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Original article

Synthesis and preliminary pharmacological evaluation of imidazo[2,1-*f*]purine-2,4-dione derivatives

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ABSTRACT

A series of N-8-arylpiperazinylpropyl derivatives of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (**2**–**10**) and amide derivatives of 1,3-dimethyl-6,7-dihydroimidazo[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione-7-carboxylic acid (**11**–**13**) were synthesized. Compounds (**2**–**10**) evaluated in vitro were potent 5-HT_{1A} receptor ligands. Preclinical studies indicated that 8-[3-(N4-phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (**2**) exerts anxiolytic-like activity in the four-plate test in mice; however its effect was weaker, than that produced by Diazepam. This compound and 8-[3-(N4-2′-metoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (**3**) behaved like antidepressants in the forced swimming test in mice; and their activity in that model was comparable with the effect of Imipramine. The obtained results suggested that the long-chain arylpiperazines (LCAPs) linked to tricyclic derivatives of the theophylline remain a worthy of future research for obtaining new derivatives with potential anxiolytic/antidepressant activity.

1. Introduction

A multitude of brain functions are influenced by serotonin (5-HT) including sleep, cognition, sensory perception, appetite [1]. 5-HT modulates the central nervous system through a number of receptor subtypes (5-HTR) [2–5]. At present, seven 5-HTR classes, including fourteen subtypes have been found, cloned and structure described. Due to the receptor-binding studies and molecular biology, close attention has been focused on site-selective agonists and antagonists of 5-HTR, used as tool substances and as potential new therapeutic drugs.

Long-chain arylpiperazines (LCAPs) have been extensively studied especially as 5-HT_{1A} and 5-HT_{2A} receptor ligands due to their potential antianxiety or antidepressant properties. Since the launch of Buspirone, a large number of arylpiperazine derivatives have been synthesized and some neuropsychiatric drugs e.g. Gepirone [6], Ziprasidone [7], Aripiprazole [8], and compounds with high therapeutical potential e.g. Adatanserin [9], Flesinoxan [10] were developed. The SAR studies revealed the role of individual fragments of this pharmacophore, essential for high affinity for 5-HT_{1A} activity and selectivity for other receptors [3]. These properties depended on the above-mentioned terminal fragments (amide or imide) and also on the N4 aryl substituent as well as on the length of the alkylen linker [11–15].

The incorporation of an appropriate long-chain substituent at the basic nitrogen of the piperazine ring resulted a higher affinity and selectivity [16]. From two to four methylene units can be found in amide-arylpiperazine derivatives as optimal for 5-HT_{1A} receptor affinity. Furthermore, their selectivity between others' receptors depends on the length of the alkylen spacer and the nature of the terminal fragment [17]. The chemical modification of Gepirone showed, that the profile of 5-HT_{1A} intrinsic activity was sensitive to the mode of substitution in the aromatic part of the pharmacophore [18,19]. It was found that ligands with the *o*-CH_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, *m*-Cl or *m*-CF_3 substituted derivatives show agonistic or partial agonistic properties [20]. Furthermore, the selectivity problem for the D_2 and α_1 receptors is not yet clearly determined due to the high degree of homology between these receptors [21].

Tricyclic theophylline derivatives, annelated six or seven-membered heterocyclic ring at 7,8-position of theophylline generally demonstrated a different profile of its central nervous system activity,

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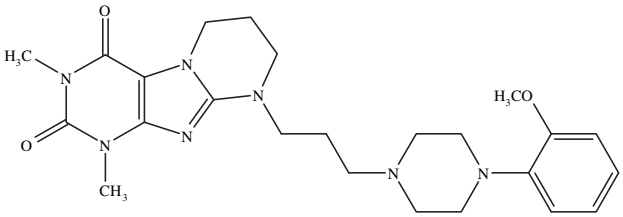
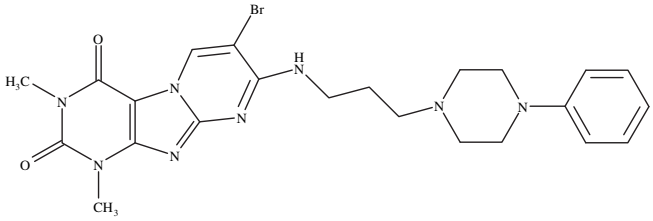


5-HT_{1A} K_i = 2,8 ± 0,3 nM, 5-HT_{2A} K_i = 486 ± 31 nM [23]



5-HT_{1A} K_i = 11 ± 0,1 nM, 5-HT_{2A} K_i = 297 ± 20 nM, D_2 K_i = 414 ± 120 nM
agonist of presynaptic and partial agonist of postsynaptic 5-HT_{1A} receptors [11]

Fig. 1. Chemical structure and binding affinities of previously synthesized LCAPs with amide part based on tricyclic theophylline derivatives.

in comparison to the reference compound (theophylline) [11,22,23]. The pharmacological evaluation of a series of tricyclic theophylline derivatives with a pyrimido- or diazepino-moiety demonstrated their sedative, hypothermizing and neuroleptic-like effects on the CNS [22]. Derivatives of pyrimido[2,1-*f*]theophylline with various LCAPs moiety showed high or very high 5-HT_{1A} receptor affinity and diversified pharmacological profile. Some of the behavioral models demonstrated that 1,3-dimethyl-9-{3-[4-(2′-methoxyphenyl)-piperazin-1-yl]-propyl}-2,4-dioxo-1,3,6,7,8,9-hexahydropyrimido[2,1-*f*]purine (Fig. 1) was classified as presynaptic agonist and postsynaptic partial agonist of 5-HT_{1A} receptor while its unsaturated analog was a pre- and postsynaptic agonist [23].

The most potent for 5-HT receptors were the compounds with aromatic six membered ring annelated at 7,8-position of the theophylline [22] and the profile of their functional activity can be attributed to π-system present in an annelated third ring 1,3-Dimethyl-7-bromo-8-[3-(4-phenylpiperazin-1-yl)-propylamino]-(1*H*,3*H*)-pyrimido[2,1-*f*]purine-2,4-dione (5-HT_{1A} $K_i = 11 \pm 0.1$ nM, 5-HT_{2A} $K_i = 297 \pm 20$ nM, D_2 $K_i = 414 \pm 120$ nM) (Fig. 1) behaved like an agonist of presynaptic and as a partial agonist of postsynaptic 5-HT_{1A} receptors and resembled Ipsapirone in terms of functional intrinsic activity [11]. The change of pyrimido moiety at 7,8-position of the theophylline, into 1,3-diazepine ring in LCAPs derivatives, resulted in a 12-fold decrease in 5-HT_{1A} affinity [23]. It showed that the third ring fused to theophylline plays an important role in binding affinity and 5-HT_{1A}/5-HT_{2A} selectivity of these compounds.

The aim of the present work was the design, synthesis and biological evaluation of LCAPs with terminal part based on imidazo[2,1-*f*]theophylline fragment (Fig. 2). We were particularly interested whether the presence of imidazole ring and/or exocyclic amide moiety in tricyclic theophylline derivatives can influence the profile of pharmacological activity in comparison to described earlier compounds.

2. Chemistry

7-Acetic-8-bromotheophylline aldehyde (**1a**), 7-acetonyl-8-bromotheophylline (**1b**) and 7-phenacyl-8-bromotheophylline (**1c**) were obtained according to the previously described procedures [24].

The final 8-substituted derivatives of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (**2**–**10**) were obtained in the cyclocondensation reaction of **1a**,**1b**,**1c**, with double amount of appropriate arylpiperazinylpropylamine [25], in boiling 2-methoxyethanol (Scheme 1).

The amide derivatives of 1,3-dimethyl-6,7-dihydroimidazo[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione-7-carboxylic acid (**11**–**13**) were obtained from **1d** [26] with equimolar amount of appropriate arylpiperazinylarylamine and HOBt in the presence of DCC in dichloromethane (Scheme 1).

The structures of the compounds were confirmed by ^{1}H NMR and MS spectral as well as by elemental (C, H, N) (see Section 5) analyses. For binding studies the synthesized free bases were converted into hydrochloride salts.

3. Pharmacology

3.1. In vitro studies

The affinities of newly synthesized compounds for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors were determined by standard competitive

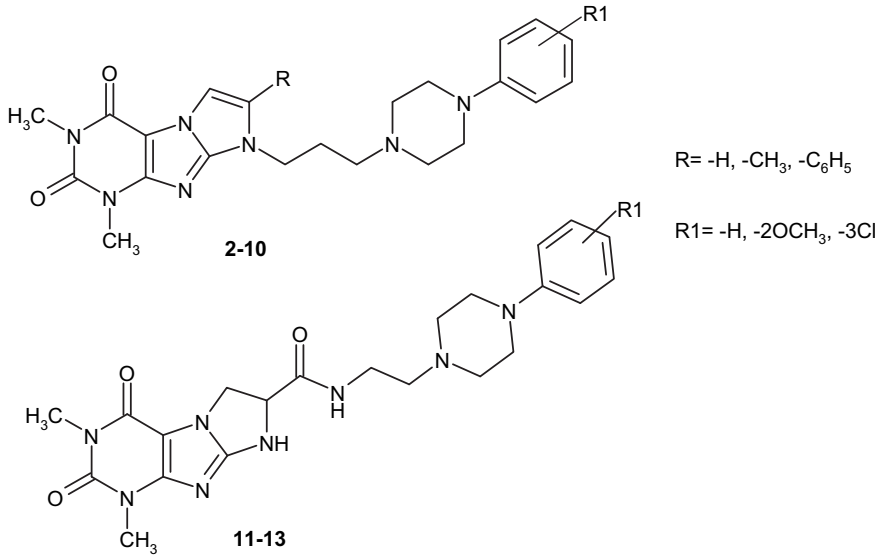
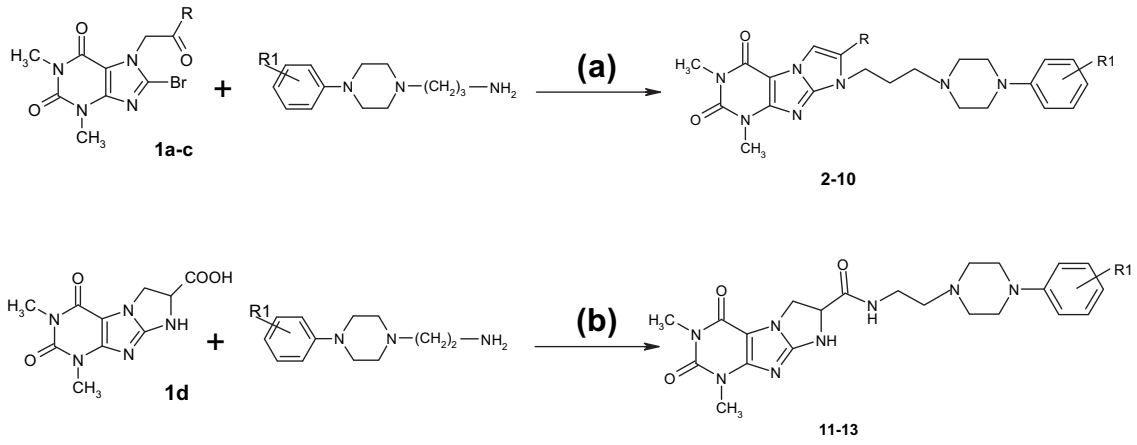


Fig. 2. General structure of the synthesized compounds.



cmpd	2	3	4	5	6	7	8	9	10	11	12	13
R	-H	-H	-H	$-CH_3$	$-CH_3$	$-CH_3$	$-C_6H_5$	$-C_6H_5$	$-C_6H_5$	-	-	-
R1	-H	-2-OMe	-3-Cl	-H	-2-OMe	-3-Cl	-H	-2-OMe	-3-Cl	-H	-2-OMe	-3-Cl

Scheme 1. Synthesis of imidazo[2,1-*f*]purine-2,4-dione derivatives. Reagents and conditions: (a) 2-methoxyethanol, reflux 6 h; (b) HOBt/DCC in DCM, stirring 12 h, room temp.

displacement assays. The results of in vitro binding studies of the newly synthesized compounds (**2**–**13**) are shown in Table 1.

Compounds **2**, **3** and **5**–**9** are potent 5-HT_{1A} receptor ligands with K_i within the range of 5.6–96.5 nM and demonstrate lack of affinity for 5-HT_{2A} subtype (except **4** K_i in micromolar value). Compounds **11**–**13** demonstrate lack of affinity for 5-HT_{1A} subtype. Compounds **3**, **6**, **7** and **9** demonstrated low or very low affinity for D_2 receptors (from $K_i = 143 \pm 10.6$ nM to 839.0 ± 61.0 nM) or did not bind to those receptors (K_i in micromolar range **2**, **4**, **5**, **8**).

3.2. In vivo studies

Selected compounds (**2**, **3**, **6**, **7** and **9**) with the highest affinity for 5-HT_{1A} receptors (K_i down to 30 nM) were further tested in vivo.

To examine the functional activity of the compounds the commonly used models were employed for measuring the responses evoked by the activation of pre- and post-synaptic 5-HT_{1A} receptors. 8-Hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) – a full 5-HT_{1A} receptor agonist – evoked a hypothermic effect in mice, a model representative of activity at presynaptic 5-HT_{1A} receptors [27] and lower lip retraction (LLR) in rats, a model reflecting 5-HT_{1A} postsynaptic activity [28,29]. Both 8-OH-DPAT-induced effects were abolished by 5-HT_{1A} receptor antagonists such as WAY 100635 [30,31]. Thus, the decrease in body temperature in mice induced by the investigated compounds and reduced by WAY 100635, was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity. Similarly, the ability of the tested compounds to induce LLR in rats was regarded as postsynaptic 5-HT_{1A} receptor agonistic activity. The antagonistic 5-HT_{1A} receptor activity of the tested compounds was assessed on the basis of the blockade of the above-mentioned 8-OH-DPAT-induced in vivo effects. All the compounds tested in vivo administered alone – like 8-OH-DPAT, a 5-HT_{1A} receptor agonist – produced a dose-dependent decrease in mouse body temperature, the maximal effects were observed at 30 min after their administration.

Table 1
Binding affinities of compounds **2**–**13**.

K_i nM (±S.E.M.)			
Cmpd	5-HT_{1A} ([^{3}H]-OH-DPAT)	5-HT_{2A} ([^{3}H]-Ketanserin)	D_2 ([^{3}H]-Spiperone)
2	24.8 ± 1.3	>1000	>1000
3	25.4 ± 6.7	>1000	839 ± 61
4	>1000	91.5 ± 1.7	>1000
5	76.4 ± 21.0	>1000	>1000
6	16.8 ± 7.6	>1000	344.7 ± 41
7	17.1 ± 0.7	403 ± 40	767.2 ± 20.5
8	96.5 ± 16	813.5 ± 54.8	>1000
9	5.6 ± 0.7	>1000	143.0 ± 10.6
10	Nd	>1000	Nt
11	>1000	Nt	Nt
12	>1000	Nt	Nt
13	>1000	Nt	Nt

Nd not detected; Nt not tested.

The functional profile of the investigated compounds, suggests that some of them may show anxiolytic- and/or antidepressant-like activity. It is well established that 5-HT_{1A} receptor ligands possess such properties [32], but their underlying mechanism of action is still unclear [33,34]. To date, there are a lot of evidences indicating that partial agonists (e.g. Buspirone), as well as full agonist (e. g. Flesinoxan) show intense activity in animal models of anxiety and depression [32,35,36]. Considering the above described premises we selected compounds **2** (a 5-HT_{1A} receptor partial agonist), **3** and **9** (full 5-HT_{1A} receptor agonists) for further in vivo preclinical studies.

3.2.1. Body temperature in mice

All the investigated compounds **2**, **3**, **6**, **7** and **9** (5–20 mg/kg) decreased rectal body temperature in mice in dose-dependent manner. The maximum hypothermic effect was observed at 30 min after their administration (Table 2). The hypothermia induced by **2**, **3**, **7**, **9** or 8-OH-DPAT was reduced by 5-HT_{1A} receptor antagonist WAY 100635 (Table 3). At the same time, WAY 100635 did not change the hypothermic effect induced by **6**.

3.2.2. Lower lip retraction (LLR) in rats

Compounds **2**, **3** and **9** (10–20 mg/kg) given alone induced weak LLR in rats, while **6** and **7**, in the same dose did not mimic the effect of 8-OH-DPAT in that test (Table 4). The LLR induced by 8-OH-DPAT was partially reduced by **2** and **7**, the remaining compounds (**3**, **6** and **9**) did

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

Treatment	Dose mg/kg	Δt ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	–	−0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	−0.1 ± 0.1
2	5	−0.6 ± 0.2	−0.4 ± 0.1	−0.2 ± 0.1	0.0 ± 0.2
	10	−1.2 ± 0.1[b]	−0.4 ± 0.2	−0.4 ± 0.2	−0.2 ± 0.2
3	10	−0.7 ± 0.2[b]	−0.6 ± 0.2[a]	−0.3 ± 0.2	0.1 ± 0.1
	20	−1.8 ± 0.2[b]	−1.3 ± 0.2[b]	−1.0 ± 0.1[b]	−1.1 ± 0.1[b]
Vehicle	–	−0.1 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	0.0 ± 0.0
6	5	−0.4 ± 0.1	−0.4 ± 0.2	−0.3 ± 0.1	−0.1 ± 0.1
	10	−0.9 ± 0.2[b]	−0.5 ± 0.2	−0.6 ± 0.2	−0.2 ± 0.1
7	5	−0.5 ± 0.2	−0.4 ± 0.1	−0.5 ± 0.2	−0.1 ± 0.1
	10	−1.0 ± 0.1[b]	−0.7 ± 0.2[a]	−0.8 ± 0.2[a]	−0.1 ± 0.2
Vehicle	–	0.0 ± 0.1	0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
9	5	−0.7 ± 0.3[a]	0.0 ± 0.2	0.1 ± 0.1	0.0 ± 0.1
	10	−2.0 ± 0.3[b]	−0.6 ± 0.1[b]	−0.6 ± 0.2[a]	−0.5 ± 0.2
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 initial body temperatures were within a range of 36.1 ± 0.5 °C.
[a] $p < 0.05$.
[b] $p < 0.01$ vs. vehicle.

not change effects of 8-OH-DPAT in this test; while 5-HT_{1A} receptor antagonist WAY 100635 almost completely blocked that effect.

The results of the in vivo studies described above demonstrated that the tested compounds present a diversified 5-HT_{1A} receptor functional activity (Table 5).

3.2.3. Four-plate test in mice

Compound **2** (at doses 10–20 mg/kg) increase of spontaneous punished crossings in the four-plate test in mice. However its effect was weaker, in terms of its potency and active doses, than that produced by Diazepam, used as reference drug (Table 6). Compounds **3** and **9** were inactive in that test.

Table 3
Effect of WAY 100635 on the hypothermia induced by compounds **2**, **3**, **6**, **7**, **9** or 8-OH-DPAT in mice.

Treatment and dose (mg/kg)	Δt ± SEM (°C)	
	30 min	60 min
Vehicle + vehicle	0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **2** (10)	−1.2 ± 0.2[a]	−0.3 ± 0.1
WAY 100635 (0.1) + **2** (10)	−0.3 ± 0.1[b]	−0.1 ± 0.1
Vehicle + vehicle	0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **3** (20)	−1.8 ± 0.2[a]	−1.3 ± 0.2[a]
WAY 100635 (0.1) + **3** (20)	−0.9 ± 0.2[a,b]	−0.5 ± 0.2[b]
Vehicle + vehicle	0.0 ± 0.1	0.1 ± 0.1
Vehicle + **6** (10)	−0.9 ± 0.1[a]	−0.6 ± 0.1[a]
WAY 100635 (0.1) + **6** (10)	−1.0 ± 0.2[a]	−0.9 ± 0.1[a]
Vehicle + vehicle	0.0 ± 0.1	−0.1 ± 0.1
Vehicle + **7** (10)	−1.3 ± 0.2[a]	−1.2 ± 0.2[a]
WAY 100635 (0.1) + **7** (10)	−0.9 ± 0.1[a]	−0.5 ± 0.1[b]
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1
Vehicle + **9** (10)	−2.0 ± 0.3[a]	−0.6 ± 0.1[a]
WAY 100635 (0.1) + **9** (10)	−0.9 ± 0.2[a,b]	0.1 ± 0.1[b]
Vehicle + 8-OH-DPAT (5)	−1.2 ± 0.1[a]	−0.8 ± 0.2[a]
WAY 100635 (0.1) + 8-OH-DPAT (5)	−0.1 ± 0.1[b]	0.1 ± 0.1[b]

WAY 100635 was administered (sc) 15 min before the investigated compounds (ip) or 8-0H-DPAT (sc). The absolute mean initial body temperatures were within a range of 36.4 ± 0.5 °C.
[a] $p < 0.01$ vs. vehicle + vehicle.
[b] $p < 0.01$ vs. vehicle + investigated compound.

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

Treatment	Dose mg/kg	Mean ± SEM LLR score	
		A	B
Vehicle	–	0.0 ± 0.0	2.8 ± 0.1
2	10	0.2 ± 0.1	1.7 ± 0.1[a]
	20	0.8 ± 0.2[a]	1.8 ± 0.3[a]
3	10	0.9 ± 0.2[a]	2.9 ± 0.1
	20	1.5 ± 0.2[a]	2.8 ± 0.1
9	10	0.8 ± 0.2[a]	2.3 ± 0.2
	20	0.8 ± 0.2[a]	2.3 ± 0.3
Vehicle	–	0.0 ± 0.0	2.8 ± 0.2
6	10	0.1 ± 0.1	2.7 ± 0.2
	20	0.1 ± 0.1	2.7 ± 0.2
7	10	0.0 ± 0.0	1.6 ± 0.1[a]
	20	0.0 ± 0.0	1.0 ± 0.3[a]
WAY 100635	0.1	0.1 ± 0.1	0.3 ± 0.2[a]

The investigated compounds (ip) and WAY 100635 (sc) were administrated 15 min before the test (A), or 45 min before 8-OH-DPAT (1 mg/kg, sc) (B).
[a] $p < 0.01$ vs. vehicle (A) or vs. vehicle + 8-OH-DPAT (B).

3.2.4. Forced swimming test in rats

Compounds **2** and **3** (10 and 20 mg/kg) reduced the immobility time in mice. Activity of **2** and **3** in that model was comparable with the effect of Imipramine (Table 7). Compound **9** (20–30 mg/kg) was practically inactive in that test.

3.2.5. Locomotor activity in mice

Compound **2** at doses active in the four-plate and forced swimming tests (20–30 mg/kg), potently reduced locomotor activity of mice (during 6-min, as well as 30-min experimental sessions). In this test compound **3** induced sedation only at the dose of 30 mg/kg (Table 8).

4. Result and discussion

The in vitro radioligand binding studies with arylpiperazines containing a terminal imidazo[2,1-*f*]theophylline fragment and trimethylene aliphatic spacer (**2**–**9**) showed that these compounds were potent ligands at 5-HT_{1A} recognition sites. The radioligand binding data on the 5-HT_{1A} receptor affinities showed large differences and depend on both, 1-arylpiperazinylpropyl moiety and the fragment of imidazo[2,1-*f*]theophylline. The chemical structure of the terminal imidazo[2,1-*f*]theophylline moiety influences the binding parameters. The affinity of 2-methoxy- and 3-chloro-phenylpiperazinylpropyl derivatives for 5-HT_{1A} receptor was the lowest for unsubstituted at 7-position of 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione derivatives (**2**–**4**) and one inactive compound **4** belonged to this group ($K_i = 677.7$ nM). The most potent for 5-HT_{1A} receptor was compound with phenyl moiety at 7-position of the 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (**9**), and with substituted phenylpiperazine ring ($K_i = 5.6$ nM). Replacement of phenyl (**9**) by methyl moiety (**6**) in 7-position of the terminal tricyclic fragment afforded the 3-fold less

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

Cmpd	5-HT_{1A} activity	
	Presynaptic	Postsynaptic
2	Agonist	Partial agonist
3	Agonist	Agonist
6	Non-active	Non-active
7	Agonist	Antagonist
9	Agonist	Agonist

Table 6
The effect of compounds **2**, **3**, **9** and Diazepam in the four-plate test in mice.

Treatment	Dose mg/kg	Number of punished crossings mean ± SEM
Vehicle	–	3.8 ± 0.4
2	10	4.7 ± 0.4
	20	6.3 ± 0.6[b]
		$F(2.27) = 7.490$
		$p < 0.01$
Vehicle	–	3.0 ± 0.3
3	20	2.5 ± 0.3
	30	2.5 ± 0.4
		$F(2.27) = 0.681$
		Ns
Vehicle	–	3.0 ± 0.3
9	20	3.5 ± 0.4
	30	3.2 ± 0.6
		$F(2.27) = 0.328$
		Ns
Vehicle[c]	–	3.5 ± 0.4
Diazepam	1.25	5.5 ± 0.5[a]
	2.5	6.8 ± 0.6[b]
	5	6.7 ± 0.6[b]
		$F(3.36) = 9.514$
		$p < 0.001$

The investigated compounds were administered 30 min, while Diazepam 60 min before the test. $n = 10$ mice per group.
[a] $p < 0.05$.
[b] $p < 0.01$ vs. vehicle. Ns: not significant.
[c] Data taken from ref [42].

of affinity for those sites ($K_i = 16.8$ nM). The amide derivatives of 1,3-dimethyl-6,7-dihydroimidazo[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione-7-carboxylic acid (**11**–**13**) were generally not active at 5-HT_{1A} recognition sites.

The 5-HT_{2A} receptor affinity of the target compounds was low $K_i > 1000$ nM, whereas **4** displayed higher affinity for that receptor subtype than for 5-HT_{1A} ($K_i = 91.5$ nM). Compounds **2**–**9** evaluated for their D_2 receptor affinity, were demonstrated low or very low affinity (from $K_i = 143 \pm 10.6$ nM to 839.0 ± 61 nM), or did not bind to those receptors (K_i in micromolar range). The present study indicates that the five-membered heterocyclic ring at 7,8-position of theophylline improved 5-HT_{1A} receptor affinity especially in the case of 2-methoxyphenylpiperazinylpropyl derivatives.

The results of the in vivo studies demonstrated that the hypothermia induced by **2**, **3**, **7**, **9** or 8-OH-DPAT was reduced by WAY 100635, a 5-HT_{1A} receptor antagonist, hence those compounds may be classified as presynaptic 5-HT_{1A} receptor agonists. At the same time, WAY 100635 did not change the hypothermic effect induced by **6**, so it seems that a contribution of presynaptic 5-HT_{1A} receptor to this effect should be excluded.

In behavioral experiment used to assess the function at postsynaptic 5-HT_{1A} receptors, compounds **2**, **3** and **9** given alone induced weak LLR in rats, while **6** and **7** did not mimic the effect of 8-OH-DPAT in that test. The LLR induced by 8-OH-DPAT was partially reduced by **2** and **7**, the remaining compounds (**3**, **6** and **9**) did not change effects of 8-OH-DPAT in this test; while 5-HT_{1A} receptor antagonist WAY 100635 almost completely blocked that effect. The results obtained in the LLR model indicate that compounds **3** and **9** might be regarded as potential agonists of postsynaptic 5-HT_{1A} receptors, whereas compound **2** behaves like partial agonist and compound **7** like antagonist of these sites (**6** was inactive in that model).

The obtained results indicate that compound **2**, but not **3** and **9**, exerts anxiolytic-like activity in the four-plate test in mice; however its effect was weaker, than that produced by Diazepam, used as reference anxiolytic drug. The results of our successive experiments also show that compounds **2** and **3**, but not **9**, behaved like antidepressants in the forced swimming test in mice; both compounds – like a typical antidepressant Imipramine – shortened the immobility time in mice. It is noteworthy that activity of **2** and **3** in that model was comparable with the effect of Imipramine. It has also been demonstrated that **2**, at doses active in the four-plate and

Table 7
The effect of compounds **2**, **3**, **9** and Imipramine in the forced swimming test in mice.

Treatment	Dose mg/kg	Immobility time (s), mean ± SEM
Vehicle	–	159.9 ± 11.4
2	10	147.5 ± 22.4
	20	87.9 ± 17.3[a]
	30	88.9 ± 15.3[a]
		$F(3.28) = 4.966$
		$p < 0.01$
Vehicle	–	166.3 ± 6.5
3	10	134.2 ± 13.0
	20	104.8 ± 18.4[a]
	30	101.3 ± 5.2[a]
		$F(3.28) = 6.363$
		$p < 0.01$
Vehicle	–	166.6 ± 11.6
9	20	169.3 ± 8.2
	30	172.0 ± 9.3
		$F(2.21) = 0.075$
		Ns
Vehicle	–	167.1 ± 6.7
Imipramine	10	149.1 ± 10.7
	20	107.8 ± 12.4[a]
	30	76.4 ± 7.1[a]
		$F(3.36) = 18.200$
		$p < 0.001$

The investigated compounds and Imipramine were administered 30 min before the test. $n = 8$–10 mice per group.
[a] $p < 0.01$ vs. vehicle.

Table 8
The effect of compounds **2**, **3**, Diazepam and Imipramine on the locomotor activity of mice.

Treatment	Dose mg/kg	Locomotor activity: number of crossings during:	
		6 min	30 min
Vehicle	–	360.9 ± 19.0	973.1 ± 70.5
2	20	146.4 ± 17.9[b]	255.6 ± 25.2[b]
	30	139.2 ± 21.9[b]	221.0 ± 31.8[b]
		$F(2.24) = 41.03$	$F(2.24) = 81.956$
		$p < 0.001$	$p < 0.001$
Vehicle	–	324.3 ± 19.7	931.1 ± 101.5
3	20	353.4 ± 15.5	925.8 ± 114.2
	30	198.0 ± 20.1[b]	567.6 ± 50.8[a]
		$F(2.21) = 19.850$	$F(2.21) = 5.018$
		$p < 0.001$	$p < 0.05$
Vehicle			917.9 ± 59.1
Diazepam	1.25	NT	686.3 ± 72.1
	2.5		666.8 ± 87.3
	5		589.5 ± 76.7[a]
			$F(3.36) = 3.477$
			$p < 0.05$
Vehicle		340.6 ± 34.1	892.7 ± 76.6
Imipramine	10	338.0 ± 36.4	876.8 ± 67.0
	20	314.9 ± 24.1	625.3 ± 53.6[a]
	30	300.9 ± 34.5	602.3 ± 71.6[a]
		$F(3.36) = 0.3398$	$F(3.36) = 5.361$
		ns	$p < 0.01$

The investigated compounds and Imipramine were administered 30 min, while Diazepam 60 min before the test. $n = 8$–10 mice per group. NT: not tested. ns: not significant.
[a] $p < 0.05$.
[b] $p < 0.01$ vs. vehicle.

forced swimming tests, potently reduced locomotor activity of mice during a 6-min, as well as 30-min experimental sessions, while **3** induced sedation only at the higher dose used. In comparison, Diazepam produces sedative effect in mice at dose 4-fold higher than that evoking a minimal, but statistically significant, anxiolytic-like effects, whereas Imipramine at doses active in the forced swimming test induces a weak sedation in mice only when locomotor activity was recorded for 30 min. The sedative effects exerted by compounds **2** and **3** excluded these ligands from being regarded as potential drugs.

5. Conclusion

The present study indicates that the reduction of the ring size from six to five member at 7,8-position of the theophylline had limited influence on receptors affinity and the binding profile, in comparison to the pyrimido[2,1-*f*]purine analogs. The impact of a double bond of the five-membered ring of imidazo[2,1-*f*]theophylline moiety was evaluated. We established in turn that the double bond in imidazo[2,1-*f*]theophylline had the most favourable influence on the affinity of derivatives for the 5-HT_{1A} receptor. The arylpiperazinylalkyl amides of imidazo[2,1-*f*]theophylline-7-carboxylic acid were inactive for 5-HT_{1A} receptors.

Compounds showed high in vitro activity at 5-HT_{1A} receptors and its intrinsic in vivo activity at this receptor subtype was diversified. 8-[3-(N4-Phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (2) exerts anxiolytic-like activity in the four-plate test in mice; however its effects were weaker, in terms of its potency and active doses, than that produced by Diazepam, used as reference anxiolytic drug. This compound and 8-[3-(N4-2′-methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione (3) behaved like antidepressants in the forced swimming test in mice and their activity in that model was comparable with the effect of Imipramine. It has also been demonstrated the sedative effects exerted by those compounds at doses active in the four-plate and forced swimming tests. The obtained results suggested that the LCAPs linked to tricyclic derivatives of the theophylline remain a worthy of future research for obtaining new derivatives with potential anxiolytic/antidepressant activity.

6. Experimental protocols

6.1. Chemistry

6.1.1. General remarks

All the chemicals and solvents were purchased from Merck (Darmstad, Germany) or Fluka (Buchs, Switzerland) and were used without further purification. Melting points (M.p.) were measured in open capillaries on an Electrothermal 9100 melting point apparatus without corrections. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Kieselgel 60 F_{254} plates (Merck) and the solvents: benzene/acetone (7:3) and chloroform/methanol/99.5% acetic acid (60:20:10) were used. Flash column chromatography was carried out on SilicaGel 100-Fluka using the solvent acetic acid/ethanol (1:2). All the compounds used (bases **2**–**13** and hydrochlorides **2a**–**13a**) were subjected to a quantitative elemental (C, H, N) analysis by a micro-method using the elemental Vario EI III Elementar analyzer (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values. ^{1}H NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 300 MHz. Chemical shifts are expressed in parts per million (δ) downfield of tetramethylsilane (TMS) used as an internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), d (doublet), dd (double doublet), m (multiplet), t (triplet), br s (broad singlet). The mass spectra (MS) were recorded on MDX SCIEX API 2000 (Concord, ON, Canada).

6.1.2. General procedure for the synthesis of 8-N4-arylpiperazin-N1-yl-propyl derivatives of 1,3-dimethyl-(1H,8H)-imidazo[2,1-f] purine-2,4-dione (**2**–**10**)

A mixture of compound **1a**–**c** [24] (0.005 mol) and 0.01 mol of suitable arylpiperazinylpropylamine in 20 ml of 2-methoxyethanol was refluxed for 6 h. The products were isolated from the mixture according to the method: *A* or *B*. *Method A*: A 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 ethanol. *Method B*: From the reaction mixture the solvent was distilled 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.

*6.1.2.1. 8-[3-(N4-phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**2**).* Method A, yield 60%, M.p. 189–190 °C, ^{1}H NMR ($CDCl_3$) δ: 2.13–2.20 (m, 2H, $CH_2CH_2CH_2$), 2.45–2.47 (t, $J = 13.2$ Hz, 2H, $CH_2CH_2CH_2$), 2.49–2.64 (m, 4H, $(CH_2)_2N1_{pip}$), 3.23–3.26 (m, 4H, $N4_{pip}(CH_2)_2$), 3.47 (s, 3H, N3CH_3), 3.64 (s, 3H, N1CH_3), 4.19–4.24 (t, $J = 13.7$ Hz, 2H, N8CH_2), 6.86–6.98 (m, 4H, $H_{aromat.}$), 7.27–7.33 (m, 2H, $H_{aromat.}$ + C7*H*), 7.44 (d, $J = 2.4$ Hz, 1H, C6*H*). MS ($M + H^+$): 423.5. Anal. calcd. for $C_{22}H_{27}N_7O_2$: C, 62.69; H, 6.45; N, 23.26%. Found: C, 62.33; H, 6.58; N, 23.02%.

*6.1.2.2. 8-[3-(N4-2′-methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**3**).* Method A, yield 73%, M.p. 230–231 °C, ^{1}H NMR ($CDCl_3$) δ: 2.12–2.17 (m, 2H, $CH_2CH_2CH_2$), 2.45–2.48 (t, $J = 13.7$ Hz, 2H, $CH_2CH_2CH_2$), 2.50–2.68 (m, 4H, $(CH_2)_2N1_{pip}$), 3.13–3.20 (m, 4H, $N4_{pip}(CH_2)_2$), 3.47 (s, 3H, N3CH_3), 3.64 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.19–4.23 (t, $J = 13.7$ Hz, 2H, N8CH_2), 6.87–7.07 (m, 5H, $H_{aromat.}$ + C7*H*), 7.43–7.44 (d, $J = 2.4$ Hz, 1H, C6*H*). MS ($M + H^+$): 452.5. Anal. calcd. for $C_{23}H_{29}N_7O_3$: C, 61.18; H, 6.47; N, 21.71%. Found: C, 60.88; H, 6.51; N, 21.72%.

*6.1.2.3. 8-[3-(N4-3′-chlorophenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**4**).* Method A, yield 69%, M.p. 244–245 °C, ^{1}H NMR ($CDCl_3$) δ: 1.95–2.03 (m, 2H, $CH_2CH_2CH_2$), 2.50–2.53 (t, $J = 13.3$ Hz, 2H, $CH_2CH_2CH_2$), 2.55–2.70 (m, 4H, $(CH_2)_2N1_{pip}$), 3.13–3.20 (m, 4H, $N4_{pip}(CH_2)_2$), 3.47 (s, 3H, N3CH_3), 3.64 (s, 3H, N1CH_3), 4.15–4.21 (t, $J = 13.7$ Hz, 2H, N8CH_2), 6.83–7.07 (m, 5H, $H_{aromat.}$ + C7*H*), 7.44 (d, $J = 2.3$ Hz, 1H, C6*H*). MS ($M + H^+$): 456.9. Anal. calcd. for $C_{22}H_{26}N_7O_2Cl$: C,57.95; H,5.74; N,21.50%. Found: C, 57.88; H, 5.95; N, 21.72%.

*6.1.2.4. 7-Methyl-8-[3-(N4-phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**5**).* Method B, yield 87%, M.p. 197–198 °C, ^{1}H NMR ($CDCl_3$) δ: 2.12–2.16 (m, 2H, $CH_2CH_2CH_2$), 2.38 (s, 3H, 7CH_3) 2.43–2.48 (t, $J = 13.3$ Hz, 2H, $CH_2CH_2CH_2$), 2.61–2.64 (m, 4H, $(CH_2)_2N1_{pip}$), 3.21–3.25 (m, 4H, $N4_{pip}(CH_2)_2$), 3.46 (s, 3H, N3CH_3), 3.63 (s, 3H, N1CH_3), 4.12–4.16 (t, $J = 13.3$ Hz, 2H, N8CH_2), 6.87–6.97 (m, 3H, $H_{aromat.}$), 7.18 (s, 1H, C6*H*), 7.26–7.30 (m, 2H, $H_{aromat.}$). MS ($M + H^+$): 437.9. Anal. calcd. for $C_{23}H_{29}N_7O_2$: C, 63.38; H, 6.71; N, 22.51%. Found C, 63.58; H, 6.94; N, 22.59%.

*6.1.2.5. 7-Methyl-8-[3-(N4-2′-methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**6**).* Method B, yield 85%, M.p. 195–196 °C, ^{1}H NMR ($CDCl_3$) δ: 2.11–2.16

(m, 2H, $CH_2CH_2CH_2$), 2.39 (s, 3H, 7CH_3) 2.39–2.47 (t, $J = 13.3$ Hz, 2H, $CH_2CH_2CH_2$), 2.49–2.67 (m, 4H, $(CH_2)_2N1_{pip}$), 3.05–3.1 (m, 4H, $N4_{pip}(CH_2)_2$), 3.46 (s, 3H, N3CH_3), 3.63 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.12–4.16 (t, $J = 13.7$ Hz, 2H, N8CH_2), 6.88–7.07 (m, 4H, $H_{aromat.}$), 7.18 (s, 1H, C6*H*). MS ($M + H^+$): 466.5. Anal. calcd. for $C_{24}H_{31}N_7O_3$: C, 61.9; H, 6.71; N, 21.06%. Found: C, 61.72; H, 6.56; N, 20.82%.

*6.1.2.6. 7-Methyl-8-[3-(N4-3′-chlorophenyl)-piperazin-N1-yl-propyl] -1,3-dimethyl-(1*H,*8*H*)-imidazo[2,1-f]purine-2,4-dione (**7**).* Method B, yield 80%, M.p. 194–195 °C, ^{1}H NMR ($CDCl_3$) δ: 2.10–2.16 (m, 2H, $CH_2CH_2CH_2$), 2.39 (s, 3H, 7CH_3) 2.45–2.52 (m, 2H, $CH_2CH_2CH_2$), 2.58–2.70 (m, 4H, $(CH_2)_2N1_{pip}$), 3.20–3.30 (m, 4H, $N4_{pip}(CH_2)_2$), 3.47 (s, 3H, N3CH_3), 3.62 (s, 3H, N1CH_3), 4.19–4.25 (t, $J = 12.7$ Hz, 2H, N8CH_2), 6.87–6.97 (m, 3H, $H_{aromat.}$), 7.18 (s, 1H, C6*H*), 7.28–7.30 (m, 1H, $H_{aromat.}$). MS ($M + H^+$): 470.9. Anal. calcd. for $C_{23}H_{28}N_7O_2Cl$: C, 58.78; H, 6.00; N, 20.86%. Found: C, 58.72; H, 6.26; N, 20.74%.

*6.1.2.7. 7-Phenyl-8-[3-(N4-phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1*H,*8*H*)-imidazo[2,1-f]purine-2,4-dione (**8**).* Method B, yield 49%, M.p. 161–162 °C, ^{1}H NMR ($CDCl_3$) δ: 1.99–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.36–2.45 (m, 6H, $CH_2CH_2CH_2 + (CH_2)_2N1_{pip}$), 3.05–3.10 (m, 4H, $N4_{pip}(CH_2)_2$), 3.48 (s, 3H, N3CH_3), 3.67 (s, 3H, N1CH_3), 4.24–4.29 (t, $J = 14.5$ Hz, 2H, N8CH_2), 6.87–6.94 (m, 3H, $H_{aromat.}$), 7.26–7.29 (m, 2H, $H_{aromat.}$), 7.46 (s, 1H, C6*H*), 7.49–7.55 (m, 5H, $H_{aromat.}$). MS ($M + H^+$): 499.6. Anal. calcd. for $C_{28}H_{31}N_7O_2$: C, 67.59; H, 6.28; N, 19.70%. Found: C, 67.83; H, 6.18; N, 19.79%.

*6.1.2.8. 7-Phenyl-8-[3-(N4-2′-methoxyphenyl)-piperazin-N1-yl-propyl] -1,3-dimethyl-(1*H,*8*H*)-imidazo[2,1-f]purine-2,4-dione (**9**).* Method B, yield 54%, M.p. 171–172 °C, ^{1}H NMR ($CDCl_3$) δ: 2.00–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.36–2.45 (m, 6H, $CH_2CH_2CH_2 + (CH_2)_2N1_{pip}$), 3.05–3.1 (m, 4H, $N4_{pip}(CH_2)_2$), 3.48 (s, 3H, N3CH_3), 3.67 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.24–4.29 (t, $J = 13.8$ Hz, 2H, N8CH_2), 6.87–6.94 (m, 3H, $H_{aromat.}$), 7.26–7.29 (m, 1H, $H_{aromat.}$), 7.46 (s, 1H, C6*H*), 7.49–7.55 (m, 5H, $H_{aromat.}$). MS ($M + H^+$): 528.6. Anal. calcd. for $C_{29}H_{33}N_7O_3$: C, 67.07; H, 6.30; N, 18.58%. Found: C, 66.94; H, 6.56; N, 18.72%.

*6.1.2.9. 7-Phenyl-8-[3-(N4-3′-chlorophenyl)-piperazin-N1-yl-propyl] -1,3-dimethyl-(1*H,*8*H*)-imidazo[2,1-f]purine-2,4-dione (**10**).* Method B, yield 51%, M.p. 228–229 °C, ^{1}H NMR ($CDCl_3$) δ: 1.99–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.36–2.45 (m, 6H, $CH_2CH_2CH_2 + (CH_2)_2N1_{pip}$), 3.05–3.1 (m, 4H, $N4_{pip}(CH_2)_2$), 3.48 (s, 3H, N3CH_3), 3.67 (s, 3H, N1CH_3), 4.24–4.29 (t, $J = 13.3$ Hz, 2H, N8CH_2), 6.87–6.94 (m, 3H, $H_{aromat.}$), 7.26 (s, 1H, $H_{aromat.}$), 7.46 (s, 1H, C6*H*), 7.49–7.55 (m, 5H, $H_{aromat.}$). MS ($M + H^+$): 533.0. Anal. calcd. for $C_{28}H_{30}N_7O_2Cl$: C, 63.79; H, 5.91; N, 17.95%. Found: C, 63.94; H, 6.06; N, 18.02%.

*6.1.3. General procedure for the synthesis of **11**–**13***

1-Hydroxy-1*H*-benzotriazole (HOBt) (0.8 g, 5.8 mmol) was added to the mixture of acid **1d** [26] (1.0 g, 3.8 mmol) and 1.0 g arylpiperazinylalkylamine in 20 ml of dichloromethane. A mixture was stirred for 30 min in 0 °C then N,N′-dicyclohexylcarbodiimide (DCC) (1.2 g, 5.8 mmol) was added and the mixture was stirred at room temperature for 12 h. The solvent was distilled, the solid residue was washed with 10% NaOH (30 ml) and filtered off. The precipitate was treated with 20 ml of acetic acid and the mixture was stirred at room temperature for 15 min, diluted with water (20 ml), the precipitate was filtered off. The filtrate was evaporated to the oily residue which was purified by flash column chromatography on silica gel, using acetic acid/ethanol (1:2 v/v) as eluent. The obtained products, in the form of acetic acid salt, were treated with an excess of triethylamine and free bases were recrystallized from acetone.

*6.1.3.1. N-2-[(N4-phenyl)-piperazin-N1-yl]-ethyl amide of 1,3-dimethyl-6,7-dihydroimidazo[2,1-f]purine-2,4-(1*H,*3*H*)-dione-7-carboxylic acid (**11**).* Yield 51%, M.p. 215–216 °C, ^{1}H NMR ($CDCl_3$) δ: 2.50–2.53 (t, $J = 13.3$ Hz, 2H, CH_2CH_2), 2.64–2.7 (m, 4H, $(CH_2)_2N1_{pip}$), 3.06–3.12 (m, 4H, $N4_{pip}(CH_2)_2$), 3.20–3.30 (m, 6H, N3$CH_3 + CH_2CH_2 +$ C7$H_{imidazole}$), 3.55 (s, 3H, N1CH_3), 4.21 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 4.44–4.5 (m, 1H, N$H_{imidazole}$), 4.86 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 6.67–6.80 (m, 3H, $H_{aromat.}$), 7.06–7.10 (m, 2H, $H_{aromat.}$), 8.22 (br s, 1H, N*H*). MS ($M + H^+$): 453.52. Anal. calcd. for $C_{22}H_{28}N_8O_3$: C, 58.39; H, 6.23; N, 24.76%. Found: C, 58.33; H, 6.30; N, 24.62%.

*6.1.3.2. N-2-[N4-(2-methoxy)-phenyl-piperazin-N1-yl]-ethyl amide of 1,3-dimethyl-6,7-dihydroimidazo[2,1-f]purine-2,4-(1*H,*3*H*)-dione-7-carboxylic acid (**12**).* Yield 51%, M.p. 217–218 °C, ^{1}H NMR ($CDCl_3$) δ: 2.50–2.53 (t, $J = 13.3$ Hz, 2H, CH_2CH_2), 2.64–2.7 (m, 4H, $(CH_2)_2N1_{pip}$), 3.06–3.12 (m, 4H, $N4_{pip}(CH_2)_2$), 3.20–3.30 (m, 6H, N3$CH_3 + CH_2CH_2 +$ C7$H_{imidazole}$), 3.55 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.21 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 4.44–4.5 (m, 1H, N$H_{imidazole}$), 4.86 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 6.67–6.80 (m, 3H, $H_{aromat.}$), 7.06–7.10 (m, 2H, $H_{aromat.}$), 8.22 (br s, 1H, N*H*). MS ($M + H^+$): 483.5. Anal. calcd. for $C_{23}H_{30}N_8O_4$: C, 57.25; H, 6.27; N, 23.22%. Found: C, 57.03; H, 5.98; N, 22.91%.

*6.1.3.3. N-2-[N4-(3-chloro)-phenyl-piperazin-N1-yl]-ethyl amide of 1,3-dimethyl-6,7-dihydroimidazo[2,1-f]purine-2,4-(1*H,*3*H*)-dione-7-carboxylic acid (**13**).* Yield 58%, M.p. 203–205 °C, ^{1}H NMR (DMSO) δ: 2.7 (s, 3H, N1CH_3), 3.06–3.12 (m, 9H, $(CH_2)_2N1_{pip} +$ N3$CH_3 + CH_2CH_2$), 3.31–3.42 (m, 7H, $N4_{pip}(CH_2)_2 + CH_2CH_2 +$ C7$H_{imidazole}$), 4.21 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 4.22–4.32 (m, 1H, N$H_{imidazole}$), 4.86 (dd, $J = 9.9$ Hz, $J = 5.9$ Hz, 1H, C6$H_{imidazole}$), 6.67–6.94 (m, 2H, $H_{aromat.}$), 7.16–7.20 (m, 2H, $H_{aromat.}$), 8.24 (br s, 1H, N*H*). MS ($M + H^+$): 487.5. Anal. calcd. for $C_{22}H_{27}N_8O_3Cl$: C, 54.26; H, 5.59; N, 23.01%. Found: C, 54.16; H, 5.22; N, 22.86%.

*6.1.4. Preparation of hydrochlorides (**2a**–**13a**)*

The bases (**2**–**13**) were dissolved in an excess (2 ml per 0.001 mol) of conc. HCl. Then anhydrous ethanol was added until salt precipitation was observed. The product was recrystallized from anhydrous ethanol.

6.2. In vitro experiments

Radioligand binding 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 the published procedures [37,38,39]. The following radioligands were used: [^{3}H]-8-Hydroxy-2-(di-*n*-propylamino)-tetralin ([^{3}H]-8-OH-DPAT, 106 Ci/mmol, NEN Chemicals), [^{3}H]-Ketanserin (60 Ci/mmol, NEN Chemicals) and [^{3}H]-Spiperone (15 Ci/mmol, NEN). Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. K_i values were determined on the basis of 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. Radioligand binding data were analyzed using interactive curve fitting routines (GraphPAD/Prism, Version 3.0, San Diego, CA, USA).

6.2.1. 5-HT_{1A} receptor-binding experiments

The membrane preparation and the assay procedure were carried out according to the published procedure [37] with slight modifications. [^{3}H]-8-OH-DPAT was used for labeling 5-HT_{1A}

receptors. Briefly, the cerebral cortex tissue was homogenized in 20 vol. of 50 mM tris–HCl buffer (pH 7.7 at 25 °C) using ULTRA TURAX homogeniser, and was then centrifuged at 32000 × *g* for 10 min. The supernatant fraction was discarded, and pellet was resuspended in the same volume of tris–HCl buffer and was then centrifuged. Before the third centrifugation, the samples were incubated at 37 °C for 10 min. The final pellet was resuspended in tris–HCl buffer containing 10 μM Pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. One milliliter of the tissue suspension (9 mg of wet weight), 100 μl of 10 μM serotonin (for unspecific binding), 100 μl of [^{3}H]-8-OH-DPAT and 100 μl of analyzed compound were incubated at 37 °C for 15 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and was when washed 3 times with 5 ml of a cold buffer (50 mM tris–HCl, pH 7.7) using a Brandel cell harvester. The final [^{3}H]-8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-4} M.

6.2.2. 5-HT_2 receptor-binding experiments

The assay was performed according to the method of Laysen et al. [38] with slight modifications. [^{3}H]-ketanserin was used for labeling 5-HT_2 receptors. The cerebral cortex tissue was homogenized in 20 vol. of 50 mM tris–HCl buffer (pH 7.7 at 25 °C) and centrifuged at 32000 × *g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer, preincubated at 37 °C for 10 min and centrifuged for 20 min. The final pellet was resuspended in 50 vol. of the same buffer. One milliliter of the tissue suspension, 100 μl of 1 μM Mianserin (displacer), 100 μl of [^{3}H]-Ketanserin and 100 μl of the analyzed compound were incubated at 37 °C for 20 min, followed by a rapid vacuum filtration through Whatman GF/B glass filters, and were then washed three times with 5 ml of a cold tris–HCl buffer. The final [^{3}H]-Ketanserin concentration was 0.6 nM and the concentrations of analyzed compounds ranged from 10^{-10} to 10^{-4} M.

6.2.3. D-2 receptor-binding experiments

The assay was performed according to the method described before [39]. Rat striatum tissue was thawed in 50 vol. of ice-cold 50 mM potassium phosphate buffer, pH 7.4, homogenized and centrifuged at 20000 × *g* for 20 min. The resulting pellet was resuspended in the same quantity of the buffer, and centrifuged again for 20 min. Assay tubes contained membrane suspension (3 mg of wet weight), [^{3}H]-Spiperone (15 Ci/mmol, NEN) in concentration of 1 nM, (+)Butaclamol (10 μM) or analyzed compound in a final volume of 0.5 ml. Samples were incubated for 30 min in 37 °C. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/B) using Brandel manifold. The filters were then washed 2 times with 5 ml ice-cold buffer. The filters obtained from the above-described procedures were placed in scintillation vials with liquid scintillation cocktail.

6.3. In vivo experiments

The experiments were performed on male Wistar rats (290–310 g) or male Albino Swiss mice (24–28 g). Before the experiment animals were kept at a room temperature of 20 ± 1 °C, and had free access to food (standard laboratory pellets) and tap water. All the investigations were conducted in the light phase, on a natural day–night cycle (from February to May), between 9 a.m. and 2 p.m. All the experimental procedures were approved by the local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (hydrobromide, 8-OH-DPAT, Tocris, Cookson Ltd., UK) was dissolved in saline, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexanecarboxamide (trihydrochloride, WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) and Imipramine (hydrochloride, Polfa-Starogard, Poland) were dissolved in distilled water. Diazepam (Polfa-Poznań, Poland) and the investigated compounds (**2**, **3**, **6**, **7** and **9**) were suspended in 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc), Diazepam, Imipramine and the tested compounds were given intraperitoneally (ip) in a volume of 2 ml/kg (rats) or 10 ml/kg (mice). Each experimental group consisted of six to ten animals, and all the animals were used only once.

6.3.1. Body temperature in mice

Effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In separate experiment the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **2**, **3**, **6**, **7** and **9** 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.

6.3.2. Lower lip retraction (LLR) in rats

LLR was assessed according to the method described by Berendsen et al. [28]. 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, 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 **2**, **3**, **6**, **7**, **9** and WAY 100635 were administered 45 min and 15 min respectively before 8-OH-DPAT and the animals were scored 15, 30 and 45 min after 8-OH-DPAT administration.

6.3.3. Four-plate test in mice

The box was made of an opaque plastic and was rectangular (25 cm × 18 cm × 16 cm) in shape. The floor was covered with four rectangular metal plates (11 cm × 8 cm), separated by a 4 mm gap. The plates were connected to a source of continuous current which enabled a 120 V difference of potential between two adjacent plates for 0.5 s when the experimenter pressed the switch. Individual mice were placed gently onto the plate, and were allowed to explore for 15 s. Afterwards, each time a mouse passed from one plate to another, the experimenter electrified the whole floor which evoked a visible flight reaction of the animal. If the animal continued running, it received no new shocks for the following 3 s. The episodes of punished crossing were counted for 60 s [40].

6.3.4. Forced swimming test in mice

The experiment was carried out according to the method of Porsolt et al. [41]. Briefly, mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 6 cm of water maintained at 23–25 °C, and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

6.3.5. Locomotor activity in mice

The spontaneous locomotor activity of mice was recorded by Opto-M3 multi-channel activity monitor (MultiDevice Software v.

1.30, Columbus Instruments). The mice were placed individually in plastic cages and the number of crossing for each channel (ambulation) was counted twice: during the first 6 min, i.e. at the time equal to the observation period in the forced swimming test, and during 30-min experimental sessions.

6.4. Statistics

The obtained data were analyzed by one-way analysis of variance followed by Dunnett's test (when only one drug was given) or by Newman–Keuls test (when two drugs were administered).

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