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New Arylpiperazinylalkyl Derivatives of 8-Alkoxy-purine-2,6-dione and Dihydro[1,3]oxazolo[2,3-*f*]purinedione Targeting the Serotonin 5-HT_{1A}/5-HT_{2A}/5-HT_7 and Dopamine D_2 Receptors

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To obtain potential antidepressants and/or antipsychotics, a series of new long-chain arylpiperazine derivatives of 8-alkoxy-purine-2,6-dione (**10**–**24**) and dihydro[1,3]oxazolo[2,3-*f*]purinedione (**30**–**34**) were synthesized and their serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7) and dopamine (D_2) receptor affinities were determined. The study allowed the identification of some potent 5-HT_{1A}/5-HT_7/D_2 ligands with moderate affinity for 5-HT_{2A} sites. The binding mode of representative compounds from both chemical classes (**11** and **31**) in the site of 5-HT_{1A} receptor was analyzed in computational studies. In functional *in vitro* studies, the selected compounds **15** and **16** showed antagonistic properties for the evaluated receptors. 8-Methoxy-7-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-purine-2,6-dione (**15**) showed a lack of activity in terms and under the conditions of the forced swim, four plate and amphetamine-induced hyperactivity tests in mice, probably as a result of its high first pass effect in the liver.

Keywords: Arylpiperazines / Depression / Serotonin receptor ligands

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Introduction

A comprehensive study of mental disorders, based upon the international diagnostic classification system [1], which covers 19 major groups [2] revealed that neuropsychiatric illnesses represent a larger disease burden on society than either cardiovascular disease or cancer. Nowadays, the investment in research and development to create better psychotropic agents, especially for affective disorders (major depression, bipolar disorder) and schizophrenia, has declined [3, 4].

Over the past 50 years, enormous progress has been made in the clinical understanding of psychiatric disorders and biochemical brain mechanisms, leading to the development of effective drug treatments, for example, tricyclic antidepressants, serotonin reuptake inhibitors, benzodiazepines, and atypical antipsychotic drug. Since the introduction of aripiprazole in 2002 and then paliperidone, iloperidone, asenapine, and lurasidone, there has been a relative hiatus in the development of new atypical antipsychotic drugs. From the chemical point of view, the new antidepressants and atypical antipsychotics are based around arylpiperazine or arylpiperidine core structures, which may explain their interaction with a large number of G-protein coupled receptors (Rs), including 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7 as well

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as D_2, D_3, and D_4 receptors. Clues from the clinic that atypical antipsychotics agents can be used as augmentation therapy in patients with poor responses to antidepressants led to the creation of the multitarget strategy for development of CNS active drugs.

Extending the concept of mixed 5-HT/D Rs targeting agents as novel antipsychotics, a series of 1-aryl-4-(biarylmethylene)-piperazines were discovered [5], which resulted in the identification of bifeprunox (Fig. 1), a potential atypical antipsychotic with reduced extrapyramidal side effects [6]. The structure–activity relationships in the group of 1-aryl-4-(phenylarylmethyl)piperazines [7] led to obtaining SLV 313 (adoprazine) (Fig. 1) [8]. The multireceptor strategy has also been explored in a series of isoquinoline- and quinoline-amide- and sulfonamide-derivatives of long chain arylpiperazines (LCAPs) exemplified by structures **A** and **B** (Fig. 1) with potential antipsychotic, antidepressant, and anxiolytic properties [9–11].

For several years, we have been interested in developing LCAPs derivatives of 1,3-dimethyl-purine-2,6-dione, which were mainly evaluated toward 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and recently also for 5-HT_6 Rs [12–20]. The 8-unsubstituted or 8-alkoxy- and 8-morpholinyl-purine-2,6-dione derivatives showed the features of dual $5HT_{1A}$/$5HT_{2A}$ or 5-HT_{1A}/$5HT_7$ Rs ligands with a moderate affinity toward D_2 sites. Some of them (compounds **I**–**VI**, Fig. 2) produced antidepressant-like and/or specific anxiolytic effects in forced swim and four plate tests in mice [15–20].

Continuing our investigation in a group of purine-2,6-dione derivatives of LCAPs, and to extend the studies aimed at verifying the impact of the alkoxy moiety in an 8 position of purine-2,6-dione system on the selected serotoninergic and dopaminergic Rs affinity, we designed and synthesized a novel series of 8-methoxy analogs with the most thoroughly studied arylpiperazines. In comparison to the previously evaluated 8-alkoxy derivatives [15], we extended the length of the linker between purine-2,6-dione core and arylpiperazine fragment from four to five carbon units to check the influence of this modification on the receptor activity. Moreover, a series of LCAPs derivatives of dihydro[1,3]-oxazolo[2,3-*f*]purinediones, which may be regarded as 8-methoxy-purine-2,6-dione analogs with methoxy group built into the cyclic oxygen ring system, were obtained. Herein, we report on their synthesis and evaluation for the selected serotoninergic (5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7) and dopaminergic (D_2) Rs as well as determination of their intrinsic activity for validation of the multireceptor approach. Additionally, computational studies were carried out to rationalize the obtained binding data for representative compounds from both chemical classes. Moreover, the potential antidepres-

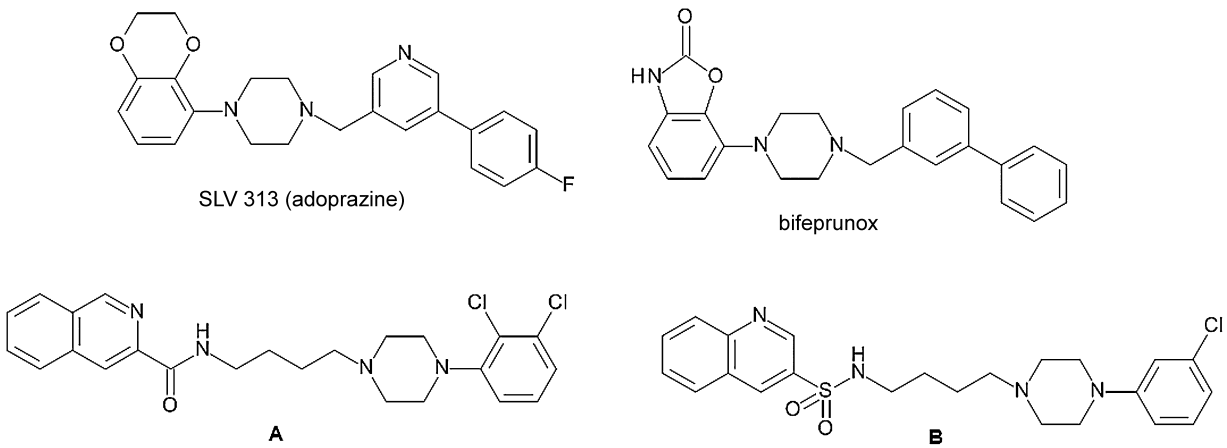
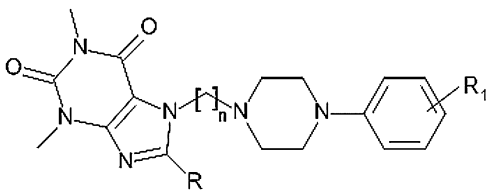


Figure 1. Structures of mixed 5-HT/D receptor agents: SLV313 (adoprazine) [8], bifeprunox [6], and isoquinoline-amide (**A**) [9] and quinoline-sulfonamide (**B**) [10] LCAPs derivatives.



I R = OC_2H_5,	R_1 = 2-OCH_3,	n = 4
II R = OC_3H_7,	R_1 = 3-Cl,	n = 4
III R = OC_3H_7,	R_1 = 3-Cl,	n = 3
IV R = morpholin-4-yl,	R_1 = 3-Cl,	n = 4
V R = H,	R_1 = 3-Cl,	n = 4
VI R = H,	R_1 = 2-OCH_3,	n = 5

Figure 2. The structures of the pharmacologically active compounds **I**–**VI** from earlier studies.

sant-, anxiolytic-, and antipsychotic-like properties of the most interesting compound **15** were evaluated in the behavioral tests in mice.

Results and discussion

Chemistry

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

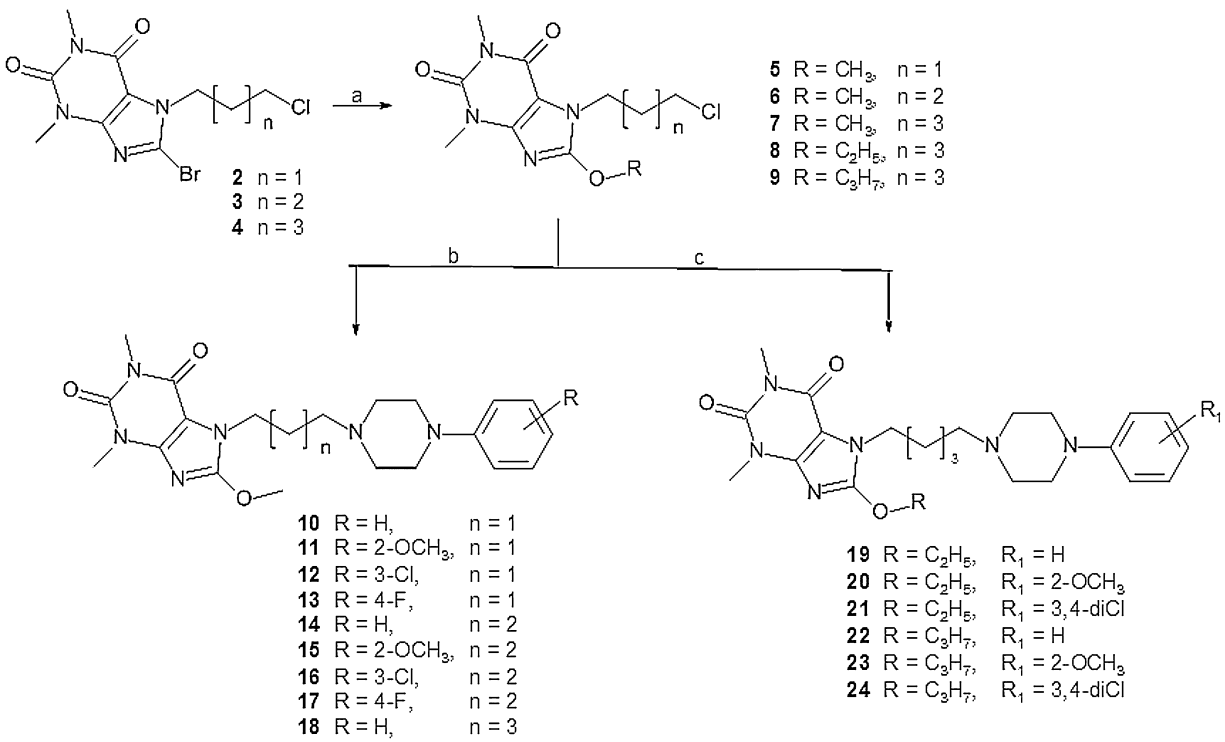
The starting 8-bromo-7-(3-chloropropyl)-1,3-dimethyl-purine-2,6-dione (**2**) [21], 8-bromo-7-(4-chlorobutyl)-1,3-dimethyl-purine-2,6-dione (**3**) [15], and the new 8-bromo-7-(5-chloropentyl)-1,3-dimethyl-purine-2,6-dione (**4**) were prepared in a reaction of 8-bromo-1,3-dimethyl-purine-2,6-dione (**1**) [22] with the appropriate bromochloroalkane according to the previously described method [15]. The 7-ω-chloroalkyl-8-alkoxy-1,3-dimethyl-purine-2,6-diones **5**–**9** were obtained in a reaction of **2**, **3**, or **4** with the appropriate sodium alcoholate in a corresponding alcohol medium [15]. The designed derivatives **10**–**24** were synthesized by nucleophilic substitution of **5**–**9** with the appropriate arylpiperazines in the presence of K_2CO_3 in toluene (**10**–**18**) and in 1-propanol (**19**–**24**). Compounds **10**–**24** were separated by column chromatography as free bases. Compounds **15**, **19**–**24** were then converted into water-soluble salts using conc. hydrochloric acid in acetone.

The synthesis of arylpiperazinylmethyl-dihydro[1,3]oxazolo[2,3-*f*]purinediones was accomplished as shown in Scheme 2. 8-Bromo-1,3-dimethyl-purine-2,6-dione reacted with the respective substituted oxiranes in the presence of a catalytic amount of pyridine in anhydrous 1-propanol.

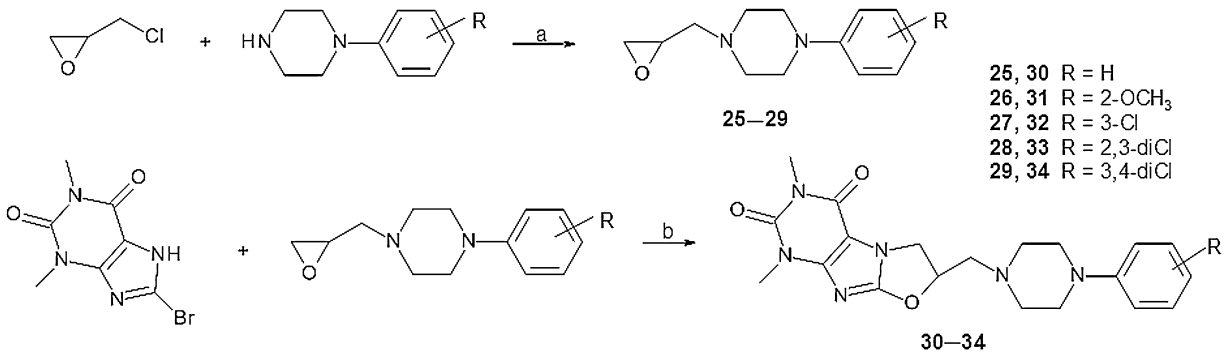
The elemental analysis data and some physical properties of these compounds are reported in the Experimental section.

In vitro evaluation

Radioligand binding assays were employed to determine the affinity and the selectivity profiles of the new synthesized compounds **10**–**24** and **30**–**34** for serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and dopamine D_2 Rs. For compounds **10**–**24** as close analogs of previously reported 8-alkoxypurine-2,6-diones, the inhibition constant (K_i) values were determined in 7–9 compound concentrations, according to the previously published procedures [11, 23, 24]. The affinity data of the compounds **10**–**24** are presented in Table 1. For compounds **30**–**34** as derivatives of the dihydro[1,3]oxazolo[2,3-f]purinedione system, never before evaluated for these targets, preliminary *in vitro* evaluation in two concentrations (10^{-6} M and 10^{-7} M) was provided to establish percent of specific binding according to described procedure [25].

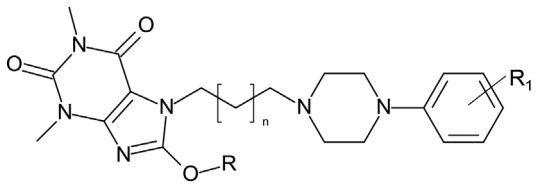


Scheme 1. The synthesis of 7-arylpiperazinylalkyl-8-alkoxy-purine-2,6-diones **10**–**24**. Reagents and conditions: (a) RONa, ROH, reflux; (b) arylpiperazine derivatives, toluene, K_2CO_3, reflux; (c) arylpiperazine derivatives, 1-propanol, K_2CO_3, reflux.



Scheme 2. The synthesis of arylpiperazinylmethyl oxiranes **25–29** and arylpiperazinylmethyl derivatives of dihydro[1,3]oxazolo[2,3-*f*]purinediones **30–34**. Reagents and conditions: (a) epichlorohydrin; (b) pyridine, 1-propanol.

Table 1. The binding data of the 10–24 for 5-HT and D_2 Rs.



Comp.	*R*	R_1	*n*	K_i (nM) ± SEM				
				5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7	D_2
10	CH_3	H	1	688 ± 55	154 ± 14	>30,000	747 ± 69	1303 ± 106
11	CH_3	2-OCH_3	1	106 ± 9	498 ± 61	>20,000	283 ± 33	216 ± 25
12	CH_3	3-Cl	1	72 ± 6	116 ± 9	>20,000	147 ± 9	432 ± 41
13	CH_3	4-F	1	3643 ± 428	22 ± 3	>10,000	447 ± 51	4012 ± 473
14	CH_3	H	2	20 ± 2	245 ± 15	>20,000	69 ± 8	196 ± 23
15	CH_3	2-OCH_3	2	4 ± 1	695 ± 75	>20,000	12 ± 1	24 ± 3
16	CH_3	3-Cl	2	10 ± 2	60 ± 7	2927 ± 264	23 ± 2	60 ± 7
17	CH_3	4-F	2	222 ± 17	157 ± 20	2330 ± 2715	283 ± 23	404 ± 31
18	CH_3	H	3	66 ± 7	289 ± 37	>20,000	204 ± 11	87 ± 8
19	C_2H_5	H	3	57 ± 6	331 ± 39	>10,000	190 ± 22	35 ± 4
20	C_2H_5	2-OCH_3	3	6 ± 1	412 ± 27	6979 ± 835	90 ± 7	8 ± 1
21	C_2H_5	3,4-diCl	3	91 ± 8	178 ± 23	719 ± 73	71 ± 6	34 ± 5
22	C_3H_7	H	3	36 ± 4	429 ± 41	4796 ± 571	106 ± 9	13 ± 2
23	C_3H_7	2-OCH_3	3	7 ± 1	333 ± 29	>10,000	53 ± 4	4 ± 1
24	C_3H_7	3,4-diCl	3	79 ± 10	72 ± 8	262 ± 17	59 ± 7	17 ± 3
Compound **I**				11 ± 1	253 ± 14	NT	54 ± 2	NT
Compound **II**				15 ± 1	28 ± 2	NT	125 ± 9	NT
Compound **III**				288 ± 18	25 ± 2	NT	267 ± 21	NT
Compound **IV**				22 ± 2	21 ± 2	207 ± 26	112 ± 10	155 ± 19
Buspirone				20 ± 2	–	–	–	–
Olanzapine				–	4 ± 0.9	7 ± 0.8	–	7 ± 0.6
Clozapine				–	–	–	18 ± 2	–

NT, not tested.

Generally the synthesized derivatives **10–24** displayed high-to-moderate affinity for selected serotonin: 5-HT_{1A} (5−688 nM), 5-HT_{2A} (22−695 nM), 5-HT_7 (12−747 nM), and D_2 (4−432 nM) Rs and, except **21** and **24**, a lack of activity for 5-HT_6 sites (Table 1). The binding results have showed a positive influence of the methoxy moiety in comparison with the previously reported 8-alkoxy analogs [15]. Compounds **14–16** were 2- to 4-fold and 5- to 13-fold more active for 5-HT_7 sites, respectively, than 8-ethoxy analogs and 5- to 13-fold more active than 8-propoxy analogs [15]. At the same time, this modification only slightly increased 5-HT_{1A} and decreased 5-HT_{2A} Rs affinity.

The elongation of the linker length from three- to four-carbon units significantly increased the affinity for 5-HT_{1A} (6- to 34-fold), 5-HT_7 (6- to 23-fold), and D_2 (6- to 9-fold) sites. The five-member alkyl spacer did not affect the binding to evaluated Rs (**14** vs. **18**). In comparison to the previously reported analogs [15], the elongation of the linker length from four- to five-carbon units increased 5-HT_{1A} but decreased 5-HT_{2A} affinity. The influence on the affinity for 5-HT_7 Rs is not so decisive; however, **22** and **23** are more active for these sites than their previously reported analogs with a four-methylene spacer [15].

Compounds with unsubstituted phenyl ring display a lower affinity for 5-HT_{1A}, 5-HT_7, and D_2 Rs than their 2-OCH_3 and 3-Cl analogs (e.g. **10** vs. **11**, **12** or **14** vs. **15** and **16**). The introduction of 4-F into the phenyl ring generally did not improve the affinity for evaluated Rs, except **12** which appeared to be a selective 5-HT_{2A} ligand (Table 1). On the other hand, the introduction of 3,4-diCl moiety (**21** and **24**) significantly increased 5-HT_6 affinity (Table 1). It is noteworthy that all of the tested LCAPs derivatives of dihydro[1,3]oxazolo-[2,3-*f*]purinedione **30–34** were inactive in the screening binding assays. The percentages of specific binding for most of these compounds were up to 90% at both concentrations studied (Supporting Information). This resulted in exclusion of these compounds from the subsequent phases of the study. The above results have conclusively proven that a change in the chemical character of the cyclic amide fragment affects the receptor affinity. Summing up, **15** containing 2-methoxyphenylpiperazine moiety could be regarded as a 5-HT_{1A}/5-HT_7 Rs ligand with D_2 dopaminergic Rs activity. In turn, **16** with 3-chlorophenylpiperazinyl fragment showed features of a 5HT_{1A}/5HT_{2A}/5-HT_7 Rs ligand. Hence, in the series of the tested 8-methoxy derivatives, the four-methylene group alkyl spacer was preferable to obtain potent 5-HT_{1A}/5-HT_{2A}/5-HT_7 ligands with a high-to-moderate affinity for D_2 Rs.

Molecular modeling

Computational studies have been engaged to predict binding mode of the presented compounds, as well as to explain pronounced differences in the receptor binding affinity between compounds representative for both chemical classes. Compound **11** from the 8-alkoxy-purine-2,6-dione class and its closest analog **31** (the derivative of oxazolo[2,3-*f*]purine-dione) were docked to a set of homology models of 5-HT_{1A} receptor, which simulates the conformational flexibility of the protein. This procedure allowed us to capture important ligand–receptor interactions, differentiating their receptor affinity. The arylpiperazine fragment, common for both compounds, provided significant interactions with Asp3.32 (charge-reinforced hydrogen bond), as well as with Phe6.52 (π–π stacking) and Lys191 (π-cation). The 8-alkoxy-purine-2,6-dione moiety of compound **11** reached additional stabilizing interactions with Tyr2.64 (π–π stacking) and Gln2.65 (H-bond) (Fig. 3A). Such beneficial interactions were inaccessible for any enantiomer of compound **31** (Fig. 3B). Moreover, the unfavorable conformation of the molecule determined by the oxazole ring fused to the purine-2,6-dione scaffold, caused steric hindrance in the binding sites of some homology models, mirroring the lack of *in vitro* affinity for the target protein.

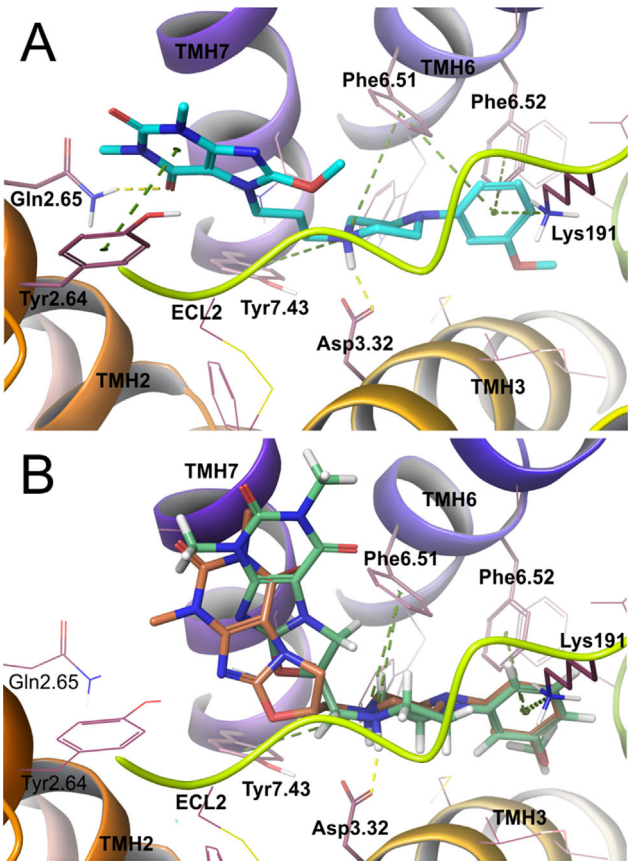


Figure 3. Binding modes of compounds **11** (A) and **31** (B) in the site of 5-HT_{1A} receptor. Compound **31** is represented by *R*-(orange) and *S*- (green) enantiomers. Amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are displayed as sticks, whereas those forming H-bonds (dotted yellow lines) or π–π stacking/π-cation interactions (dotted green lines) are represented as thick sticks. For the sake of clarity, a part of ECL2 and its residues were hidden. TMH, transmembrane helix; ECL, extracellular loop.

Functional *in vitro* evaluation

The preliminary functional activity of the selected compounds **15** and **16** on evaluated Rs, on intracellular cAMP levels, was determined at Cerep (Le Bois l'Eveque, 86600 Celle L'Evescault, France) according to the previously published methods [26–30]. The assays were carried out in HEK-293 and CHO cells which stably express human 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2 Rs, respectively, with WAY 10063, ketanserin, methiothepin, and butaclamol (antagonistic effect) and 8-OH-DPAT, and 5-HT (agonistic effect) as reference compounds.

The compound **15** showed a strong 5-HT_{1A} and D_2 Rs antagonistic effect and a weak 5-HT_7 antagonistic activity (Table 2). In turn, **16** exerted strong 5-HT_{1A} antagonistic activity as well as displayed 73% of control agonistic response to this type of Rs. This compound revealed weak antagonistic properties for 5-HT_{2A} and 5-HT_7 Rs (Table 2).

Behavioral evaluation

Both the receptor profile and functional activity of **15** (Tables 1 and 2) have prompted us to evaluate its potential antidepressant- and anxiolytic-like properties, using forced swim and four-plate test in mice, respectively [31, 32].

Serotonin 5-HT_{1A} R is well known to mediate antidepressant- and anxiolytic-like activity in rodents and humans [33]. In the tests conducted, **15** in comparison with diazepam and imipramine, used as reference drugs, showed neither antidepressant- nor anxiolytic-like activity (Table 3). The previously reported 8-ethoxy analog **I** (Fig. 1) with a similar receptor 5-HT_{1A} affinity but a different agonistic functional

Table 2. **The functional *in vitro* evaluation of 15 and 16.**

Comp.	% inhibition of control agonistic response (antagonistic effect)				% of control agonistic response (agonistic effect)	
	5-HT_{1A}	5-HT_{2A}	5-HT_7	D_2	5-HT_{1A}	5-HT_7
15[a]	105	NT	44	94	NT	NT
16	110	42	47	NT	73	5.0

NT, not tested.
[a]Test concentration: 10^{-6} M.

Table 3. **Effects of 15 in the forced swim test (A) and four-plate test (B) in mice.**

Treatment	Dose [mg/kg]	Mean ± SEM
(A)		Immobility time [s]
Vehicle	–	179.4 ± 5.0
15	0.625	164.4 ± 13.5
	1.25	168.0 ± 4.2
	2.5	167.2 ± 7.8
	5	173.5 ± 8.4
	10	184.3 ± 13.4 F(5,49) = 0.54752, ns
Vehicle	–	162.7 ± 6.8
Imipramine	5	170.4 ± 10.9
	10	119.6 ± 13.0[a]
	20	77.8 ± 12.2[b], F(3,36) = 16.757, $p < 0.0001$
(B)		Number of punished crossings/60 s
Vehicle	–	2.6 ± 0.3
15	2.5	1.3 ± 0.2
	5	2.7 ± 0.4
	10	2.3 ± 0.4, F(3,34) = 3.0414, $p < 0.05$
Vehicle	–	4.2 ± 0.4
Diazepam	1.25	5.8 ± 0.3[a]
	2.5	6.4 ± 0.5[b]
	5	6.6 ± 0.4[b], F(3,36) = 6.455 $p < 0.01$

Compound **15** and diazepam were administered i.p. 60 min, while imipramine 30 min before the test; $n = 8$–10 mice per group.
[a]$p < 0.05$.
[b]$p < 0.01$ vs. vehicle.

profile at 5-HT_{1A} sites produced antidepressant- and anxiolytic-like effects in mice [15]. The above information indicates that the impact of an alkoxy moiety on the affinity to the 5-HT Rs studied, the functional profile, and psychotropic activity is not decisive.

D-Amphetamine produces locomotor hyperactivity in animals as a result of increased dopaminergic activity in mesolimbic system [34, 35] and its effect is blocked by antipsychotics having antagonist properties toward dopamine Rs [36]. Taking into account the above information, the influence of **15** on the amphetamine-induced hyperactivity in mice was studied. The obtained results indicate that **15**, given at doses of 1.25–10 mg/kg *i.p.*, did not attenuate D-amphetamine-induced hyperactivity in mice (Supporting Information) and, therefore, showed no antipsychotic-like activity.

The metabolic studies of 15

The lack of activity *in vivo* of **15** prompted as to examine its metabolism using mouse liver microsomes. The samples for analysis were taken at three different time points, that is, after 5, 15, and 30 min of incubation. At the first time point five major metabolites (M1–M5) were observed, M6 was found after 15 and M7 after 30 min from the start of experiment. On the basis of ions fragmentation (MS/MS) the metabolic fate, the chemical structures, and percentage content of metabolites M1–M7 were proposed (Fig. 4). The exemplary liquid chromatography results and the ions present in LC/MS fragmentation spectra of **15** were also presented (Supporting Information).

From the time course of disappearance of the parent compound during incubation with microsomes, the half-life ($t_{0.5}$) of **15** was determined and then its intrinsic clearance (CL_{int}) was estimated. The evaluated compound demonstrated a high CL_{int} (122.0 μL/mg/min, and a short $t_{0.5} = 7.1$ min), which suggests that a high hepatic first pass effect may occur in mice following intraperitoneal administration of **15**. Noteworthy, the value of CL_{int} of **15** is significantly higher than that exhibited by an antidepressant imipramine ($CL_{int} = 0.02$ μL/mg/min with $t_{0.5} = 66$ min) [37]. It, therefore, seems probable that the lack of activity *in vivo* of **15** may be related to its high first pass effect. Another explanation might be a poor distribution to the site of action. Further pharmacological and pharmacokinetic studies are warranted to explain this phenomenon.

Experimental

General

Reagents and organic solvents were from Sigma–Aldrich, Alfa Aesar, and Chempur. Monitoring of the reaction was carried out by TLC performed on silica gel 60 F_{254} aluminum sheets (Merck) with the following solvents: dichloromethane/methanol: A: 9.5:0.5, B: 9:1, C: dichloromethane/triethylamine 9:1, D-ethyl acetate/methanol 9:1. Spots were detected by their absorption under UV light ($\lambda = 254$ nm). ^{1}H-NMR spectra were taken on a Varian Mercury-VX (300 MHz) spectrometer in $CDCl_3$ (**4**, **7**–**18**, and **25**–**34**) or DMSO-d_6 (**19**–**24**) solutions, using signals of solvents residual ^{1}H atoms as internal standard ($\delta = 7.26$ ppm and 2.48 ppm, respectively). Chemical shifts were expressed in δ (ppm) and the coupling constants J in Hertz (Hz). The splitting patterns were designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). The UPLC-MS/MS system consisted of a Waters Acquity UPLC (BEH, C_{18} column; 2.1 mm × 100 mm, and 1.7 μm particle size) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). The column was maintained at 40°C, and eluted under gradient conditions from 95% to 0% of eluent A over 10 min, at a flow

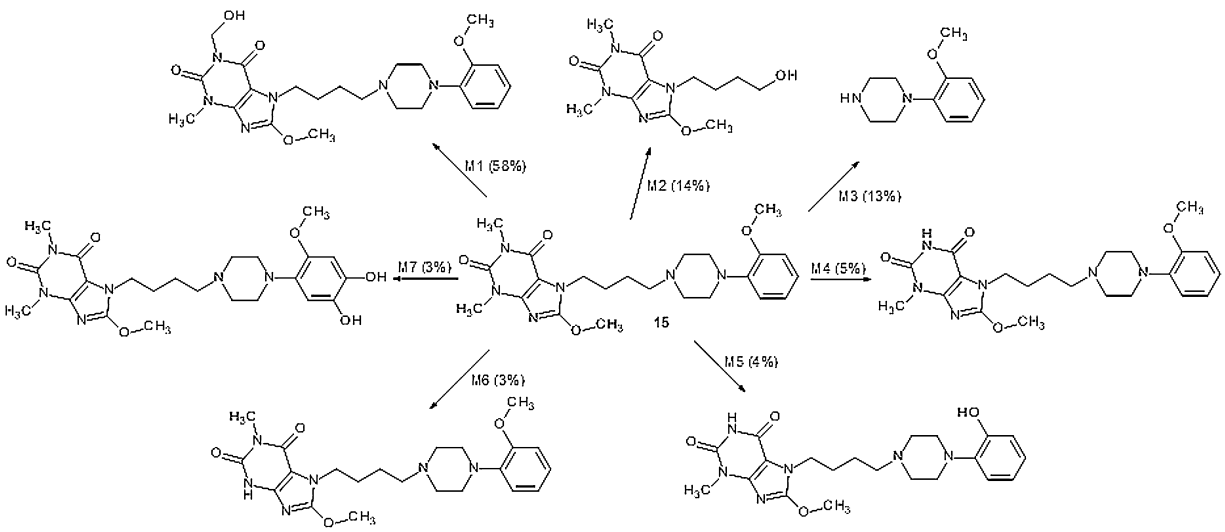


Figure 4. Proposed metabolic pathways of **15** using mouse liver microsomes.

rate of 0.3 mL/min. The eluent A consisted of water/formic acid (0.1%). A total of 10 μL of each sample were injected. The UPLC/MS purity of all the investigated compounds was determined to be over 98%. Elemental analyses were taken with Elementar Vario EL III apparatus and were found within ±0.4% of the theoretical values. Melting points (mp) were determined with a Büchi apparatus and were uncorrected. Column chromatography separations were carried out on column with Merck Kieselgel 60 using solvents A–D.

*Synthesis of compounds **1–4***

The starting 8-bromo-1,3-dimethyl-purine-2,6-dione (**1**) was synthesized by a procedure published elsewhere [22]. 8-Bromo-7-(3-chloropropyl)-1,3-dimethyl-purine-2,6-dione (**2**) [21], 8-bromo-7-(4-chlorobutyl)-1,3-dimethyl-purine-2,6-dione (**3**) [15], and new 8-bromo-7-(5-chloropentyl)-1,3-dimethyl-purine-2,6-dione (**4**) were obtained from **1** according to the previously described method [13].

*8-Bromo-7-(5-chloropentyl)-1,3-dimethyl-purine-2,6-dione **4***

Yield 81%, mp 111–112°C, $R_f = 0.82$ (A), ^{1}H-NMR δ: 1.38–1.58 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.72–2.02 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 3.40 (s, 3H, N1–CH_3), 3.56 (s, 3H, N3–CH_3), 3.51–3.62 (m, 2H, CH_2CH_2–Cl), 4.34 (t, $J = 7.2$ Hz, 2H, N7–CH_2CH_2). LC/MS: *m/z* calc. 363.02, found 363.26. Anal. ($C_{12}H_{16}BrClN_4O_2$) C, H, N.

*Synthesis of compounds **5–8***

The 7-(3-chloropropyl)-8-methoxy-1,3-dimethyl-purine-2,6-dione (**5**) [15], 7-(4-chlorobutyl)-8-methoxy-1,3-dimethyl-purine-2,6-dione (**6**) [15], and new 7-(5-chloropentyl)-8-alkoxy-1,3-dimethyl-purine-2,6-diones (**7–9**) were prepared by a published procedure [15].

*7-(5-Chloropentyl)-8-methoxy-1,3-dimethyl-purine-2,6-dione **7***

Yield 80%, mp 79–81°C, $R_f = 0.49$ (A), ^{1}H-NMR δ: 1,44–1.49 (m, 2H, N7–$(CH_2)_2CH_2CH_2$), 1.75–1.85 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 3.38 (s, 3H, N1–CH_3), 3.52 (s, 3H, N3–CH_3), 3.55 (t, 2H, CH_2CH_2Cl), 4.11 (t, 2H, N7–CH_2CH_2), 4.12 (s, 3H, OCH_3). LC/MS: *m/z* calc. 315.12, found 314.88. Anal. ($C_{13}H_{19}ClN_4O_3$) C, H, N.

*7-(5-Chloropentyl)-8-ethoxy-1,3-dimethyl-purine-2,6-dione **8***

Yield 74%, mp 84–86°C, $R_f = 0.75$ (A), ^{1}H-NMR δ: 1.44–1.49 (m, 5H, N7–$(CH_2)_2CH_2CH_2$ and O–CH_2CH_3), 1.75–1.83 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 3.38 (s, 3H, N1–CH_3); 3.53 (s, 3H, N3–CH_3), 3.49–3.52 (m, 2H, CH_2Cl), 4.05–4.10 (t, $J = 6.9$ Hz, 2H, N7–CH_2), 4.50–4.53 (q, $J = 7.2$ Hz, O–CH_2CH_3). LC/MS: *m/z* calc. 329.13, found 328.92. Anal. ($C_{14}H_{21}ClN_4O_3$) C, H, N.

*7-(5-Chloropentyl)-1,3-dimethyl-8-propoxy-purine-2,6-dione **9***

Yield 84%, mp 48–50°C, $R_f = 0.79$ (A), ^{1}H-NMR δ: 1.03 (t, $J = 7.2$ Hz, 3H, O–$CH_2CH_2CH_3$), 1.41–1.46 (m, 2H, N7 $(CH_2)_2CH_2CH_2$), 1.77–1.82 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 1.85–1.92 (m, 2H, O–$CH_2CH_2CH_3$), 3.37 (s, 3H, N1–CH_3), 3.51 (s, 3H, N3–CH_3), 3.55 (t, $J = 6.9$ Hz, 2H, CH_2Cl), 4.42–4.48 (t, $J = 6.8$ Hz, 2H, O–$CH_2CH_2CH_3$). LC/MS: *m/z* calc. 343.15, found 343.47. Anal. ($C_{15}H_{23}ClN_4O_3$) C, H, N.

*General procedure for the synthesis of 7-arylpiperazinylalkyl-8-alkoxy-purine-2,6-dione derivatives **10–24***

The appropriate 8-alkoxy-purine-2,6-diones **5–9** (50 mmol), respective arylpiperazines (50 mmol), and anhydrous K_2CO_3 (100 mmol) were refluxed in (10 mL) toluene (**10–18**) or 1-propanol (**19–24**) for 30 h .The mixture was filtered off and the solvent was evaporated under reduced pressure. The final products were purified by column chromatography (A). In case of **15** and **19–24**, free bases were converted into hydrochloride by treatment with an excess of conc. HCl to pH 3 in an acetone solution. The precipitated salts were washed with acetone and filtered off.

*8-Methoxy-1,3-dimethyl-7-[3-(4-phenylpiperazin-1-yl)-propyl]-purine-2,6-dione **10***

Yield 58%, mp 151–153°C, $R_f = 0.60$ (A), ^{1}H-NMR δ: 2.23–2.31 (m, 2H, N7–$CH_2CH_2CH_2$), 2.48–2.56 (m, 2H, N7–$CH_2CH_2CH_2$), 3.02–3.13 (m, 4H, N–$(CH_2)_2$), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 3.55–3.76 (m, 4H, $(CH_2)_2$N–Ph), 4.14 (s, 3H, O–CH_3), 4.21–4.26 (m, 2H, N7–CH_2CH_2), 6.85–6.99 (m, 3H, Ph), 7.22–7.31 (m, 2H, Ph). LC/MS: *m/z* calc. 413.22, found 413.39. Anal. ($C_{21}H_{28}N_6O_3$) C, H, N.

*8-Methoxy-7-{3-[4-(2-methoxyphenyl)-piperazin-1-yl]-propyl}-1,3-dimethyl-purine-2,6-dione **11***

Yield 49%, mp 127–129°C, $R_f = 0.50$ (A), ^{1}H-NMR δ: 2.26–2.33 (m, 2H, N7–$CH_2CH_2CH_2$), 2.50–2.58 (m, 2H, N7–$CH_2CH_2CH_2$), 3.02–3.13 (m, 4H, N–$(CH_2)_2$), 3.36 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 3.55–3.76 (m, 4H, $(CH_2)_2$N–Ph), 3.98 (s. 3H, Ph–O–CH_3), 4.15 (s, 3H, O–CH_3), 4.23–4.27 (m, 2H, N7–CH_2CH_2), 6.94–7.80 (m, 4H, Ph). LC/MS: *m/z* calc. 443.24, found 443.35. Anal. ($C_{22}H_{30}N_6O_4$) C, H, N.

*7-{3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propyl}-8-methoxy-1,3-dimethyl-purine-2,6-dione **12***

Yield 67%, mp 137–140°C, $R_f = 0.27$ (A), ^{1}H-NMR δ: 2.23–2.31 (m, 2H, N7–$CH_2CH_2CH_2$), 2.48–2.56 (m, 2H, N7–$CH_2CH_2CH_2$), 3.02–3.13 (m, 4H, N–$(CH_2)_2$), 3.36 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 3.55–3.76 (m, 4H, $(CH_2)_2$N–Ph), 4.15 (s, 3H, O–CH_3), 4.21–4.26 (m, 2H, N7–CH_2CH_2), 7.10–7.43 (m, 4H, Ph). LC/MS: *m/z* calc. 447.19, found 447.43. Anal. ($C_{21}H_{27}ClN_6O_3$) C, H, N.

*7-{3-[4-(4-Fluorophenyl)-piperazin-1-yl]-propyl}-8-methoxy-1,3-dimethyl-purine-2,6-dione **13***

Yield 48%, mp 119–121°C, $R_f = 0.26$ (A), ^{1}H-NMR δ: 1.73–1.88 (m, 2H, N7–$CH_2CH_2CH_2$), 2.37–2.49 (m, 2H, N7–$CH_2CH_2CH_2$), 2.56–2.63 (m, 4H, N–$(CH_2)_2$), 3.07–3.14 (m, 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.12 (s, 3H, O–CH_3), 4.05–4.17 (m, 2H, N7–CH_2CH_2), 6.81–7.01 (m, 4H, Ph). LC/MS: *m/z* calc. 431.22, found 431.33. Anal. ($C_{21}H_{27}FN_6O_3$) C, H, N.

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8-Methoxy-1,3-dimethyl-7-[4-(4-phenylpiperazin-1-yl)-butyl]-purine-2,6-dione ***14***
Yield 45%, mp 117–119°C, $R_f = 0.26$ (A), ^{1}H-NMR δ: 1.43–1.61 (m, 2H, N7–$(CH_2)_2CH_2CH_2$), 1.75–1.88 (m, 2H, N7–$CH_2CH_2(CH_2)_2$), 2.38–2.49 (m, 2H, N7–$(CH_2)_3CH_2$), 2.56–2.63 (m, 4H, $CH_2N(CH_2)_2$), 3.07–3.14 (m 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.05–4.17 (m, 5H, OCH_3 and N7–CH_2), 6.85–7.25 (m, 5H, Ph). LC/MS: *m/z* calc. 427.24, found 427.41. Anal. ($C_{22}H_{30}N_6O_3$) C, H, N.

8-Methoxy-7-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-purine-2,6-dione ***15***
Yield 43%, mp 120–123°C, $R_f = 0.28$ (A), ^{1}H-NMR δ: 1.45–1.60 (m, 2H, N7–$(CH_2)_2CH_2$–CH_2), 1.74–1.87 (m, 2H, N7–$CH_2CH_2(CH_2)_2$), 2.37–2.46 (m, 2H, N7–$(CH_2)_3CH_2$), 2.57–2.67 (m, 4H, $CH_2N(CH_2)_2$), 3.00–3.25 (m, 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.03–4.16 (m, 5H, OCH_3 and N7–CH_2), 6.83–7.01 (m, 4H, Ph). LC/MS:, *m/z* calc. 457.25, found 457.53. Anal. ($C_{23}H_{32}N_6O_4$) C, H, N. **15** · HCl: mp 169–171°C, Anal. ($C_{23}H_{32}N_6O_4$ · HCl) C, H, N.

7-{4-[4-(3-Chlorophenyl)-piperazin-1-yl]-butyl}-8-methoxy-1,3-dimethyl-purine-2,6-dione ***16***
Yield 46%, mp 82–85°C, $R_f = 0.26$ (A), ^{1}H-NMR δ: 1.48–1.63 (m, 2H, N7–$(CH_2)_2CH_2CH_2$), 1.73–1.88 (m, 2H, N7–$CH_2CH_2(CH_2)_2$), 2.35–2.49 (m, 2H, N7–$(CH_2)_3CH_2$), 2.56–2.63 (m, 4H, $CH_2N(CH_2)_2$), 3.08–3.17 (m 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.05–4.17 (m, 5H, OCH_3 and N7–CH_2), 6.85–7.03 (m, 4H, Ph). LC/MS: *m/z* calc. 461.21, found 461.32. Anal. ($C_{22}H_{29}ClN_6O_3$) C, H, N.

7-{4-[4-(4-Fluorophenyl)-piperazin-1-yl]-butyl}-8-methoxy-1,3-dimethyl-purine-2,6-dione ***17***
Yield 41%, mp 113–115°C, $R_f = 0.26$ (A), ^{1}H-NMR δ: 1.46–1.62 (m, 2H, N7–$(CH_2)_2CH_2$–CH_2), 1.73–1.88 (m, 2H, N7–$CH_2CH_2(CH_2)_2$), 2.37–2.49 (m, 2H, N7–$(CH_2)_3CH_2$), 2.56–2.63 (m, 4H, $CH_2N(CH_2)_2$), 3.07–3.14 (m 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.05–4.17 (m, 5H, OCH_3 and N7–CH_2), 6.81–7.01 (m, 4H, Ph). LC/MS: *m/z* calc. 445.23, found 445.36. Anal. ($C_{22}H_{29}FN_6O_3$) C, H, N.

8-Methoxy-1,3-dimethyl-7-[5-(4-phenylpiperazin-1-yl)-pentyl]-purine-2,6-dione ***18***
Yield 39%, mp 110–112°C, $R_f = 0.56$ (A), ^{1}H-NMR δ: 1.27–1.38 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.58–1.60 (m, 2H, N7–$(CH_2)_3CH_2CH_2$), 2.32–2.40 (m, 2H, N7–$(CH_2)_3CH_2CH_2$), 2.56–2.60 (m, 4H, $N(CH_2)_2$), 3.15–3.20 (m, 4H, $(CH_2)_2$N–Ph), 3.38 (s, 3H, N1–CH_3), 3.53 (s, 3H, N3–CH_3), 4.05 (t, $J = 6.5$ Hz 2H, N7–CH_2CH_2), 4.10 (s, 3H, O–CH_3), 6.80–6.95 (m, 3H, Ph), 7.22–7.35 (m, 2H, Ph). LC/MS: *m/z* calc. 441.26, found 44.89. Anal. ($C_{23}H_{32}N_6O_3$) C, H, N.

8-Ethoxy-1,3-dimethyl-7-[5-(4-phenylpiperazin-1-yl)-pentyl]-purine-2,6-dione hydrochloride ***19***
Yield 67%, mp 128–130°C, $R_f = 0.45$ (A), ^{1}H-NMR δ: 1.22–1.26 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.40 (t, $J = 7.1$ Hz, 3H, O–CH_2CH_3), 1.60–1.80 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 3.00–3.18 (m, 6H, $(CH_2)_2$N-Ph and N7–$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.38 (s, 3H, N3–CH_3), 3.40–3.56 (m, 2H, $N(CH_2)_2$ axial), 3.75–3.85 (m, 2H, $N(CH_2)_2$ equat.), 4.10 (t, $J = 6.5$ Hz 2H, N7–CH_2CH_2), 4.50 (q, $J = 7.1$ Hz, 2H, O–CH_2CH_3), 6.80–7.00 (m, 3H, Ph), 7.24–7.32 (m, 2H, Ph), 10.37 (s, 1H, H^+) LC/MS: *m/z* calc. 455.27, found 454.99. Anal. ($C_{24}H_{34}N_6O_3 \cdot HCl$) C, H, N.

8-Ethoxy-7-{5-[4-(2-methoxyphenyl)-piperazin-1-yl]-pentyl}-1,3-dimethyl-purine-2,6-dione hydrochloride ***20***
Yield 62%, mp 157–159°C, $R_f = 0.49$ (A), ^{1}H-NMR δ: 1.21–1.26 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.38 (t, $J = 7.1$ Hz, 3H, O–CH_2CH_3), 1.60–1.80 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 2.82–3.18 (m, 6H, $(CH_2)_2$N–Ph and N7-$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.37 (s, 3H, N3–CH_3), 3.40–3.58 (m, 2H, $N(CH_2)_2$), 3.76 (s, 3H, Ph–OCH_3), 4.00 (t, $J = 6.5$ Hz 2H, N7–CH_2CH_2), 4.50 (q, $J = 7.0$ Hz, 2H, O–CH_2CH_3), 6.82–7.02 (m, 4H, Ph), 10.77 (s, 1H, H^+). LC/MS: *m/z* calc. 485.28, found 484.97. Anal. ($C_{25}H_{36}N_6O_4$ · HCl) C, H, N.

7-{5-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-pentyl}-8-ethoxy-1,3-dimethyl-purine-2,6-dione hydrochloride ***21***
Yield 64%, mp 169–171°C, $R_f = 0.41$ (A), ^{1}H-NMR δ: 1.18–1.28 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.38 (t, $J = 7.1$ Hz, 3H, O–CH_2CH_3), 1.60–1.80 (m, 4H, N7–$CH_2CH_2CH_2CH_2CH_2$), 3.00–3.17 (m, 6H, $(CH_2)_2$N–Ph and N7–$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.37 (s, 3H, N3–CH_3), 3.40–3.57 (m, 2H, $N(CH_2)_2$ axial), 3.80–3.85 (m, 2H, $N(CH_2)_2$ equat.), 4.00 (t, $J = 6.5$ Hz, 2H, N7–CH_2CH_2), 4.50 (q, $J = 7.1$ Hz, 2H, O–CH_2CH_3), 7.00 d, 1H, Ph), 7.22 (s, 1H, Ph), 7.43 (d, 1H, Ph), 10.30 (s, 1H, H^+). LC/MS: *m/z* calc. 523.19, found 523.44. Anal. ($C_{24}H_{32}Cl_2N_6O_3 \cdot HCl$) C, H, N.

1,3-Dimethyl-7-[5-(4-phenylpiperazin-1-yl)-pentyl]-8-propoxy-purine-2,6-dione hydrochloride ***22***
Yield 56%, mp 179–180°C, $R_f = 0.42$ (A), ^{1}H-NMR δ: 0.97 (t, $J = 7.4$ Hz, 3H, O–CH_2CH_3), 1.20–1.38 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.60–1.80 (m, 6H, N7–$CH_2CH_2CH_2CH_2CH_2$ and O–$CH_2CH_2CH_3$), 2.95–3.18 (m, 6H, $(CH_2)_2$N–Ph and N7–$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.37 (s, 3H, N3–CH_3), 3.40–3.60 (m, 2H, $N(CH_2)_2$ axial), 3.72–3.85 (m, 2H, $N(CH_2)_2$ equat.), 4.00 (t, $J = 6.3$ Hz, 2H, N7–CH_2CH_2), 4.40 (t, $J = 7.1$ Hz, 2H, O–$CH_2CH_2CH_3$), 6.84 (t, $J = 7.2$ Hz, 1H, Ph), 7.00 (d, $J = 8.0$ Hz, 2H, Ph), 7.20–7.30 (m, 2H, Ph), 10.18 (s, 1H, H^+). LC/MS: *m/z* calc. 469.29, found 468.96. Anal. ($C_{25}H_{36}N_6O_3 \cdot$ HCl) C, H, N.

7-{5-[4-(2-Methoxyphenyl)-piperazin-1-yl]-pentyl}-1,3-dimethyl-8-propoxy-purine-2,6-dione hydrochloride ***23***
Yield 59%, mp 190–192°C, $R_f = 0.45$ (A), ^{1}H-NMR δ: 0.97 (t, $J = 7.4$ Hz, 3H, O–$(CH_2)_2CH_3$), 1.18–1.26 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.59–1.84 (m, 6H, N7–$CH_2CH_2CH_2CH_2CH_2$ and O–$CH_2CH_2CH_3$), 2.95–3.18 (m, 6H, $(CH_2)_2$N–Ph and N7–$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.37 (s, 3H, N3–CH_3), 3.41–3.55 (m, 4H, $N(CH_2)_2$), 3.76 (s, 3H, Ph–OCH_3), 4.05 (t, $J = 6.0$ Hz, 2H, N7–$CH_2(CH_2)_4$), 4.42 (t, $J = 6.5$ Hz, 2H, O-$CH_2C_2H_5$), 6.86–7.02 (m, 4H, Ph), 10.42 (s, 1H, H^+). LC/MS: *m/z* calc. 499.30, found 499.13. Anal. ($C_{26}H_{38}N_6O_4 \cdot HCl$) C, H, N.

7-{5-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-pentyl}-8-ethoxy-1,3-dimethyl-8-propoxy-purine-2,6-dione hydrochloride ***24***

Yield 65%, mp 201–203°C, $R_f = 0.43$ (A), ^{1}H-NMR δ: 0.98 (t, $J =$ 7.4 Hz, 3H, O–$(CH_2)_2CH_3$), 1.20–1.28 (m, 2H, N7–$(CH_2)_2CH_2(CH_2)_2$), 1.60–1.80 (m, 6H, N7–$CH_2CH_2CH_2CH_2CH_2$ and O–$CH_2CH_2CH_3$), 2.96–3.20 (m, 6H, $(CH_2)_2$N–Ph and N7–$(CH_2)_3CH_2CH_2$), 3.20 (s, 3H, N1–CH_3), 3.37 (s, 3H, N3–CH_3), 3.46 (d, $J = 9.7$ Hz, 2H, N$(CH_2)_2$ axial), 3.85 (d, $J = 10.5$ Hz, 2H, N$(CH_2)_2$ equat.), 4.04 (t, $J = 6.0$ Hz, 2H, N7–$CH_2(CH_2)_4$), 4.41 (t, $J = 6.3$ Hz, 2H, O–$CH_2C_2H_5$), 6.97 (d, 1H, Ph), 7.21 (s, 1H, Ph), 7.42 (d, 1H, Ph), 10.65 (s, 1H, H^+). LC/MS: *m/z* calc. 537.48, found 537.03. Anal. ($C_{25}H_{34}Cl_2N_6O_3 \cdot$ HCl) C, H, N.

General procedures for the preparation of derivatives of phenylpiperazinylmethyloxiranes ***25–29*** *[38]*

Epichlorohydrin (1.9 mmol) was added all at once and with vigorous stirring to corresponding phenylpiperazines (1.9 mmol) containing 0.6 mL of water. The reaction mixtures were maintained between 18 and 35°C (1 h), and next heated to 75°C and treated during 15 min with 2.31 g of aqueous NaOH (38.4%). The cooled solutions were filtered and the final compounds were purified by column chromatography (C).

3-(4-Phenyl-piperazinyl)-1,2-epoxypropane ***25***

Yield 83%; mp 82–84°C; $R_f = 0.71$ (C); ^{1}H-NMR δ: 2.29–2.35 (m, 1H, NCH_2), 2.50–2.53 (m, 1H, CH_2CH), 2.63–2.70 (m, 2H, CH_2CH+NCH_2), 2.74–2.85 (m, 4H, N$(CH_2)_2$), 3.11–3.27 (m, 5H, N$(CH_2)_2$+CH), 6.80–6.94 (m, 3H, Ph), 7.24–7.29 (m, 2H, Ph). LC/MS: *m/z* calc. 218.14, found 219.30 $(M+H)^+$. Anal. ($C_{13}H_{18}N_2O$) C, H, N.

3[4-(2′-Methoxyphenyl)-piperazinyl]-1,2-epoxypropane ***26***

Yield 90%; mp 76–77°C; $R_f = 0.71$ (C); ^{1}H-NMR δ: 2.34–2.39 (m, 1H, NCH_2), 2.51–2.53 (m, 1H, CH_2CH), 2.60–2.89 (m, 6H, CH_2CH+NCH_2+N$(CH_2)_2$), 3.12–3.24 (m, 5H, N$(CH_2)_2$+CH), 3.86 (s, 3H, OCH_3), 6.85–7.00 (m, 4H, Ph). LC/MS: *m/z* calc. 248.15, found 249.33 $(M+H)^+$. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

3[4-(3′-Chlorophenyl)-piperazinyl]-1,2-epoxypropane ***27***

Yield 90%; mp 139–141°C; $R_f = 0.71$ (C); ^{1}H-NMR δ: 2.48–2.59 (m, 2H, CH_2CH+NCH_2), 2.62–2.85 (m, 4H, N$(CH_2)_2$), 3.18–3.29 (m, 6H, N$(CH_2)_2$+CH_2CH+NCH_2), 3.59–3.61 (m, 1H, CH), 6.76–6.90 (m, 3H, Ph), 7.14–7.19 (m, 1H, Ph). LC/MS: *m/z* calc. 252.10, found 253.73 $(M+H)^+$. Anal. ($C_{13}H_{17}ClN_2O$) C, H, N.

3[4-(2′,3′-Dichlorophenyl)-piperazinyl]-1,2-epoxypropane ***28***

Yield 87%; mp 145–146°C, $R_f = 0.68$ (C); ^{1}H-NMR δ: 2.34–2.88 (m, 6H, N$(CH_2)_2$+CH_2CH+NCH_2), 3.14–3.16 (m, 4H, N$(CH_2)_2$), 3.44–3.62 (m, 3H, CH_2CH+NCH_2+CH), 6.93–6.98 (m, 1H, Ph), 7.14–7.16 (m, 2H, Ph). LC/MS: *m/z* calc. 286.06, found 288.19 $(M+H)^+$. Anal. ($C_{13}H_{16}Cl_2N_2O$) C, H, N.

3[4-(3′,4′-Dichlorophenyl)-piperazinyl]-1,2-epoxypropane ***29***

Yield 82%; mp 156–157°C, $R_f = 0.68$ (C); ^{1}H-NMR δ: 2.42–2.86 (m, 6H, N$(CH_2)_2$+CH_2CH+NCH_2), 3.10–3.22 (m, 5H, N$(CH_2)_2$+CH_2CH), 3.44–3.69 (m, 2H, CH_2CH+NCH_2), 6.68–6.78 (m, 1H, Ph), 6.89–6.98 (m, 1H, Ph), 7.23–7.28 (m, 1H, Ph). LC/MS: *m/z* calc. 286.06, found 288.19 $(M+H)^+$. Anal. ($C_{13}H_{16}Cl_2N_2O$) C, H, N.

General procedures for the preparation of derivatives of 7-arylpiperazinylmethyldihydro[1,3]oxazolo[2,3-f]-purinediones ***30–34***

A mixture of 8-bromo-1,3-dimethyl-purine-2,6-dione (10 mmol) and 20 mmol of the corresponding substituted oxiranes was heated under reflux in anhydrous 1-propanol in the presence of catalytic amount of pyridine (0.3 mL) for 10 h. After cooling, the solvent was evaporated, the residue was mixed with 10% NaOH, and washed with water. The final compounds were purified by column chromatography using C (**30**, **31**, **34**), B (**32**) or D (**33**) as solvent.

7-[(4-Phenyl)-piperazin-1-yl-methylene]-1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-f]-purine-2,4(1H,3H)-dione ***30***

Yield 89%; mp 123–124°C, $R_f = 0.4$ (C); ^{1}H-NMR δ: 3.21–3.40 (m, 8H, N$(CH_2)_4$N, 3.45 (s, 3H, N3–CH_3), 3.55 (s, 3H, N1–CH_3), 4.20 (t, $J = 8.02$ Hz, 1H, CH_2), 4.40–4.52 (m, 1H, CH_2), 4.55–4.68 (m, 2H, CH_2), 5.92–6.05 (m, 1H, CH), 6.84–6.98 (m, 3H, Ph), 7.25–7.34 (m, 2H, Ph). LC/MS: *m/z* calc. 396.19, found 397.45 $(M+H)^+$. Anal. ($C_{20}H_{24}N_6O_3$) C, H, N.

7-[(4-(-2′-Methoxyphenyl)-piperazin-1-yl-methylene]-1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-f]-purine-2,4-(1H,3H)-dione ***31***

Yield 90%; mp 132–133°C, $R_f = 0.34$ (C); ^{1}H-NMR δ: 3.25–3.48 (m, 8H, N$(CH_2)_4$N, 3.42 (s, 3H, N3–CH_3), 3.58 (s, 3H, N1–CH_3), 3.80 (s, 3H, OCH_3), 4.17 (t, $J = 8.02$ Hz, 1H, CH_2), 4.46–4.54 (m, 1H, CH_2), 4.58–4.67 (m, 2H, CH_2), 5.87–6.05 (m, 1H, CH), 6.85–7.04 (m, 4H, Ph). LC/MS: *m/z* calc. 426.20, found 427.45 $(M+H)^+$. Anal. ($C_{21}H_{26}N_6O_4$) C, H, N.

7-[(4-(-3′-Chlorophenyl)-piperazin-1-yl-methylene]-1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-f]-purine-2,4-(1H,3H)-dione ***32***

Yield 90%; mp 178–179°C, $R_f = 0.68$ (B); ^{1}H-NMR δ: 3.29–3.44 (m, 8H, N$(CH_2)_4$N, 3.46 (s, 3H, N3–CH_3), 3.58 (s, 3H, N1–CH_3), 4.21 (t, $J = 8.02$ Hz, 1H, CH_2), 4.47–4.54 (m, 1H, CH_2), 4.59–4.70 (m, 2H, CH_2), 5.94–6.08 (m, 1H, CH), 6.76–6.91 (m, 3H, Ph), 7.15–7.23 (m, 1H, Ph). LC/MS: *m/z* calc. 430.15, found 431.90 $(M+H)^+$. Anal. ($C_{20}H_{23}ClN_6O_3$) C, H, N.

7-[(4-(-2′,3′-Dichlorophenyl)-piperazin-1-yl-methylene]-1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-f]-purine-2,4-(1H,3H)-dione ***33***

Yield 90%; mp 166–167°C, $R_f = 0.28$ (D); ^{1}H-NMR δ: 3.28–3.46 (m, 8H, N$(CH_2)_4$N, 3.51 (s, 3H, N3–CH_3), 3.61 (s, 3H, N1–CH_3), 4.20–4.32 (t, $J = 5.5$Hz, 1H, CH_2), 4.48–4.56 (m, 1H, CH_2), 4.57–4.67 (m, 2H, CH_2), 5.95–6.04 (m, 1H, CH), 6.93–6.98 (m, 1H, Ph), 7.16–7.18 (m, 2H, Ph). LC/MS: *m/z* calc. 464.11, found 466.34 $(M+H)^+$. Anal. ($C_{20}H_{22}Cl_2N_6O_3$) C, H, N.

7-[(4-(-3′,4′-Dichlorophenyl)-piperazin-1-yl-methylene]-1,3-dimethyl-6,7-dihydro-1,3-oxazolo[2,3-f]-purine-2,4 (1H,3H)-dione ***34***

Yield 90%; mp 155–156°C, $R_f = 0.7$ (C); ^{1}H-NMR δ: 3.32–3.49 (m, 8H, N(CH_2)$_4$N, 3.56 (s, 3H, N_3–CH_3), 3.64 (s, 3H, N_1–CH_3), 4.23–4.31 (t, $J = 5.5$Hz, 1H, CH_2), 4.54–4.61 (m, 1H, CH_2), 4.66–4.75 (m, 2H, CH_2), 6.03–6.11 (m, 1H, C*H*), 6.71–6.80 (m, 1H, Ph), 6.89–7.01 (m, 1H, Ph), 7.22–7.28 (m, 1H, Ph). LC/MS: *m/z* calc. 464.11, found 466.34 $(M+H)^+$. Anal. ($C_{20}H_{22}Cl_2N_6O_3$) C, H, N.

In vitro radioligand binding assays

Compounds **10**–**24** were tested in competition binding experiments by displacement of respective radioligands from cloned human Rs, all stably expressed in HEK-293 cells: [^{3}H]-8-OH-DPAT, [^{3}H]-ketanserin), [^{3}H]-LSD, [^{3}H]-5-CT, and [^{3}H]-raclopride for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and dopamine D_2 Rs, respectively, according to the previously published procedures [11, 23, 24]. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated based on the method described in literature [39]. Results were expressed as means of at least two separate experiments.

Compounds **30**–**34** were tested in a screening assay at two final concentrations: 10^{-6} M and 10^{-7} M. Detailed conditions of the assays for the respective Rs were previously reported [25]. Results were expressed as a percentage of specific binding.

Molecular modeling

The homology models of human 5-HT_{1A} serotonin receptor used for docking studies were described in previously published papers [20, 40, 41].

The homology models were built and optimized according to a validated method [42] on the basis of β_2 adrenergic receptor crystal structure (PDB ID: 2RH1) [43]. Ligand structures were optimized using LigPrep and Jaguar tools. Glide XP flexible docking was carried out using default parameters, setting H-bond constraints on Asp3.32.

Glide, Jaguar, and LigPrep were implemented in Small-Molecule Drug Discovery Suite (Schroödinger, Inc.), which was licensed for Jagiellonian University Medical College.

In vivo experiments

The experiments were performed on male Albino Swiss or CD-1 mice (22–28 g). Detailed conditions of the procedures and reagents were previously reported [20]. D-Amphetamine (Sigma–Aldrich) was administered subcutaneously (*sc*), 30 min before the test; D-amphetamine and **15** were dissolved in distilled water. All the experimental procedures were approved by the Local Ethics Committee for Animal Experiments of Jagiellonian University in Cracow.

Forced swim (Porsolt) test in Swiss Albino mice

The experiment was carried out according to the method of Porsolt et al. [31] and procedures published before [20]. 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.

Four-plate test in Swiss Albino mice

The experiment was assessed according to the method of Aron et al. [32]. The method and conditions of experiment were previously reported [20]. The number of punished crossings received by an animal was recorded during the 60 s period.

D-Amphetamine-induced hyperactivity in CD-1 mice

Locomotor activity was recorded with an Opto M3 multichannel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). The mice were individually placed in plastic cages (22 cm × 12 cm × 13 cm) for 30 min habituation period, and then the crossings of each channel (ambulation) were counted during 1 h with data recording every 5 min.

The metabolic studies in mouse liver microsomes

The incubation systems were composed of the tested compound (20 μM in 100 mM potassium phosphate buffer, pH 7.4), mouse liver microsomes (Sigma–Aldrich) (0.8 mg/mL), and NADPH-regenerating system [44]. The resulting mixture was incubated for 5, 15, or 30 min at 37°C. Then, an internal standard (levallorphan, 20 μM) was added. The incubation was terminated at different time points by the addition of perchloric acid (69–72%, by volume). Proteins were sedimented by centrifugation. The resulting supernatant was analyzed using UPLC/MS in order to determine the quantity of starting material left in solution and possible metabolites. The tests without NADPH-regenerating system were conducted in parallel. All probes were done in duplicate [45, 46].

Statistical analysis

All the data are presented as the mean ± SEM. The statistical significance of results was evaluated by a one-way analysis of variance (ANOVA) followed by Bonferroni's comparison test. A $p < 0.05$ was considered statistically significant.

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