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7-Arylpiperazinylalkyl and 7-tetrahydroisoquinolinylalkyl derivatives of 8-alkoxy-purine-2,6-dione and some of their purine-2,6,8-trione analogs as 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_7$ serotonin receptor ligands

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Abstract—On the basis of our earlier studies with the serotonin receptor ligands in the group of 1,3-dimethyl-3,7-dihydropurine-2,6-dione derivatives, a series of new arylpiperazinylalkyl and tetrahydroisoquinolinylalkyl analogs of 8-alkoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (**10**–**25**) and 1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione (**26**–**30**) were synthesized and their 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_7$ receptor affinities were determined. The new compounds **17**, **18**, **20**, and **21** were found to be highly active 5-HT$_{1A}$ receptor ligands (K_i = 11–19 nM) with diversified affinity for 5-HT$_{2A}$ receptors (K_i = 15–253 nM). Compounds **12**, **13**, **15**, and **19** were moderately potent 5-HT$_{2A}$ ligands (K_i = 23–57 nM), whereas **17**, **18**, **24**, and **25** showed distinct affinity for 5-HT$_7$ receptors (K_i = 51–83 nM). Purine-2,6,8-triones showed weak affinities for 5-HT$_{1A}$ and 5-HT$_7$ receptors; among them, **27** and **29** were classified as 5-HT$_{2A}$ receptor ligands.
The selected compounds **17** and **21** were pharmacologically evaluated to determine their functional activities at pre-(hypothermia in mice) and post-(lower lip retraction in rats) synaptic 5-HT$_{1A}$ receptors. Compound **17** showed features of a potential agonist of pre- and post-synaptic 5-HT$_{1A}$ receptors, whereas **21** was classified as a potential, weak partial agonist of postsynaptic sites. Last of all, the most interesting compound **17** tested in behavioral models showed potential anxiolytic and antidepressant activities.

1. Introduction

It is well known that central serotoninergic receptors play an essential role in a number of psychiatric disorders such as anxiety, depression or schizophrenia. Apart from the well-established role of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors, the most recently identified serotonin receptor subtype—5-HT$_7$, is also implicated in the control of many CNS functions, for example, thermoregulation, circadian rhythms or learning and memory, and dysfunctions, like migraine and depression.[1] Among several classes of 5-HT$_7$ receptor ligands some common substructures have been distinguished; a diversified amide or sulfonamide fragment connected by different length alkylen spacer with terminal tertiary amine fragment, often aryl-piperazinyl, and piperidyl or tetrahydroisoquinolinyl moieties (Fig. 1).[2] It is worth noting that these structural features remain similar to the well-known pharmacophoric fragments for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor ligands.

For several years we have been interested in developing agents generally classified as long-chain arylpiperazines (LCAPs) containing a different amide/imide terminal fragment, which were mainly evaluated toward 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors.[3,4] Some of our previous structure–affinity and structure–intrinsic activity studies were concerned with chemical modifications in a group of compounds containing tricyclic theophyllines with an anellated heterocyclic ring of lactam or non-lactam structure, that is, pyrimido[2,1-*f*]purine, diazepino[2,1-*f*]purine.[5–7]

Keywords: Purine-2,6-diones; Purine-2,6,8-triones; Long-chain arylpiperazines; 5-HT$_{1A}$; 5-HT$_{2A}$; 5-HT$_7$ receptor ligands.
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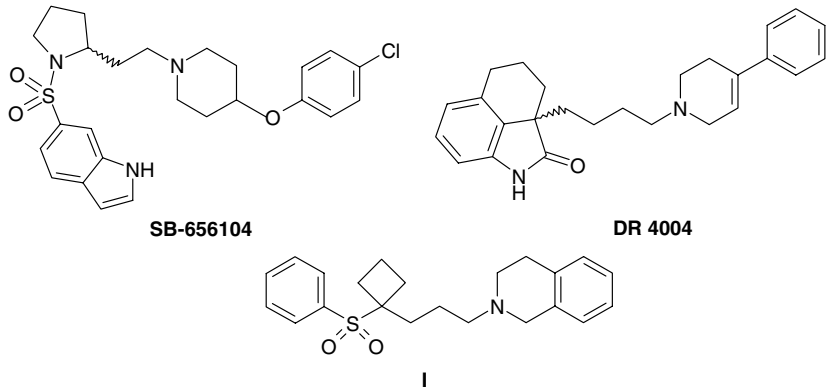


Figure 1.

Recently we have developed two series of arylpiperazine derivatives, connected by a different length alkyl spacer to 7-alkyl, 7-arylalkyl or 7-alkylcarboxylic acid ester substituted theophylline (1,3-dimethyl-3,7-dihydropurine-2,6-dione) moiety.[8–10] Among them, the most interesting 7-arylalkyl derivatives displayed high-to-moderate affinity for 5-HT_{1A} receptors and moderate-to-low affinity for 5-HT_{2A} sites (Fig. 2). Those compounds, examined in functional in vivo models, behaved like postsynaptic 5-HT_{1A} receptor antagonists.[9]

To continue our research with a class of purine-2,6-dione analogs, we designed and synthesized a novel series of arylpiperazines with molecular diversity in the complex terminal amide/imide fragment. Comparing to the previous work,[9] structural modifications consisted in shifting the arylpiperazinylalkyl substituent from the 8- to the 7-position of 1,3-dimethyl-3,7-dihydropurine-2,6-dione and introducing simultaneously an alkoxy moiety to the 8-position. Further, by replacing an alkoxy moiety by a 8-oxo one, we obtained 1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione core as a new cyclic amide group. The influence of the structural modifications on the activity for serotonin receptors was investigated in classic arylpiperazine (R = H, *o*-OCH_3, *m*-Cl) or 1,2,3,4-tetrahydroisoquinoline derivatives with three or four methylene group linkers. All the new compounds were tested for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptor affinity; in the case of selected compounds displaying the highest receptor affinity, a 5-HT_{1A} functional profile was determined. Finally, the most interesting compound was pharmacologically evaluated in preclinical animal models of anxiety and depression.

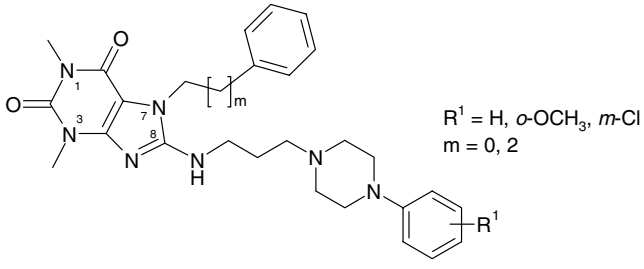


Figure 2.

2. Chemistry

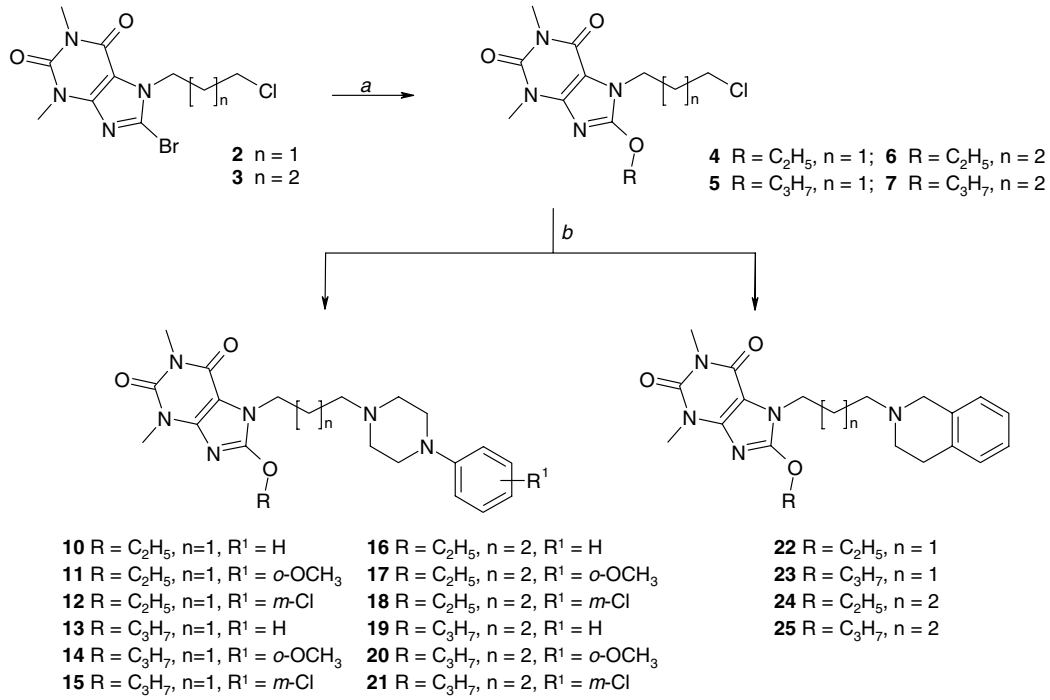
The structures of the investigated compounds and their syntheses are presented in Schemes 1 and 2.

The starting 7-(3-chloropropyl)-8-bromo-1,3-dimethyl-3,7-dihydropurine-2,6-dione (**2**) and the new higher homolog (**3**) were prepared in a reaction of 8-bromo-1,3-dimethyl-3,7-dihydropurine-2,6-dione with the appropriate 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane according to the previously described method.[11] The 7-ω-chloroalkyl-8-alkoxy-1,3-dimethyl-3,7-dihydropurine-2,6-diones **4**–**9** were obtained in a reaction of **2** or **3** with the appropriate sodium alcoholate in a corresponding alcohol medium. The designed purine-2,6-dione derivatives were synthesized by nucleophilic substitution of **4**–**7** with the appropriate phenylpiperazines or 1,2,3,4-tetrahydroisoquinoline (THIQ) in the presence of K_2CO_3, yielding compounds **10**–**21** and **22**–**25**, respectively (Scheme 1). Under the same conditions 8-methoxy derivatives **8** and **9** did not yield desired purine-2,6-diones analogs; instead, purine-2,6,8-trione derivatives **26**–**30** were isolated (Scheme 2). Regarding 8-alkoxy derivatives **4**–**9** as iminoethers or uric acid esters, the phenomenon observed may be attributed to the lower stability of 8-methoxy derivatives than 8-ethoxy and 8-propoxy ones under reaction condition described. Final compounds **10**–**16** were isolated from the reaction mixtures as free bases and were then converted into hydrochloride salts in a reaction with conc. HCl in EtOH, while compounds **17**–**30** were directly isolated from the reaction mixtures as hydrochloride salts.

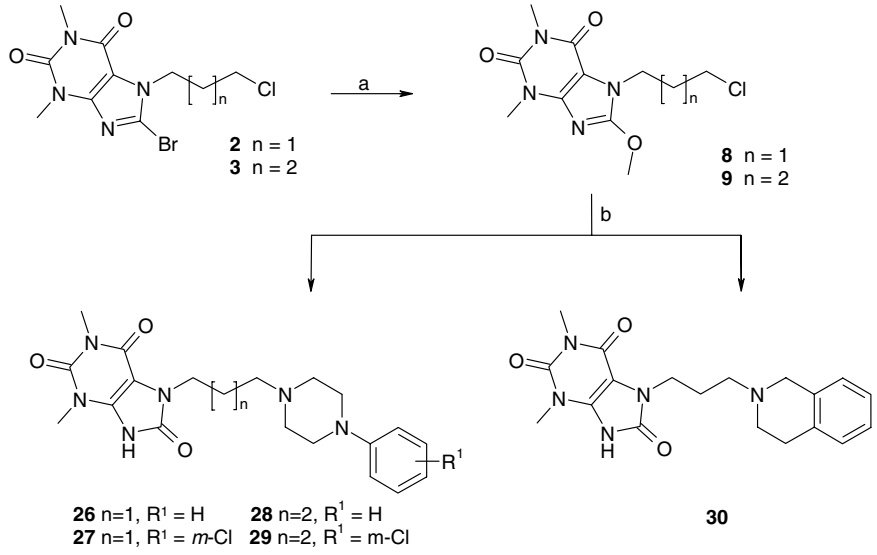
The structures of the newly synthesized compounds (**3**–**30**) were confirmed by ^{1}H NMR spectra and an elemental analysis. For compounds **18** and **29**, also HMBC spectral data were analyzed. The investigated compounds **10**–**30** were pharmacologically tested as hydrochloride salts.

3. Pharmacology

The compounds were tested in competition binding experiments for rats serotonin 5-HT_{1A}, 5-HT_{2A}, and



Scheme 1. Reagents and conditions: (a) RONa, ROH, reflux; (b) 1-arylpiperazine or 1,2,3,4-tetrahydroisoquinoline, *n*-PrOH, K_2CO_3, reflux.



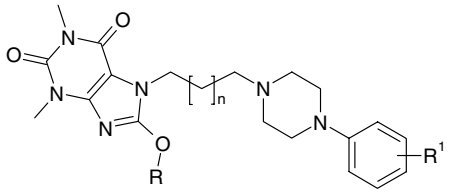
Scheme 2. Reagents and conditions: (a) CH_3ONa, MeOH, reflux; (b) 1-arylpiperazine or 1,2,3,4-tetrahydroisoquinoline, *n*-PrOH, K_2CO_3, reflux.

5-HT_7 receptors. The affinity data are shown in Tables 1 and 2.

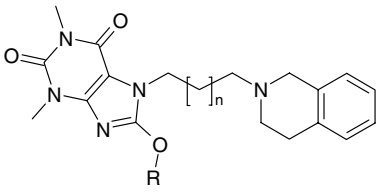
The functional activity of the selected compounds (**17**, **21**) at 5-HT_{1A} receptors was tested in several commonly used in vivo models. It has been previously demonstrated that the hypothermia induced by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice depended primarily on activation of presynaptic 5-HT_{1A} receptors[15] and was abolished by selective 5-HT_{1A} receptor antagonists, for example, by *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(pyridin-2-yl)-cyclohexanecarboxamide (WAY 100635).[16] Thus the ability of the tested compounds to induce hypothermia (blocked by WAY 100635) in mice was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity.

To determine a postsynaptic 5-HT_{1A} receptor agonistic effect of the tested compounds, their ability to induce lower lip retraction (LLR) in rats was tested. It has been commonly accepted that the 8-OH-DPAT-induced LLR in rats is connected with the stimulation of postsynaptic 5-HT_{1A} receptors[17,18]; moreover, it has been shown that this symptom is sensitive to 5-HT_{1A} receptor antagonists.[16] Hence the ability of the tested compounds to

Table 1. Structure and binding affinity data on serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors of the investigated purine-2,6-dione derivatives



10 - 21

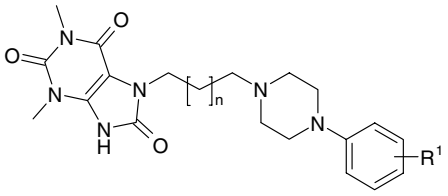


22 - 25

Compound	R	*n*	R^1	K_i (nM) ± SEM		
				5-HT_{1A}	5-HT_{2A}	5-HT_7
10	C_2H_5	1	H	1300 ± 100	176 ± 26	477 ± 48
11	C_2H_5	1	*o*-OCH_3	83 ± 6	980 ± 60	410 ± 20
12	C_2H_5	1	*m*-Cl	190 ± 28	23 ± 2	130 ± 10
13	C_3H_7	1	H	800 ± 40	51 ± 5	980 ± 60
14	C_3H_7	1	*o*-OCH_3	314 ± 17	540 ± 40	328 ± 15
15	C_3H_7	1	*m*-Cl	288 ± 18	25 ± 2	267 ± 21
16	C_2H_5	2	H	67 ± 5	131 ± 15	197 ± 11
17	C_2H_5	2	*o*-OCH_3	11 ± 1	253 ± 14	54 ± 2
18	C_2H_5	2	*m*-Cl	12 ± 2	15 ± 1	51 ± 4
19	C_3H_7	2	H	130 ± 12	57 ± 3	680 ± 40
20	C_3H_7	2	*o*-OCH_3	19 ± 2	150 ± 12	160 ± 22
21	C_3H_7	2	*m*-Cl	15 ± 1	28 ± 2	125 ± 9
22	C_2H_5	1	—	1070 ± 80	590 ± 18	5750 ± 130
23	C_3H_7	1	—	1010 ± 76	2610 ± 80	4400 ± 120
24	C_2H_5	2	—	430 ± 15	1930 ± 40	83 ± 5
25	C_3H_7	2	—	332 ± 17	920 ± 70	57 ± 5
	Buspirone[a]			14	851	375
	Ketanserin[a]			1993	1.4	265
	SB-656104[b]			562	63	2
	Compound **I**[c]			35	65	8

[a] Data taken from Ref. 12.
[b] Data taken from Ref. 13.
[c] Data taken from Ref. 14.

Table 2. Structure and binding affinity data on serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors of the investigated purine-2,6,8-trione derivatives



Compound	*n*	R^1	K_i (nM) ± SEM		
			5-HT_{1A}	5-HT_{2A}	5-HT_7
26	1	H	4210 ± 110	230 ± 30	13940 ± 200
27	1	*m*-Cl	1110 ± 50	58 ± 3	1880 ± 60
28	2	H	1500 ± 50	560 ± 20	1820 ± 30
29	2	*m*-Cl	260 ± 20	60 ± 4	3670 ± 6
30	1	a[a]	6320 ± 50	7960 ± 118	16480 ± 800

[a] Tetrahydroisoquinolin-2-yl instead of 4-phenylpiperazin-1-yl.

inhibit the LLR induced by 8-OH-DPAT was regarded as postsynaptic 5-HT_{1A} receptor antagonistic activity.

The potential anxiolytic and antidepressant activity of compound **17** was evaluated by the four-plate test in mice[19] and by the forced swimming test in mice,[20] respectively. Moreover, its effect on the spontaneous locomotor activity of mice was also tested.

4. Results and discussion

4.1. In vitro evaluation

The newly synthesized derivatives of purine-2,6-diones and purine-2,6,8-triones showed a diversified level of affinity for 5-HT_{1A} receptors, ranging from 11 to 6320 nM, and displayed high-to-low affinity for

5-HT_{2A} receptors (15–7960 nM). The receptor binding properties of the new compounds are presented in Tables 1 and 2.

Generally, in comparison with the previously reported derivatives (Fig. 2),[9,10] the shifting of the arylpiperazinylalkyl moiety from the 8- to the 7-position of 1,3-dimethyl-3,7-dihydro-purine-2,6-dione did not significantly affect the binding to 5-HT_{1A} receptors; at the same time, it increased the affinity for 5-HT_{2A} sites.

A strong positive influence of the alkoxy moiety in the 8-position of purine-2,6-diones in comparison with the 8-oxo moiety in purine-2,6,8-trione derivatives on the affinity for 5-HT_{1A} receptors should be pointed out. The 8-ethoxy and 8-propoxy derivatives **10**–**25** were 3- to 22-fold more active for 5-HT_{1A} sites than their 8-oxo analogs (**26**–**30**); on the other hand, the introduction of the 8-oxo moiety only slightly decreased the affinity of **26**–**30** for 5-HT_{2A} receptors. The above observations give support to our concept that the change in the chemical character of the cyclic amide fragment from 8-alkylamino-purine-2,6-diones to 8-alkoxy-purine-2,6-diones (regarded as uric acid esters) or 8-oxo-purine-2,6-diones (regarded as uric acids) may influence receptor binding. Moreover, the higher affinity of alkoxy derivatives for the 5-HT_{1A} and 5-HT_{2A} sites confirms the role of hydrophobic properties as additional factors influencing receptor affinity. It is worth noting that the influence of the kind of an alkoxy moiety on the affinity of the 5-HT receptors studied is not decisive.

The elongation of the linker length between purine-2,6-dione or purine-2,6,8-trione cores and arylpiperazine fragment from three- to four-carbon units generally increased 5-HT_{1A} affinity. At the same time, it only slightly increased 5-HT_{2A} affinity (by up to threefold, e.g., **14** vs **20**), or an opposite effect was observed for **22**, **26**, and **27**. Hence, in the series tested, the four-methylene group alkyl spacer was preferable to obtain potent 5-HT_{1A} ligands.

The results presented in Tables 1 and 2 have conclusively proven that the introduction of *o*-OCH_3 into the phenylpiperazine moiety increases the affinity for 5-HT_{1A} receptors, while *m*-Cl substituted phenylpiperazines prefer binding to 5-HT_{2A} sites. Within a set of respective structural analogs, unsubstituted phenylpiperazines always displayed lower affinity for 5-HT_{1A} receptors (e.g., **10**–**12** and **19**–**21**), while *o*-OCH_3 derivatives were less active for 5-HT_{2A} ones (e.g., **13**–**15** and **16**–**18**). Compounds **17**, containing an *o*-OCH_3 substituent, and **27** containing *m*-Cl turned out to be the most selective for 5-HT_{1A} and 5-HT_{2A} sites, respectively. It was also found that compounds **18** and **21** could be regarded as potent dual 5-HT_{1A}/5-HT_{2A} receptor ligands.

It has recently been reported that differently substituted arylpiperazine and tetrahydroisoquinoline moieties are often incorporated into the structure of 5-HT_7 receptor ligands.[14,21] Hence, at the following stage, all the purine-2,6-diones and purine-2,6,8-triones were evaluated for their in vitro affinity for 5-HT_7 receptors. 8-Alkoxy derivatives showed a diversified level of affinity ranging from 51 nM for **18** to 5750 nM for **22**, while purine-2,6,8-triones (**26**–**30**) were almost inactive at those sites (K_i = 1815–16,480 nM). It was also found that the introduction of a four-member alkyl spacer turned out to be strongly beneficial for 5-HT_7 binding. That modification resulted in an almost 8-fold increase in 5-HT_7 affinity in the case of **11** (yielding **17**), and even a 70 times higher increase in the case of **22**. Interestingly, **17** and **18** containing *o*-OCH_3 and *m*-Cl substituents appeared to have almost the same 5-HT_7 receptor affinity. It was also found that the replacement of an arylpiperazinyl moiety by tetrahydroisoquinolinyl in the group of purine-2,6-diones (**17**, **18** vs **24**, and **20**, **21** vs **25**) resulted in a 5–6-fold increase in 5-HT_7 receptor selectivity over 5-HT_{1A} sites.

4.2. In vivo assays

The selected compounds **17** and **21** (with the highest affinity for 5-HT_{1A} receptors) were tested in vivo in mice and rats to establish their functional activity at pre- and post-synaptic 5-HT_{1A} receptors. As shown in Table 3, compounds **17** (2.5–5 mg/kg) and **21** (5–10 mg/kg)—like 8-OH-DPAT, a 5-HT_{1A} receptor agonist—produced a dose-dependent decrease in mice body temperature.

The hypothermia produced by the tested compounds was observed up to 120 min after their administration, the maximum effect being seen after 30 min. The hypothermic effect evoked by **17** (2.5 mg/kg) was reduced by WAY 100635 (0.1 mg/kg), a 5-HT_{1A} receptor antagonist (Table 4), hence this compound may be classified as a potential agonist of presynaptic 5-HT_{1A} receptors. At the same time, WAY 100635 did not change the hypothermic effect induced by **21** (10 mg/kg), so it seems that the presynaptic 5-HT_{1A} receptor activity of this compound is negligible in this experimental paradigm.

In the experiment used to evaluate postsynaptic 5-HT_{1A} receptor activity, compounds **17** and **21** given alone in the higher dose used (20 mg/kg) induced LLR in rats, but the effect was considerably weaker than that observed after 8-OH-DPAT (1 mg/kg) administration (Table 5).

The LLR induced by 8-OH-DPAT was partly reduced by **21** (10–20 mg/kg), whereas compound **17** (10–20 mg/kg) practically did not change the effect of 8-OH-DPAT in that test (Table 5). Therefore the results obtained in the LLR model in rats indicate that compound **17** showed properties of a potential and weak agonist of postsynaptic 5-HT_{1A} receptors, whereas **21** behaved like a weak partial agonist of these sites.

The results obtained in vivo indicate that of the tested 5-HT_{1A} receptor ligands, compound **17** shows features of a potential agonist of pre- and post-synaptic 5-HT_{1A} receptors, whereas **21** can be classified as a potential partial agonist of postsynaptic sites. Therefore it seems that in comparison with the previously described series of 7-phenylalkyl-purine-2,6-diones (Fig. 2),[9] classified as 5-HT_{1A} postsynaptic antagonists, the position of an arylpiperazinylalkyl fragment in the purine-2,6-dione

Table 3. The effect of the tested compounds on the body temperature of mice

Treatment	Dose (mg/kg)	Δ*t* ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	—	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
17	2.5	−1.5 ± 0.2[b]	−1.0 ± 0.2[b]	−0.9 ± 0.2[b]	−0.7 ± 0.1[a]
	5	−2.5 ± 0.3[b]	−1.9 ± 0.3[b]	−1.5 ± 0.2[b]	−1.3 ± 0.1[b]
Vehicle	—	−0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1
21	5	−0.7 ± 0.1[a]	−0.6 ± 0.1[a]	−0.6 ± 0.1[a]	−0.2 ± 0.2
	10	−1.7 ± 0.2[b]	−1.1 ± 0.1[b]	−1.1 ± 0.1[b]	−1.1 ± 0.1[b]

The tested compounds were administered 30 min before the test. The absolute mean initial body temperatures were within a range of 36.0 ± 0.6 °C; $n = 8$ mice per group.
[a] $p < 0.05$.
[b] $p < 0.01$ versus respective vehicle group.

Table 4. The effect of WAY 100635 on the hypothermia induced by the tested compounds in mice

Treatment and dose (mg/kg)	Δ*t* ± SEM (°C)	
	30 min	60 min
Vehicle + vehicle	−0.1 ± 0.1	0.0 ± 0.1
Vehicle + **17** (2.5)	−1.5 ± 0.2[a]	−1.0 ± 0.2[a]
WAY 100635 (0.1) + **17** (2.5)	−0.5 ± 0.1[c]	−0.3 ± 0.1[b]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **21** (10)	−1.7 ± 0.2[a]	−1.1 ± 0.1[a]
WAY 100635 (0.1) + **21** (10)	−1.4 ± 0.1[a]	−0.9 ± 0.2[a]
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT (5)	−1.0 ± 0.1[a]	−0.7 ± 0.1[a]
WAY 100635 (0.1) + 8-OH-DPAT (5)	−0.1 ± 0.1[c]	0.1 ± 0.1[c]

WAY 100635 was administered 15 min before the tested compounds. The absolute mean initial body temperatures were within a range of 36.0 ± 0.5 °C; $n = 8$ mice per group.
[a] $p < 0.01$ versus respective vehicle + vehicle group.
[b] $p < 0.05$.
[c] $p < 0.01$ versus respective vehicle + tested compound group.

Table 5. Induction of lower lip retraction (LLR) by the tested compounds (A) and their effect on the 8-OH-DPAT-induced LLR (B) in rats

Treatment	Dose (mg/kg)	Mean ± SEM LLR score	
		A	B
Vehicle	—	0.0 ± 0.0	2.8 ± 0.1
17	10	0.3 ± 0.2	2.1 ± 0.3
	20	1.3 ± 0.2[a]	2.1 ± 0.2
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
21	10	0.4 ± 0.3	1.7 ± 0.2[a]
	20	0.8 ± 0.2[a]	1.5 ± 0.2[a]
WAY 100635	0.1	0.1 ± 0.1	0.3 ± 0.2[a]

The tested compounds were administered 15 min before the test (A) or 45 min before 8-OH-DPAT (1 mg/kg) (B); $n = 6$ rats per group.
[a] $p < 0.01$ versus vehicle (A) or versus vehicle + 8-OH-DPAT (B).

moiety is responsible for the postsynaptic 5-HT_{1A} intrinsic activity of the compounds.

Regarding the many classes of 5-HT_{1A} receptors described in the central nervous system, much attention has been devoted to the role of the 5-HT_{1A} receptor subtype in such psychiatric disorders as anxiety and depression. In fact, several authors have shown that 5-HT_{1A} receptor ligands exert anxiolytic- and/or antidepressant-like effects.[22–24] Taking into account the functional profile of the investigated derivatives, we selected **17** (a 5-HT_{1A} receptor agonist) as a potential anxiolytic and/or antidepressant compound for further in vivo preclinical studies. Our results indicated that compound **17** at a dose of 5 mg/kg (but not 2.5 or 10 mg/kg) exerted anxiolytic-like activity by increasing (by 70%) the number of punished crossings in a mouse four-plate test (Table 6A). Interestingly, the effect of **17** was weaker in terms of its potency and active doses than was the effect produced by diazepam (used as a reference anxiolytic drug). In fact, diazepam at doses of 1.25, 2.5, and 5 mg/kg significantly increased (by 57, 94, and 91%, respectively) the number of punished crossings.

Furthermore, compound **17** administered at two medium doses (5 and 10 mg/kg) reduced (by 24% and 27%, respectively) the immobility time of mice in the forced swimming test (Table 6B). The typical antidepressant imipramine (used as a reference drug) at a dose of 10 mg/kg was ineffective in that test, but higher doses (20 and 30 mg/kg) significantly shortened (by 35% and 54%, respectively) the immobility time of mice. At the same time, **17** at doses active in the four-plate test and/or the forced swimming test (5–10 mg/kg) potently and dose-dependently attenuated the spontaneous locomotor activity of mice during the initial 6-min (i.e., at the time identical to the observation period in the forced swimming test) and during the following 30-min experimental session; an almost complete reduction of mouse activity was observed after administration of the highest dose (20 mg/kg) of that compound (Table 7). In comparison, imipramine at doses producing a distinct antidepressant-like effect in the forced swimming test (20 and 30 mg/kg) slightly reduced the locomotor activity of mice during a 30-min experimental session. Similarly, diazepam at doses up to 2.5 mg/kg practically did not affect the locomotor activity of mice, but slightly decreased it at a dose of 5 mg/kg (Table 7).

Thus the sedation induced by **17** may partly explain the lack of activity of the higher doses of this compound in the four-plate test and the forced swimming test in mice. The obtained results indicate that further studies with analogs of **17** aimed at modulating anxiolytic/antidepressant properties are necessary.

Table 6. The effect of **17** in the four-plate test (A) and the forced swimming test (B) in mice

Treatment	Dose (mg/kg)	Mean ± SEM
A		Number of punished crossings
Vehicle	—	3.4 ± 0.4
17	2.5	4.1 ± 0.3
	5	5.8 ± 0.4[b]
	10	3.8 ± 0.4
		$F(3,36) = 7.371$
		$P < 0.001$
Vehicle	—	3.5 ± 0.4
Diazepam	1.25	5.5 ± 0.5[a]
	2.5	6.8 ± 0.6[b]
	5	6.7 ± 0.6[b]
		$F(3,36) = 9.514$
		$P < 0.001$
B		Immobility time (s)
Vehicle	—	168.1 ± 4.4
17	2.5	154.4 ± 5.6
	5	126.9 ± 9.7[a]
	10	122.6 ± 9.7[a]
	20	178.2 ± 16.5
		$F(4,40) = 5.598$
		$P < 0.01$
Vehicle	—	167.1 ± 6.7
Imipramine	10	149.1 ± 10.7
	20	107.8 ± 12.4[b]
	30	76.4 ± 7.1[b]
		$F(3,36) = 18.200$
		$P < 0.001$

Compound **17** and imipramine were administered 30 min, while diazepam 60 min before the test; $n = 9$–10 mice per group.
[a] $p < 0.05$.
[b] $p < 0.01$ versus respective vehicle group.

Table 7. The effect of **17** on the locomotor activity of mice

Treatment	Dose (mg/kg)	Locomotor activity: number of crossings during	
		6 min	30 min
Vehicle		289.0 ± 26.8	855.5 ± 91.2
17	5	125.5 ± 9.7[b]	325.0 ± 31.5[b]
	10	56.2 ± 9.3[b]	193.4 ± 28.3[b]
	20	11.1 ± 2.1[b]	19.5 ± 3.3[b]
		$F(3,36) = 70.552$	$F(3,36) = 51.357$
		$P < 0.001$	$P < 0.001$
Vehicle			917.9 ± 59.1
Diazepam	1.25		686.3 ± 72.1
	2.5	NT	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	$P < 0.01$

Compound **17** and imipramine ware administered 30 min, while diazepam 60 min before the test. $n = 10$ mice per group. NT—not tested.
[a] $p < 0.05$.
[b] $p < 0.01$ versus respective vehicle group.

In conclusion, we synthesized a series of arylpiperazines containing novel 8-alkoxy-purine-2,6-dione and purine-2,6,8-trione fragments. The structural modifications allowed us to select several 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptor ligands displaying agonistic properties toward postsynaptic 5-HT_{1A} receptor sites. Among them, compound **17** revealed promising activity in a preclinical model of anxiety and depression. In addition, the study showed that an 8-alkoxy-purine-2,6-dione fragment may be an interesting pharmacophoric fragment of novel serotonin receptor ligands. Further chemical and pharmacological investigations with this group of compounds are in progress and will be reported in due course.

5. Experimental

5.1. Chemistry

Melting points (mp) were determined with the Boetius apparatus and are uncorrected. ^{1}H NMR and HMBC spectra were taken with a Varian Mercury-VX (300 MHz) spectrophotometer in $CDCl_3$ (compounds **3**–**16**) or DMSO (compounds **17**–**30**) solutions with TMS as an internal standard. The spectral data of new compounds refer to their free bases (**3**–**16**), or to their hydrochloride salts (**17**–**30**). Chemical shifts were expressed in δ (ppm) and the coupling constants J in Hertz (Hz). All the compounds were routinely checked by TLC using Merck Kieselgel 60 F_{254} sheets with the following eluents: (A: benzene/acetone = 7:3, B: benzene/acetone/methanol = 1:1:1). Spots were detected by UV absorption and visualization with Dragendorff's reagent. Elemental analyses were within ±0.4% of the theoretical values.

The starting 8-bromo-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (**1**) was synthesized by a procedure published elsewhere.[25] 7-(3-Chloro-propyl)-8-bromo-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (**2**) and new 7-(4-chlorobutyl)-8-bromo-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (**3**) were obtained from **1** according to the previously described method.[9]

5.1.1. 7-(4-Chlorobutyl)-8-bromo-1,3-dimethyl-3,7-dihydropurine-2,6-dione (3). Yield 91%, mp 95–97 °C, $R_f = 0.42$ (A), ^{1}H NMR δ 1.76–2.10 (m, 4H, –CH_2–($C\underline{H}_2$)$_2$–CH_2–), 3.40 (s, 3H, N_1–$C\underline{H}_3$), 3.56 (s, 3H, N_3–$C\underline{H}_3$), 3.57 (t, $^3J = 6.4$ Hz, 2H, –$C\underline{H}_2$–Cl), 4.38 (t, $^3J = 7.2$ Hz, 2H, N_7–$C\underline{H}_2$–). Anal. ($C_{11}H_{14}BrClN_4O_2$) C, H, N.

5.1.2. A general procedure for preparing 8-alkoxy-purine-2,6-dione derivatives (4–9). Equimolar amounts (20 mmol) of the appropriate compounds **2** or **3** and the appropriate sodium alcoholate (sodium methanolate, sodium ethanolate and sodium propan-1-olate) were refluxed in corresponding alcohol (100 mL) for 6 h. The mixture was filtered off and the solvent was evaporated under reduced pressure. The oily residue was washed with water until solidification; then it was filtered off and purified by crystallization from methanol.

5.1.3. 7-(3-Chloropropyl)-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (4). From **2** in 83% yield, mp 111–112 °C, $R_f = 0.58$ (A), ^{1}H NMR δ 1.49 (t, $^3J = 7.2$ Hz, 3H, –O–CH$_2$–CH$_3$), 2.26–2.35 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 3.41 (s, 3H, N$_1$–CH$_3$), 3.55 (s, 3H, N$_3$–CH$_3$), 3.56 (t, 2H, –CH$_2$–Cl), 4.28 (t, 2H, N$_7$–CH$_2$–), 4.58 (q, $^3J = 7.2$, 2H, –O–CH$_2$–CH$_3$). Anal. ($C_{12}H_{17}ClN_4O_3$) C, H, N.

5.1.4. 7-(3-Chloropropyl)-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5). From **2** in 81% yield, mp 92–95 °C, $R_f = 0.58$ (A), ^{1}H NMR δ 1.04 (t, $^3J = 7.4$ Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.78–1.90 (m, 2H, –O–CH$_2$–CH$_2$–CH$_3$), 2.23–2.31 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 3.37 (s, 3H, N$_1$–CH$_3$), 3.50 (t, 2H, –CH$_2$–Cl), 3.51 (s, 3H, N$_3$–CH$_3$), 4.24 (t, $^3J = 6.7$ Hz, 2H, N$_7$–CH$_2$–), 4.43 (t, $^3J = 6.7$ Hz, 2H, –O–CH$_2$–C$_2$H$_5$). Anal. ($C_{13}H_{19}ClN_4O_3$) C, H, N.

5.1.5. 7-(4-Chlorobutyl)-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (6). From **3** in 98% yield: mp 69–72 °C, $R_f = 0.59$ (A), ^{1}H NMR δ 1.49 (t, $^3J = 7.2$ Hz, 3H, –O–CH$_2$–CH$_3$), 1.79–1.93 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 1.95–2.00 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 3.42 (s, 3H, N$_1$–CH$_3$), 3.55 (s, 3H, N$_1$–CH$_3$), 3.59 (t, $^3J = 6.6$ Hz, 2H, –CH$_2$–Cl), 4.16 (t, 2H, N$_7$–CH$_2$–), 4.58 (q, $^3J = 7.2$ Hz, 2H, –O–CH$_2$–CH$_3$). Anal. ($C_{13}H_{19}ClN_4O_3$) C, H, N.

5.1.6. 7-(4-Chlorobutyl)-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (7). From **3** in 85% yield, mp 59–63 °C, $R_f = 0.81$ (A), $R_f = 0.87$ (B), ^{1}H NMR δ 1.04 (t, $^3J = 4.3$ Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.75–1.99 (m, 6H, –CH$_2$–(CH$_2$)$_2$–CH$_2$–, –O–CH$_2$–CH$_2$–CH$_3$), 3.38 (s, 3H,N$_1$–CH$_3$), 3.51–3.57 (m, 2H, –CH$_2$–Cl), 3.53 (s, 3H, N$_3$–CH$_3$), 4.14 (t, $^3J = 7.0$ Hz, 2H, N$_7$–CH$_2$–), 4.45 (t, $^3J = 6.5$ Hz, 2H, –O–CH$_2$–C$_2$H$_5$). Anal. ($C_{14}H_{21}ClN_4O_3$) C, H, N.

5.1.7. 7-(3-Chloropropyl)-8-methoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (8). From **2** in 94% yield, mp 165–167 °C, $R_f = 0.85$ (A), ^{1}H NMR δ 2.27–2.36 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 3.42 (s, 3H, N$_1$–CH$_3$), 3.55 (t, 2H, –CH$_2$–Cl), 3.57 (s, 3H, N$_3$–CH$_3$), 4.18 (s, 3H, –O–CH$_3$), 4.28 (t, 2H, N$_7$–CH$_2$–). Anal. ($C_{11}H_{15}ClN_4O_3$) C, H, N.

5.1.8. 7-(4-Chlorobutyl)-8-methoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (9). From **3** in 97% yield: mp 139–140 °C, $R_f = 0.54$ (A), ^{1}H NMR δ 1.64–1.82 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 1.88–1.96 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 3.38 (s, 3H, N$_1$–CH$_3$), 3.41 (t, 2H, –CH$_2$–Cl), 3.52 (s, 3H, N$_3$–CH$_3$), 4.13 (t, 2H, N$_7$–CH$_2$–), 4.13 (s, 3H, –O–CH$_3$). Anal. ($C_{12}H_{17}ClN_4O_3$) C, H, N.

5.1.9. A general procedure for preparing 7-aminoalkyl-8-alkoxy-purine-2,6-dione derivatives (10–25). The appropriate 8-alkoxy-purine-2,6-dione derivatives **4**–**9** (55 mmol), respective phenylpiperazine derivatives (1-phenylpiperazine, 1-(2-methoxyphenyl)-piperazine, 1-(3-chlorophenyl)-piperazine) or 1,2,3,4-tetrahydroisoquinoline (THIQ), and 100 mmol anhydrous K_2CO_3 were refluxed in propan-1-ol (10 mL) for 30 h. The mixture was filtered off and the solvent was evaporated under reduced pressure. In case of compounds **10**–**16** free bases were isolated from the residue and purified by crystallization from propan-2-ol. For pharmacological assays free bases were converted into hydrochloride salts by treatment with an excess of conc. HCl in an ethanol solution. Compounds **17**–**25** were isolated directly from the reaction mixture as hydrochloride salts using the same method as that presented above. Hydrochloride salts of compounds **10**–**25** were purified by crystallization from anhydrous ethanol.

5.1.10. 7-[3-(4-Phenylpiperazin-1-yl)-propyl]-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (10). From **4** in 43% yield, mp 141–145 °C, $R_f = 0.19$ (A), ^{1}H NMR δ 1.48 (t, $^3J = 3.3$ Hz, 3H, –O–CH$_2$–CH$_3$), 1.99–2.09 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 2.49 (t, $^3J = 6.9$ Hz, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 2.60–2.63 (m, 4H, N(CH$_2$)$_2$), 3.19–3.92 (m, 4H, (CH$_2$)$_2$N–Ph), 3.43 (s, 3H, N$_1$–CH$_3$), 3.55 (s,3H, N$_3$–CH$_3$), 4.20 (t, $^3J = 7.2$ Hz, 2H, N$_7$–CH$_2$–), 4.56 (q, $^3J = 7.2$ Hz, 2H, –O–CH$_2$–CH$_3$), 6.86–6.97 (m, 3H, Ph), 7.26-7.32 (m, 2H, Ph). **10**·HCl·**0.5H$_2$O**: mp 275–280 °C. Anal. ($C_{22}H_{30}N_6O_3$·HCl·0.5H$_2$O) C, H, N.

5.1.11. 7-{3-[4-(2-Methoxyphenyl)-piperazin-1-yl]-propyl}-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (11). From **4** in 52% yield, mp 129–131 °C, $R_f = 0.21$ (A), ^{1}H NMR δ 1.44 (t, $^3J = 6.9$ Hz, 3H, –O–CH$_2$–CH$_3$), 2.04–2.17 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 2.51 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 2.68 (m, 4H, N(CH$_2$)$_2$), 3.09–3.15 (m, 4H, (CH$_2$)$_2$N–Ph), 3.38 (s, 3H, N$_1$–CH$_3$), 3.51 (s, 3H, N$_3$–CH$_3$), 3.85 (s, 3H, Ph–O–CH$_3$), 4.16 (t, $^3J = 6.9$ Hz, 2H, N$_7$–CH$_2$–), 4.53 (q, $^3J = 6.9$, 2H, –O–CH$_2$–CH$_3$), 6.84–7.03 (m, 4H, Ph). **11**·**HCl**: 174–177 °C. Anal. ($C_{23}H_{32}N_6O_4$·HCl) C, H, N.

5.1.12. 7-{3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propyl}-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (12). From **4** in 58% yield, mp 138–140 °C, $R_f = 0.35$ (A), ^{1}H NMR δ 1.46 (t, $^3J = 7.1$ Hz, 3H, –O–CH$_2$–CH$_3$), 1.52–1.69 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 2.18–2.36 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 2.55–3.07 (m, 4H, N(CH$_2$)$_2$), 3.28–3.43 (m, 4H, (CH$_2$)$_2$N–Ph), 3.38 (s, 3H, N$_1$–CH$_3$), 3.52 (s, 3H, N$_3$–CH$_3$), 4.19 (t, $^3J = 6.9$ Hz, 2H, N$_7$–CH$_2$–), 4.55 (q, $^3J = 7.0$ Hz, 2H, –O–CH$_2$–CH$_3$), 6.75–6.78 (m, 1H, Ph), 6.83–6.89 (m, 2H, Ph), 7.18 (t, $^3J = 8.3$ Hz, 1H, 5Ph). **12**·**HCl**: mp 240–246 °C. Anal. ($C_{22}H_{29}ClN_6O_3$·HCl) C, H, N.

5.1.13. 7-[3-(4-Phenylpiperazin-1-yl)-propyl]-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (13). From **5** in 33% yield, mp 120–122 °C, $R_f = 0.32$ (A), ^{1}H NMR δ 1.08 (t, $^3J = 7.4$ Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.61 (m, 2H, –O–CH$_2$–CH$_2$–CH$_3$), 1.85–1.92 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 2.1 (m, 2H, N$_7$-CH$_2$-CH$_2$-CH$_2$-), 2.66 (m, 4H, N(CH$_2$)$_2$), 3.28–3.31 (m, 4H, (CH$_2$)$_2$N–Ph), 3.42 (s, 3H, N$_1$–CH$_3$), 3.55 (s, 3H, N$_3$–CH$_3$), 4.22 (t, $^3J = 6.8$ Hz, 2H, N$_7$–CH$_2$–), 4.47 (t, $^3J = 6.6$ Hz, 2H, –O–CH$_2$–C$_2$H$_5$), 6.61–6.97 (m, 5H, Ph). **13**·**HCl**·**2H$_2$O**: mp 280–285 °C. Anal. ($C_{23}H_{32}N_6O_3$·HCl·2H$_2$O) C, H, N.

5.1.14. 7-{3-[4-(2-Methoxyphenyl)-piperazin-1-yl]-propyl}-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (14). From **5** in 44% yield, mp 105–107 °C, R_f = 0.16 (A), ^{1}H NMR δ 0.94–1.07 (m, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.66–1.73 (m, 2H, –O–CH$_2$–CH$_2$–CH$_3$), 1.80–1.89 (m, 2H, –CH$_2$–CH$_2$–CH$_2$–), 2.48–2.53 (m, 2H, N$_7$–CH$_2$–CH$_2$–CH$_2$–), 3.13–3.65 (m, 8H, piperazine), 3.37 (s, 3H, N$_1$–CH$_3$), 3.53 (s, 3H, N$_3$–CH$_3$), 3.91 (s, 3H, Ph–O–CH$_3$), 4.24 (t, ^3J = 6.8 Hz, 2H, N$_7$-CH$_2$–), 4.40–4.47 (m, 2H, –O–CH$_2$–C$_2$H$_5$), 6.91–6.98 (m, 2H, Ph), 7.16–7.27 (m, 2H, Ph). **14 · 2HCl**: mp 182–185 °C. Anal. (C$_{24}$H$_{34}$N$_6$O$_4$·2HCl) C, H, N.

5.1.15. 7-{3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propyl}-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (15). From **5** in 45% yield, mp 109–111, R_f = 0.39 (A), ^{1}H NMR δ 0.99-1.07 (m, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.48–1.72 (m, 2H, –O–CH$_2$–CH$_2$–CH$_3$), 1.79–1.91 (m, 2H, –CH$_2$–CH$_2$–CH$_2$-), 2.36–2.52 (m, 2H, N$_7$–CH$_2$–CH$_2$-CH$_2$–), 2.96-3.13 (m, 4H, N(CH$_2$)$_2$), 3.37–3.75 (m, 4H, (CH$_2$)$_2$N–Ph), 3.37 (s, 3H, N$_1$–CH$_3$), 3.52 (s, 3H, N$_3$–CH$_3$), 4.21 (t, ^3J = 6.8 Hz, 2H, N$_7$–CH$_2$–), 4.40–4.46 (m, 2H, –O–CH$_2$–C$_2$H$_5$), 6.75–6.79 (m, 1H, Ph), 6.86–6.91 (m, 2H, Ph), 7.19 (t, ^3J = 8.1 Hz, 1H, Ph). **15·HCl**: mp 210–215 °C. Anal. (C$_{23}$H$_{31}$ClN$_6$O$_3$·HCl) C, H, N.

5.1.16. 7-[4-(4-Phenylpiperazin-1-yl)-butyl]-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione (16). From **6** in 46% yield, mp 114–117 °C, R_f = 0.19 (A), R_f = 0.79 (B), ^{1}H NMR δ 1.45 (t, ^3J = 7.1 Hz, 3H, –O–CH$_2$–CH$_3$), 1.75–1.95 (m, 4H, -CH$_2$–(CH$_2$)$_2$–CH$_2$–), 2.50-3.30 (m, 6H, 4H, N(CH$_2$)$_2$, N$_7$–CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.37 (s, 3H, N$_1$–CH$_3$), 3.52 (s, 3H, N$_3$–CH$_3$), 3.30–3.60 (m, 4H, (CH$_2$)$_2$N–Ph), 4.12 (t, ^3J = 6.5 Hz, 2H, N$_7$–CH$_2$–), 4.54 (q, ^3J = 7.1 Hz, 2H, –O–CH$_2$–CH$_3$), 6.85–7.00 (m, 3H, Ph), 7.25–7.30 (m, 2H, Ph). **16·HCl·0.5H$_2$O**: mp 174–176 °C. Anal. (C$_{23}$H$_{32}$N$_6$O$_3$·HCl·0.5H$_2$O) C, H, N.

5.1.17. 7-{4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl}-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (17). From **6** in 40% yield, mp 180–183 °C, R_f = 0.35 (A), R_f = 0.60 (B), ^{1}H NMR δ 1.38 (t, ^3J = 7.1 Hz, 3H, –O–CH$_2$–CH$_3$), 1.60–1.80 (m, 4H, –CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.00–3.20 (m, 6H, 4H, (CH$_2$)$_2$N–Ph, N$_7$–CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.20 (s, 3H, N$_1$–CH$_3$), 3.37 (s, 3H, N$_3$–CH$_3$), 3.40–3.50 (m, 4H, N(CH$_2$)$_2$), 3.77 (s, 3H, Ph–O–CH$_3$), 4.00–4.10 (m, 2H, N$_7$–CH$_2$–), 4.51 (q, ^3J = 7.0 Hz, 2H, –O–CH$_2$–CH$_3$), 6.85–7.03 (m, 4H, Ph), 10.91 (s, 1H, H$^+$). **17·HCl·0.5H$_2$O**: Anal. (C$_{24}$H$_{34}$N$_6$O$_4$·HCl·0.5H$_2$O) C, H, N.

5.1.18. 7-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (18). From **6** in 49% yield, mp 198–200 °C, R_f = 0.15 (A), R_f = 0.79 (B), ^{1}H NMR δ 1.38 (t, ^3J = 7.1 Hz, 3H, –O–CH$_2$–CH$_3$), 1.60–1.80 (m, 4H, –CH$_2$–(CH$_2$)$_2$–CH$_2$–), 2.95–3.20 (m, 6H, 4H (CH$_2$)$_2$N–Ph, N$_7$–CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.20 (s, 3H, N$_1$–CH$_3$), 3.37 (s, 3H, N$_3$–CH$_3$), 3.40–3.55 (m, 2H, N(CH$_2$)$_2$ axial), 3.75–3.90 (m, 2H, N(CH$_2$)$_2$ equatorial), 4.00–4.10 (m, 2H, N$_7$–CH$_2$–), 4.51 (q, ^3J = 7.1 Hz, 2H, –O–CH$_2$–CH$_3$), 6.85 (d, ^3J = 7.7 Hz, 1H, Ph), 6.93 (d, ^3J = 8.2 Hz, 1H, Ph), 7.03 (s, 1H, Ph), 7.24 (t, ^3J = 8.1 Hz, 1H, 5-Ph), 10.30–10.45 (s, 1H, H$^+$). HMBC (ppm, δ_H, δ_C cross peaks) (1.16, 14.3), (1.60, 14.3), (1.38, 67.8), (1,70, 20.0), (1.7, 27.0), (1.52, 27.0), (1.88, 27.0), (2.97, 27.8), (3.43, 27.8), (3.20, 150.9), (3.20, 153.9), (3.14, 30.4), (3.60, 30.4), (3.37, 146.5), (3.37, 150.9), (4.05, 20.0), (4.05, 27.0), (4.05, 102.6), (4.05, 155.2), (4.51, 14.3), (4.25, 67.8), (4.77, 67.8) (4.51, 155.2), (6.85, 114.8), (6.85, 116.0), (6.85, 134.3), (6.65, 114.8), (7.21, 114.8), (6.93, 116.0), (6.93, 120.0), (7.03, 114.8), (6.76, 114.8), (7.30, 114.8), (7.03, 120.0), (7.03, 134.3), (6.97, 130.9), (7.51, 130.9), (7.24, 134.3), (7.24, 150.9). **18·HCl·0.5H$_2$O**: Anal. (C$_{23}$H$_{31}$N$_6$ClO$_3$·HCl·0.5H$_2$O) C, H, N.

5.1.19. 7-[4-(4-Phenylpiperazin-1-yl)-butyl]-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (19). From **7** in 46% yield, mp 158–161 °C, R_f = 0.16 (A), R_f = 0.82 (B), ^{1}H NMR δ 0.97 (t, ^3J = 7.4 Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.61–1.86 (m, 6H, -CH$_2$–(CH$_2$)$_2$–CH$_2$–, –O–CH$_2$–CH$_2$–CH$_3$), 2.95–3.18 (m, 6H, 4H (CH$_2$)$_2$N–Ph, N$_7$–CH$_2$– (CH$_2$)$_2$–CH$_2$–), 3.20 (s, 3H, N$_1$–CH$_3$), 3.37 (s, 3H, N$_3$–CH$_3$), 3.48 (d, ^3J = 5.6 Hz, 2H, N(CH$_2$)$_2$ axial), 3.80 (d, ^3J = 9.0 Hz, 2H, N(CH$_2$)$_2$ equatorial), 4.05 (t, ^3J = 5.9 Hz, 2H, N$_7$–CH$_2$–), 4.12 (t, ^3J = 6.5 Hz, 2H, –O–CH$_2$–C$_2$H$_5$), 6.84 (t, ^3J = 7.2 Hz, 1H, Ph), 6.97 (d, ^3J = 8.0 Hz, 2H, Ph), 7.24 (td, ^3J = 8.0 Hz, ^4J = 1.3 Hz, 2H, Ph), 10.50 (s, 1H, H$^+$). **19·HCl·H$_2$O**: Anal. (C$_{24}$H$_{34}$N$_6$O$_3$·HCl·H$_2$O) C, H, N.

5.1.20. 7-{4-[4-(2-Methoxyphenyl)-piperazin-1-yl]-butyl}-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (20). From **7** in 44% yield, mp 191–193 °C, R_f = (0.81), ^{1}H NMR δ 0.97 (t, ^3J = 7.4 Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.59–1.86 (m, 6H, –CH$_2$–(CH$_2$)$_2$–CH$_2$–, –O–CH$_2$–CH$_2$–CH$_3$), 2.97 (t, ^3J = 11.3 Hz, 2H, (CH$_2$)$_2$N–Ph axial), 3.05–3.18 (m, 4H, 2H, (CH$_2$)$_2$N–Ph equatorial, N$_7$–CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.20 (s, 3H, N$_1$–CH$_3$), 3.37 (s, 3H, N$_3$–CH$_3$), 3.41–3.55 (m, 4H, N(CH$_2$)$_2$), 3.76 (s, 3H, Ph–O–CH$_3$), 4.05 (t, 2H, N$_7$–CH$_2$–), 4.42 (t, ^3J = 6.5 Hz, 2H, –O–CH$_2$–C$_2$H$_5$), 6.88–7.03 (m, 4H, Ph), 10.36 (s, 1H, H$^+$). **20·HCl**: Anal. (C$_{25}$H$_{36}$N$_6$O$_4$·HCl) C, H, N.

5.1.21. 7-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-8-propoxy-3,7-dihydropurine-2,6-dione hydrochloride (21). From **7** in 49% yield: mp 191–192 °C, R_f = 0.35 (A), R_f = 0.83 (B), ^{1}H NMR δ 0.97 (t, ^3J = 7.4 Hz, 3H, –O–(CH$_2$)$_2$–CH$_3$), 1.60–1.80 (m, 6H, –CH$_2$–(CH$_2$)$_2$–CH$_2$–, –O–CH$_2$–CH$_2$–CH$_3$), 2.95–3.20 (m, 6H, 4H, (CH$_2$)$_2$N–Ph, N$_7$–CH$_2$–(CH$_2$)$_2$–CH$_2$–), 3.20 (s, 3H, N$_1$–CH$_3$), 3.37 (s, 3H, N$_3$–CH$_3$), 3.46 (d, ^2J = 9.7 Hz, 2H, N(CH$_2$)$_2$ axial), 3.85 (d, ^2J = 10.5 Hz, 2H, N(CH$_2$)$_2$ equatorial), 4.04 (t, ^3J = 6.0 Hz, 2H, N$_7$–CH$_2$–), 4.41 (t, ^3J = 6.3 Hz, 2H, –O–CH$_2$–C$_2$H$_5$), 6.85 (d, ^3J = 9.0 Hz, 1H, Ph), 6.94 (dd, ^4J = 1.9 Hz, ^3J = 8.3 Hz, 1H, Ph), 7.03 (s, 1H, Ph), 7.24 (t, ^3J = 8.1 Hz, 1H, Ph), 10.40 (s, 1H, H$^+$). **21·HCl**: Anal. (C$_{24}$H$_{33}$ClN$_6$O$_3$·HCl) C, H, N.

5.1.22. 7-[4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)propyl]-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (22). From **4** in 72% yield, mp 190–195 °C, R_f = 0.8 (A), ^{1}H NMR δ 1.38 (t, ^3J = 7.2 Hz, 3H, –O–

CH_2–C$\underline{H}_3$), 2.22–2.27 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–), 2.94–2.99 (m, 1H, 4-THIQ axial), 3.19–3.21 (m, 4H, N_7–CH_2–CH_2–C$\underline{H}_2$–, 3-THIQ axial, 4-THIQ equatorial), 3.21 (s, 3H, N_1–C$\underline{H}_3$), 3.38 (s, 3H, N_3–C$\underline{H}_3$), 3.61–3.64 (m, 1H, 3-THIQ equatorial), 4.12 (t, 2H, N_7–C$\underline{H}_2$–), 4.19–4.27 (m, 1H, 1-THIQ axial), 4.41–4.44 (m, 1H, 1-THIQ equatorial), 4.47–4.55 (q, 2H, –O–C$\underline{H}_2$–CH_3), 7.15–7.28 (m, 4H, 5,6,7,8-THIQ), 10.78 (s, 1H, $\underline{H}^+$). **22·HCl·0.5H_2O**: Anal. ($C_{21}H_{27}N_5O_3$·HCl·0.5H_2O) C, H, N.

5.1.23. 7-[3-(1,2,3,4-Tetrahydroisoquinolin-2-yl)-propyl]-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (23). From **5** in 51% yield mp 195–196 °C, R_f = 0.8 (A), R_f = 0.55 (B), ^{1}H NMR δ 0.97 (t, ^3J = 7.4 Hz, 3H, –O–$(CH_2)_2$–C$\underline{H}_3$), 1.72–1.84 (m, 2H, –O–CH_2–C$\underline{H}_2$–CH_3), 2.22-2.27 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–), 2.95–2.99 (m, 1H, 4-THIQ axial), 3.18–3.22 (m, 4H, N_7–CH_2–CH_2–C$\underline{H}_2$–, 3-THIQ axial, 4-THIQ equatorial), 3.21 (s, 3H, N_1–C$\underline{H}_3$), 3.38 (s, 3H, N_3–C$\underline{H}_3$), 3.52–3.64 (m, 1H, 3-THIQ equatorial), 4.12–4.14 (m, 2H, N_7–C$\underline{H}_2$–), 4.19–4.27 (m, 1H, 1-THIQ axial), 4.39–4.44 (m, 1H, 1-THIQ equatorial), 4.47–4.53 (m, 2H, –O–C$\underline{H}_2$–C_2H_5), 7.15–7.26 (m, 4H, 5,6,7,8-THIQ), 10.65 (s, 1H, $\underline{H}^+$). **23·HCl**: Anal. ($C_{22}H_{29}N_5O_3$·HCl) C, H, N.

5.1.24. 7-[4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)-butyl]-8-ethoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (24). From **6** in 44% yield: mp 176–179 °C, R_f = 0.15 (A), R_f = 0.77 (B), ^{1}H NMR δ 1.38 (t, ^3J = 7.1 Hz, 3H, –O–CH_2–C$\underline{H}_3$), 1.68–1.91 (m, 4H, –CH_2–$(C\underline{H}_2)_2$–CH_2–), 2.86–3.05 (m, 1H, 4-THIQ axial), 3.19 (s, 3H, N_1–C$\underline{H}_3$), 3.10–3.30 (m, 4H, N_7–CH_2–$(CH_2)_2$–C$\underline{H}_2$–, 3-THIQ axial, 4-THIQ equatorial), 3.36 (s, 3H, N_3–C$\underline{H}_3$), 3.50–3.68 (m, 1H, 3-THIQ equatorial), 4.02–4.05 (m, 2H, N_7–C$\underline{H}_2$–), 4.09–4.32 (m, 1H, 1-THIQ axial), 4.32–4.55 (m, 1H, 1-THIQ equatorial), 4.50 (q, ^3J = 7.0 Hz, 2H, –O–C$\underline{H}_2$–CH_3), 7.15–7.28 (m, 4H, 5,6,7,8-THIQ), 11.29 (s, 1H, $\underline{H}^+$). **24·HCl**: Anal. ($C_{22}H_{29}N_5O_3$·HCl) C, H, N.

5.1.25. 7-[4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)-butyl]-8-propoxy-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride (25). From **7** in 64% yield, mp 169–171 °C, R_f = 0.75 (A), ^{1}H NMR δ 0.97 (t, ^3J = 7.4 Hz, 3H, –O–$(CH_2)_2$–C$\underline{H}_3$), 1.72–1.84 (m, 6H, –CH_2–$(C\underline{H}_2)_2$–CH_2–, –O–CH_2–C$\underline{H}_2$–CH_3), 2.96–2.99 (m, 1H, 4-THIQ axial), 3.19 (s, 3H, N_1–C$\underline{H}_3$), 3.19–3.22 (m, 4H, N_7–CH_2–$(CH_2)_2$–C$\underline{H}_2$–, 3-THIQ axial, 4-THIQ equatorial), 3.37 (s, 3H, N_3–C$\underline{H}_3$), 3.56–3.62 (m,1H, 3-THIQ equatorial), 4.03–4.07 (m, 2H, N_7–C$\underline{H}_2$–), 4.23–4.25 (m, 1H, 1-THIQ axial), 4.39–4.47 (m, 1H, 1-THIQ equatorial), 4.42 (t, 2H, –O–C$\underline{H}_2$–C_2H_5), 7.15–7.28 (m, 4H, 5,6,7,8-THIQ), 10.79 (s, 1H, $\underline{H}^+$). **25·HCl**: Anal. ($C_{23}H_{31}N_5O_3$·HCl) C, H, N.

5.1.26. A general procedure for preparing 7-aminoalkyl-purine-2,6,8-trione derivatives (26–30). Starting with the 7-ω-chloro-alkyl-8-methoxy-purine-2,6-dione derivatives **8** or **9**, and following the method described for the preparation of 7-aminoalkyl-8-alkoxy-purine-2,6-dione derivatives, the appropriate 7-aminoalkyl-purine-2,6,8-trione derivatives **26–30** were obtained. Compounds **26–30** were isolated directly from the reaction mixture as hydrochloride salts by treating them with an excess of conc. HCl in an ethanol solution. Hydrochloride salts of the compounds were purified by crystallization from the mixture of ethanol and water (1:1).

5.1.27. 7-[3-(4-Phenylpiperazin-1-yl)-propyl]-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione hydrochloride (26). From **8** in 20% yield, mp 278–280 °C, R_f = 0.30 (B), ^{1}H NMR δ 2.05–2.20 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–), 2.75–3.25 (m, 6H, 4H $(C\underline{H}_2)_2$N–Ph, N_7–CH_2–CH_2–C$\underline{H}_2$–), 3.18 (s, 3H, N_1–C$\underline{H}_3$), 3.33 (s, 3H, N_3–C$\underline{H}_3$), 3.40–3.65 (m, 2H, N$(C\underline{H}_2)_2$ axial), 3.65–3.90 (m, 2H, N$(C\underline{H}_2)_2$ equatorial), 3.87 (t, ^3J = 6.5 Hz, 2H, N_7–C$\underline{H}_2$–), 6.83 (t, ^3J = 7.3 Hz, 1H, Ph), 6.96 (t, ^3J = 8.0 Hz, 2H, Ph), 7.23 (t, ^3J = 8.0 Hz, 2H, Ph), 10.73 (s, 1H, $\underline{H}^+$), 12.25 (s, 1H, N_9–$\underline{H}$). **26·HCl·H_2O**: Anal. ($C_{20}H_{26}N_6O_3$·HCl·H_2O) C, H, N.

5.1.28. 7-{3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propyl}-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione hydrochloride (27). From **8** in 24% yield, mp 291–293 °C, R_f = 0.56 (B), ^{1}H NMR δ 2.00-2.20 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–), 2.90–3.25 (m, 6H, 4H, $(C\underline{H}_2)_2$N–Ph, N_7–CH_2–CH_2–C$\underline{H}_2$–), 3.18 (s, 3H, N_1–C$\underline{H}_3$), 3.32 (s, 3H, N_3–C$\underline{H}_3$), 3.35–3.65 (m, 2H, N$(C\underline{H}_2)_2$ axial), 3.75–4.13 (m, 2H, N$(C\underline{H}_2)_2$ equatorial), 3.87 (t, ^3J = 6.5 Hz, 2H, N_7–C$\underline{H}_2$–), 6.85 (dd, ^3J = 8.0 Hz, ^4J = 1.3 Hz, 1H, Ph), 6.93 (dd, ^3J = 8.5 Hz, ^4J = 1.8 Hz, 1H, Ph), 7.02 (t, ^4J = 2.1 Hz, 1H, Ph), 7.24 (t, ^3J = 8.2 Hz, 1H, Ph), 10.40 (s, 1H, $\underline{H}^+$), 12.23 (s, 1H, N_9–$\underline{H}$). **27·HCl·H_2O**: Anal. ($C_{20}H_{25}N_6O_3Cl$·HCl·H_2O) C, H, N.

5.1.29. 7-[4-(4-Phenylpiperazin-1-yl)-butyl]-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione hydrochloride (28). From **9** in 21% yield, mp 289–292 °C, R_f = 0.34 (B), ^{1}H NMR δ 1.61–1.79 (m, 4H, –CH_2–$(C\underline{H}_2)_2$–CH_2–), 2.94–3.20 (m, 6H, 4H, $(C\underline{H}_2)_2$N–Ph, N_7–CH_2–CH_2–C$\underline{H}_2$–), 3.17 (s, 3H, N_1–C$\underline{H}_3$), 3.32 (s, 3H, N_3–C$\underline{H}_3$), 3.36–3.61 (m, 2H, N$(C\underline{H}_2)_2$ axial), 3.69–3.86 (m, 4H, 2H, N$(C\underline{H}_2)_2$ equatorial, N_7–C$\underline{H}_2$–), 6.84 (t, ^3J = 7.2 Hz, 1H, Ph), 6.97 (d, ^3J = 7.7 Hz, 2H, Ph), 7.24 (td, ^3J = 8.0 Hz, ^4J = 1.5 Hz, 2H, Ph), 10.50 (s, 1H, $\underline{H}^+$), 12.20 (s, 1H, N_9–$\underline{H}$). **28·HCl·2.5H_2O**: Anal. ($C_{21}H_{28}N_6O_3$·HCl·2.5H_2O) C, H, N.

5.1.30. 7-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione hydrochloride (29). From **9** in 17% yield mp 263–265 °C, R_f = 0.33 (B), ^{1}H NMR δ 1.60–1.75 (m, 4H, –CH_2–$(C\underline{H}_2)_2$–CH_2–), 2.95–3.20 (m, 6H, 4H, $(C\underline{H}_2)_2$N–Ph, N_7–CH_2–CH_2–C$\underline{H}_2$–), 3.17 (s, 3H, N_1–C$\underline{H}_3$), 3.31 (s, 3H, N_3–C$\underline{H}_3$), 3.40–3.55 (m, 2H, N$(C\underline{H}_2)_2$ axial), 3.75–3.90 (m, 4H, 2H, N$(C\underline{H}_2)_2$ equatorial, N_7–C$\underline{H}_2$–), 6.85 (dd, ^3J = 7.8 Hz, ^4J = 1.4 Hz, 1H, Ph), 6.94 (dd, ^3J = 8.5 Hz, ^4J = 1.8 Hz, 1H, Ph), 7.03 (t, ^4J = 2.1 Hz, 1H, Ph), 7.24 (t, ^3J = 8.2 Hz, 1H, Ph), 10.50 (s, 1H, $\underline{H}^+$), 12.20 (s, 1H, N_9–$\underline{H}$). HMBC (ppm, δ_H, δ_C cross peaks) (1.67, 20.0), (2.92, 28.0), (3.40, 28.0), 3.17, 150.0), (3.17, 153.5), (3.07, 31.3), (3.55, 31.3), (3.31, 137.4), (3.31, 150.0), (3.83, 20.4), (3.83, 27.0), (3.83, 97.8), (3.83, 152.2), (6.85, 114.8), (6.85, 115.2), (6.85,

134.3), (6.66, 114.8), (7.22, 114.8), (6.94, 115.2), (6.94, 120.0), (7.03, 114.8), (6.76, 115.2), (7.30, 115.2), (7.03, 120.0), (7.03, 134.3), (6.97, 131.3), (7.51, 131.3), (7.24, 134.3), (7.24, 151.3). **29·HCl·H_2O**: Anal. ($C_{21}H_{27}N_6O_3$·Cl·HCl·H_2O) C, H, N.

5.1.31. 7-{3-[1,2,3,4-Tetrahydroisoquinolin-2-yl]-propyl}-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione hydrochloride (30). From **8** in 24% yield, mp 261–263 °C, R_f = 0.24 (B), ^{1}H NMR δ 2.08–2.26 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–), 2.88–3.05 (m, 1H, 4-THIQ axial), 3.19 (s, 3H, N_1–C$\underline{H}_3$), 3.05–3.26 (m, 4H, N_7–CH_2–$(CH_2)_2$–C$\underline{H}_2$–, 3-THIQ axial, 4-THIQ equatorial), 3.33 (s, 3H, N_3–C$\underline{H}_3$), 3.49–3.78 (m, 1H, 3-THIQ equatorial), 3.90 (t, ^3J = 6.5 Hz, 2H, N_7–C$\underline{H}_2$–), 4.08–4.67 (m, 2H, 1,1-THIQ), 7.10–7.31 (m, 4H, 5,6,7,8-THIQ), 10.65 (s, 1H, $\underline{H}^+$), 12.28 (s, 1H, N_9–$\underline{H}$). **30·HCl·H_2O**: Anal. ($C_{19}H_{23}N_5O_3$·HCl·1.5H_2O) C, H, N.

5.2. In vitro radioligand binding assays

All the assays were carried out on rat brain tissues; inhibition constants (K_i) were determined from at least three separate experiments in which 8–10 drug concentrations, run in triplicate, were used. The binding effect was terminated by rapid filtration through Whatman GF/B filters, followed by three 4 mL washes with the ice-cold incubation buffer. The radioligand concentration used in competition assays was equal to the K_d values obtained in the respective saturation experiments, that is, 1, 0.6, and 0.5 nM for [^{3}H]-8-OH-DPAT, [^{3}H]-ketanserin, and [^{3}H]-5-CT, respectively.

The radioactivity retained on the filters was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 mL scintillation fluid (Akwascynt, BioCare). Binding isotherms of the tested compounds were analyzed by nonlinear regression (Prism, GraphPad Software Inc., San Diego, USA), using the Cheng–Prusoff equation to calculate K_i values.

5.2.1. Serotonin 5-HT_{1A} and 5-HT_{2A} binding assays. Radioligand studies with native 5-HT_{1A} and 5-HT_{2A} receptors were conducted according to the methods previously described.[7] Briefly, the following were used: for 5-HT_{1A} assays, rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals), and 5-HT (10 μM) for non-specific binding; [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide (1 μM) for non-specific binding.

5.2.2. Serotonin 5-HT_7 binding assays. A serotonin 5-HT_7 receptor binding assay was performed using rat hypothalamic membranes, according to the method described by Aguirre et al.[26] with minor modifications. In brief, hypothalami dissected from male Wistar rats (200–250 g) were frozen at −80 °C prior to the preparation of a radioligand binding homogenate. On the day of experiment, the hypothalami were allowed to defrost and were then immediately homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.4 at 23 °C) and centrifuged at 48,000*g* for 10 min at 4 °C. The supernatant was removed, the resulting pellet was rehomogenized and incubated at 37 °C for 15 min to remove endogenous serotonin. After incubation, the homogenate was centrifuged twice under the same conditions as before. The final pellet was resuspended in the assay buffer (50 mM) Tris–HCl containing 0.01 mM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. Membrane aliquots (10 mg—original wet tissue weight) were incubated in the presence of 3 μM (±)-pindolol (to eliminate the binding to 5-HT_{1A} and 5-HT_{1B} receptors) with 0.5 nM [^{3}H]-5-CT (specific activity, 34.5 Ci/mmol; NEN) and eight concentrations of a displacing drug. Non-specific binding was determined using 10 μM of serotonin. After incubation at 23 °C for 120 min, the reaction was terminated by rapid filtration through a Whatman GF/B filter.

5.3. In vivo experiments

The experiments were performed on male Wistar rats (290–310 g) or male Albino Swiss mice (24–28 g). The animals were kept at a room temperature of 20 ± 1 °C, and had free access to food (standard laboratory pellets) and tap water before the experiment. All the investigations were conducted in the light phase, on a natural day–night cycle (January–March), between 9.00 a.m. and 2.00 p.m. Each experimental group consisted of 6–10 animals/dose, and all the animals were used only once. 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*-(pyridin-2-yl)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. Compounds **17** and **21** were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc), imipramine and the tested compounds were given intraperitoneally (ip) in a volume of 2 mL/kg (rats) or 10 mL/kg (mice). The experimental procedures used were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. The obtained data were analyzed by a one-way analysis of variance followed by Dunnett's test (when only one drug was given), or by the Newman–Keuls test (when two drugs were administered).

5.3.1. Body temperature in mice. The effects of the tested compounds given alone on the rectal body temperature of mice (measured with an Ellab thermometer) were recorded 30, 60, 90 and 120 min after their administration. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg, sc) on the hypothermia induced by compounds **17** and **21** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the investigated compounds and 8-OH-DPAT and rectal body temperature was recorded at 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.

5.3.2. Lower lip retraction (LLR) in rats. LLR was assessed according to the method described by Berendsen et al.[17,18] The rats were individually placed in cages ($30 \times 25 \times 25$ cm) and were scored three times (at 15, 30, and 45 min after 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 number of scores was 3/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, sc) was tested. Compounds **17**, **21** and WAY 100635 were administered at 45 and 15 min, respectively, before 8-OH-DPAT and the animals were scored at 15, 30, and 45 min after 8-OH-DPAT administration.

5.3.3. Four-plate test in mice. The box was made of opaque plastic and was rectangular ($25 \times 18 \times 16$ cm) in shape. The floor was covered with four rectangular metal plates (11×8 cm), separated by a 4-mm gap. The plates were connected to the source of continuous current which enabled a 120 V difference of the potential between two adjacent plates for 0.5 s when the experimenter pressed the switch. Individual mice were gently placed on 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 assessed for 60 s.[19]

5.3.4. Forced swimming test in mice. The experiment was carried out according to the method of Porsolt et al.[20] 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 in the water making only insignificant movements to keep its head above it. The total duration of immobility was recorded during the final 4 min of a 6-min test session.

5.3.5. Locomotor activity in mice. The spontaneous locomotor activity of mice was quantified using Opto-M3 systems (Columbus Instruments). The mice were placed individually in transparent plastic cages, equipped with computer-controlled sensors which automatically monitored their movements, and consecutive IR beam breaks were scored as an ambulatory count. The locomotor activity of mice was recorded twice: during the first 6 min, that is, at the time equal to the observation period in the forced swimming test, and during 30-min experimental sessions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.05.017.

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