36.94; H, 2.50; N, 21.52.

*General procedure for the preparation of compounds (**15**–**22**)*

A mixture of 1,3-dimethyl-7-bromo-pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione (5 mmol) **14** with a twofold excess of appropriate arylpiperazinylalkylamine in 2-metoxyethanol was heated under reflux for 24 h. After evaporation of the solvent to the brown oil residues, the products were separated by column chromatography.

*7-[3-(N4-Phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**15**)*

Yield 67%; m.p. 280–281.3°C; $R_f = 0.3$ (B); ^{1}H NMR ($CDCl_3$) δ: 2.03–2.17 (m, 2H, $CH_2CH_2CH_2$), 3.00–3.20 (m, 6H, CH_2CH_2N$(CH_2)_2$), 3.43 (s, 3H, N3–CH_3), 3.50–3.60 (m, 4H, $(CH_2)_2$N), 3.53 (s, 3H, N1–CH_3), 3.65–3.77 (m, 2H, NHCH_2), 6.7–6.95 (m, 3H, arom), 7.15–7.25 (m, 2H, arom), 7.77 (t, $J = 12.05$, 1H, NHCH$_2$), 8.61 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 465.56 $(M+H)^+$. Anal. calcd. for $C_{23}H_{28}N_8O_3$: C, 59.47; H, 6.08; N, 24.12. Found: C, 59.70; H, 6.10; N, 24.24.

*7-[3-(N4-2′-Methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**16**)*

Yield 76%; m.p. 265–266°C; $R_f = 0.47$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.87–2.03 (m, 2H, $CH_2CH_2CH_2$), 2.61–2.77 (m, 6H, CH_2CH_2N$(CH_2)_2$), 3.10–3.21 (m, 4H, $(CH_2)_2$N), 3.43 (s, 3H, N3–CH_3), 3.61 (s, 3H, N1–CH_3), 3.65–3.77 (m, 2H, NHCH_2), 3.86 (s, 3H, OCH_3), 6.85–7.09 (m, 4H, arom), 7.90 (t, $J = 13.05$, 1H, NHCH$_2$), 8.57 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 495.56 $(M+H)^+$. Anal. calcd. for $C_{24}H_{30}N_8O_4$: C, 58.29; H, 6.11; N, 22.66. Found: C, 58.34; H, 6.10; N, 22.54.

*7-[3-(N4-3′-Chlorophenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**17**)*

Yield 84%; m.p. 286.7–287.1°C; $R_f = 0.48$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.97–2.05 (m, 2H, $CH_2CH_2CH_2$), 2.61–2.70 (m, 6H, CH_2CH_2N$(CH_2)_2$),

3.20–3.25 (m, 4H, (CH_2)$_2$N), 3.43 (s, 3H, N3–CH_3), 3.65 (s, 3H, N1–CH_3), 3.60–3.70 (m, 2H, NHCH_2), 6.78–6.95 (m, 3H, arom), 7.17–7.21. (m, 1H, arom), 7.44 (t, $J = 13.7$, 1H, N*H*CH$_2$), 8.59 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 499.96 (M+H)$^+$. Anal. calcd. for $C_{23}H_{27}ClN_8O_3$: C, 55.36; H, 5.45; N, 22.46. Found: C, 55.34; H, 5.10; N, 22.54.

*7-[3-(N4-2′,3′-Dichlorophenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**18**)*

Yield 86%; m.p. 299–300°C; $R_f = 0.54$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.90–2.03 (q, 2H, $CH_2CH_2CH_2$), 2.61–2.70 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.20–3.25 (m, 4H, (CH_2)$_2$N), 3.43 (s, 3H, N3–CH_3), 3.65 (s, 3H, N1–CH_3), 3.60–3.70 (m, 2H, NHCH_2), 7.17–7.21. (m, 3H, arom), 7.44 (t, $J = 12.5$, 1H, N*H*CH$_2$), 8.59 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 534.51 (M+H)$^+$. Anal. calcd. for $C_{23}H_{26}Cl_2N_8O_3$: C, 51.79; H, 4.91; N, 21.01. Found: C, 51.84; H, 5.08; N, 21.24.

*7-[4-(N4-Phenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**19**)*

Yield 72%; m.p. 258–259°C; $R_f = 0.28$ (B); ^{1}H NMR ($CDCl_3$) δ: 2.03–2.17 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.61–2.77 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.10–3.21 (m, 4H, (CH_2)$_2$N), 3.43 (s, 3H, N3–CH_3), 3.61 (s, 3H, N1–CH_3), 3.65–3.77 (m, 2H, NHCH_2), 3.86 (s, 3H, OCH_3), 6.7–6.95 (m, 3H, arom), 7.15–7.25 (m, 2H, arom), 7.90 (t, $J = 12.05$, 1H, N*H*CH$_2$), 8.57 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 479.51 (M+H)$^+$. Anal. calcd. for $C_{24}H_{30}N_8O_3$: C, 60.24; H, 6.32; N, 23.42. Found: C, 60.51; H, 6.08; N, 23.24.

*7-[4-(N4-2′-Methoxyphenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**20**)*

Yield 68%; m.p. 198.6–199.7°C; $R_f = 0.49$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.46–2.16 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.79–2.92 (m, 6H, $CH_2CH_2N(CH_2)_2$), 2.99–3.26 (m, 4H, (CH_2)$_2$N), 3.43 (s, 3H, N3–CH_3), 3.52–3.63 (m, 5H, N1–CH_3 + NHCH_2), 3.85 (s, 3H, OCH_3), 6.7–6.95 (m, 3H, arom), 7.15–7.25 (m, 2H, arom), 7.90 (t, $J = 12.8$, 1H, N*H*CH$_2$), 8.57 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 509.51 (M+H)$^+$. Anal. calcd. for $C_{25}H_{32}N_8O_4$: C, 59.04; H, 6.34; N, 22.03. Found: C, 59.11; H, 6.08; N, 22.24.

*7-[4-(N4-4′-Chlorophenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**21**)*

Yield 82%; m.p. 275–276°C; $R_f = 0.31$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.49–1.96 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.61–2.70 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.20–3.25 (m, 4H, (CH_2)$_2$N), 3.43 (s, 3H, N3–CH_3), 3.65 (s, 3H, N1–CH_3), 3.60–3.70 (m, 2H, NHCH_2), 6.94–7.02 (m, 2H, arom), 7.20–7.32. (m, 2H, arom), 7.89 (t, $J = 13.8$, 1H, N*H*CH$_2$), 8.72 (d, $J = 8.1$, C6*H*), ESI-MS (*m/z*) 513.96 (M+H)$^+$. Anal. calcd. for $C_{24}H_{29}ClN_8O_3$: C, 56.19; H, 5.70; N, 21.84. Found: C, 56.34; H, 5.54; N, 21.54.

*7-[4-(N4-3′,4′-Chlorophenyl)-piperazin-N1-yl-butyl]-1,3-dimethyl-(1H,3H,9H)-pyrimido[2,1-f]purine-2,4,8-trione (**22**)*

Yield 93%; m.p. 296.1–297°C; $R_f = 0.56$ (B); ^{1}H NMR ($CDCl_3$) δ: 1.14–1.76 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.32–2.61 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.00–3.55 (m, 10H, (CH_2)$_2$N + N3–CH_3 + N1–CH_3), 4.05–4.15 (m, 2H, NHCH_2), 7.13–7.40 (m, 3H, arom), 7.66 (t, $J = 14.08$, 1H, N*H*CH$_2$), 8.85 (m, 1H, C6*H*), ESI-MS (*m/z*) 548.63 (M+H)$^+$. Anal. calcd. for $C_{24}H_{28}Cl_2N_8O_3$: C, 52.66; H, 5.16; N, 20.47. Found: C, 52.34; H, 5.14; N, 20.54.

In vitro experiments

Experiments were conducted in the rat brain tissue (cerebral cortex tissue for 5-HT$_{1A}$, 5-HT$_{2A}$, and striatum tissue for D$_2$) according to procedures shown in the Supporting Information or to procedures published before [20–22]. The membrane preparation and the assay procedure were carried out according to the published procedure with slight modifications. The following radioligands were used: [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)-tetralin ([^{3}H]-8-OH-DPAT, 10^6 Ci/mmol, NEN Chemicals), [^{3}H]-Ketanserin (60 Ci/mmol, NEN Chemicals) and [^{3}H]-serone (15 Ci/mmol, NEN). Radioligand binding data were analyzed using interactive curve fitting routines (GraphPAD/Prism, Version 3.0, San Diego, CA, USA). Detailed conditions of the assays are shown in Supporting Information.

In vivo experiments

The experiments were performed on male Wistar rats (290–310 g) and male Albino Swiss mice (22–28 g). The animals were kept at a room temperature of 20 ± 1°C, and had free access to food (standard laboratory pellets) and tap water before the experiment. All the investigations were conducted in the light phase, on a natural day–night cycle (from February to May), between 9 a.m. and 2 p.m. All the experimental procedures were approved by the Local Ethics Commission for Animal Experiments of Jagiellonian University in Cracow. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (hydrobromide, 8-OH-DPAT, Tocris, Cookson Ltd., UK) was dissolved in saline, *N*-{2-[4-(2-methoxyphenyl)-1-erazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (trihydrochloride, WAY 100635), apomorphine (hydrochloride, Tocris), and (±)2,5-dimethoxy-4-iodoamphetamine ((±)DOI, hydrochloride, Adamed Pharmaceuticals) were dissolved in distilled water. The investigated compounds (**7** and **9**) were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT, WAY 100635, and apomorphine were injected subcutaneously (s.c.) while (±)DOI and investigated compounds were given intraperitioneally (i.p.). Each experimental group consisted of six to ten animals and all the animals were used only once.

Body temperature in mice

Effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In a separate experiment, the effect of WAY 100635 (0.3 mg/kg) on the hypothermia induced by compounds or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the compounds or 8-OH-DPAT and rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. The results were expressed as a change in body temperature (δt) with respect to the basal body temperature, as measured at the beginning of the experiment [13].

Lower lip retraction (LLR) in rats

LLR was assessed according to the method described by Berendsen et al. [11]. The rats were individually placed in cages (30 cm × 25 cm × 25 cm) and they were scored three times (at 15, 30, and 45 min) after the administration of the tested compounds or 8-OH-DPAT as follows: 0 = lower incisors not visible, 0.5 = partly visible, and 1 = completely visible. The total maximum scores

amounted to 3 for each rat. In a separate experiment, the effect of the tested compounds or WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds **7**, **9**, and WAY 100635 were administered 45 and 15 min, respectively, before 8-OH-DPAT and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

(±)DOI-induced head-twitch response of 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 in mice were induced by (±)DOI (2.5 mg/kg). Immediately after treatment, the number of head twitches (a clear, rapid, left to right, or right to left tic movement of the head of a mouse) was counted during 20-min session [15]. The investigated compounds were injected 60 min prior to (±)DOI administration.

Apomorphine-induced climbing behavior in mice

For observation, mice were placed in separate cages with walls made of metal bars. Twenty minutes after injection of apomorphine (3 mg/kg), time of climbing was determined for 2 min. Climbing time was defined as the period during which the animal held the 2, 3, or 4 paws on the wall. The investigated compounds were injected 40 min prior to the apomorphine administration.

Statistical analysis

The obtained data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni comparison test (when only one drug was given) or by Newman–Keuls test (when two drugs were administered). $p < 0.05$ was considered statistically significant.

This study was supported by statutory funds from Jagiellonian University Medical College (K/ZDS/001990 and K/ZDS/120093).

The authors have declared no conflict of interest.

References

[1] S. Leucht, C. Corves, D. Arbter, R. R. Engel, C. Li, J. M. Dacis, *Lancet* **2009**, *373*, 31–41.

[2] M. D. Stahl, *J. Clin. Psychiatry* **2003**, *5* (3), 9–13.

[3] B. L. Roth, D. Sheffler, S. G. Potkin, *Clin. Neurol. Res.* **2003**, *3*, 108–117.

[4] M. Leopoldo, E. Lacivita, F. Berardi, R. Perrone, P. B. Hedlund, *Pharmacol. Ther.* **2011**, *129*, 120–148.

[5] A. Czopek, H. Byrtus, M. Kołaczkowski, M. Pawłowski, M. Dybała, G. Nowak, E. Tatarczyńska, A. Wesołowska, E. Chojnacka-Wójcik, *Eur. J. Med. Chem.* **2010**, *45*, 1295–1303.

[6] P. Zajdel, K. Marciniec, A. Maślankiewicz, G. Satała, B. Duszyńska, A. J. Bojarski, A. Partyka, M. Jastrzębska-Więsek, D. Wróbel, A. Wesołowska, M. Pawłowski, *Bioorg. Med. Chem.* **2012**, *20*, 1545–1556.

[7] U. Geis, B. Grahner, M. Pawłowski, A. Drabczyńska, M. Gorczyca, C. E. Müller, *Pharmazie* **1995**, *50* (5), 333–336.

[8] S. Jurczyk, M. Kołaczkowski, E. Maryniak, P. Zajdel, M. Pawłowski, E. Tatarczyńska, A. Kłodzińska, E. Chojnacka-Wójcik, A. J. Bojarski, S. Charakchieva-Minol, B. Duszyńska, G. Nowak, D. Maciąg, *J. Med. Chem.* **2004**, *47*, 2659–2666.

[9] A. Zagórska, S. Jurczyk, M. Pawłowski, M. Dybała, G. Nowak, E. Tatarczyńska, A. Nikiforuk, E. Chojnacka-Wójcik, *Eur. J. Med. Chem.* **2009**, *44*, 4288–4296.

[10] M. J. Millan, *Pharmacol. Ther.* **2006**, *110*, 135–370.

[11] H. H. G. Berendsen, F. Jenck, C. L. E. Broekkamp, *Pharmacol. Biochem. Behav.* **1989**, *33*, 821–827.

[12] H. H. G. Berendsen, C. L. E. Broekkamp, A. M. L. van Delft, *Behav. Neural Biol.* **1991**, *55*, 214–226.

[13] E. A. Forster, I. A. Cliffe, D. J. Bill, G. M. Dover, D. Jones, Y. Reilly, A. Fletcher, *Eur. J. Pharmacol.* **1995**, *281*, 81–88.

[14] W. Koek, M. B. Assié, G. Zernig, C. France, *Psychopharmacology* **2000**, *149*, 377–387.

[15] N. A. Darmani, B. R. Martin, U. Pandey, R. A. Glennon, *Pharmacol. Biochem. Behav.* **1990**, *36*, 901–906.

[16] P. Protais, J. Costentin, J. C. Schwartz, *Psychopharmacology* **1976**, *50*, 1–6.

[17] A. J. Bojarski, M. H. Paluchowska, B. Duszyńska, R. Bugno, A. Kłodzińska, A. E. Tatarczyńska, E. Chojnacka-Wójcik, *Bioorg. Med. Chem.* **2006**, *14*, 1391–1402.

[18] S. Gabriel, *Chem. Ber.* **1887**, *20*, 2224.

[19] Y. V. Strokin, F. S. Zarudii, A. A. Kremzer, N. M. Nazinow, N. V. Chvalyuk, D. N. Lazarewa, *Pharm. Chem. J. (Engl. Transl.)* **1987**, *21*, 338–341.

[20] J. F. J. Deakin, *Psychopharmacology* **1993**, *7*, 283–289.

[21] D. N. Middlemiss, J. R. Fozard, *Eur. J. Pharmacol.* **1983**, *90*, 151–153.

[22] J. E. Leysen, C. J. Niemegeers, J. M. Van Nueten, P. M. Laduron, *Mol. Pharmacol.* **1982**, *21* (2), 301–314.

