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New 8-aminoalkyl derivatives of purine-2,6-dione with arylalkyl, allyl or propynyl substituents in position 7, their 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptor affinity and pharmacological evaluation

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Abstract:

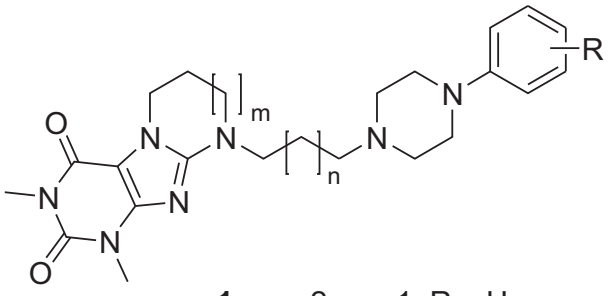
Background: Our previous studies in a group of arylpiperazine derivatives of 1,3-dimethyl-3,7-dihydro-purine-2,6-diones, aimed at chemical diversification of the purine-2,6-dione by introduction of hydrophobic substituent in a 7- or 8- position or elongation of the linker length between arylpiperazine and purine core, allowed a selection of potent 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptor ligands displaying anxiolytic and antidepressant properties. Continuing our research in this field, in the present studies we designed a new series of 8-aminoalkylamino (**15**–**35**) and 8-arylpiperazinylpropoxy (**36**–**42**) derivatives of 7-substituted 1,3-dimethyl-3,7-dihydro-purine-2,6-dione as potential 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptor ligands with potential psychotropic activity.

Methods: Radioligand binding assays were employed for determining the affinity and the selectivity profile of the synthesized compounds for native 5-HT_{1A}, 5-HT_{2A}, and cloned 5-HT_6 and 5-HT_7 receptors. The functional activity of the selected compounds at 5-HT_{1A} and 5-HT_{2A} receptors was tested in the commonly used *in vivo* models. Antidepressant and anxiolytic properties were evaluated in the forced swim (FST) and the four-plate test (FPT) in mice, respectively.

Results: Among the evaluated series, selected 7-benzyl-8-((4-(4-(3-chlorophenyl)piperazin-1-yl)butyl)amino)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**21**), a mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 receptor ligand, produced an antidepressant-like effect in FST, and exerted anxiolytic-like activity in FPT. Another pharmacologically evaluated compound **42** (a mixed 5-HT_{1A}/5-HT_7 ligand) slightly, but non-significantly attenuated the immobility time of mice in FST and was devoid of activity in FPT.

Conclusions: Study revealed advantage of mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 receptor ligands over 5-HT_{1A}/5-HT_7 agents to display antidepressant- and anxiolytic-like activity. Modification of arylalkyl/allyl substituent in position 7 of purine-2,6-dione opens possibility for designing new 5-HT ligands with preserved π electron system and lower molecular weight.

Key words:
purine-2,6-diones, 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 receptor ligands, depression, forced swim test, anxiety, four-plate test



1: m = 2, n = 1, R = H
2: m = 1, n = 1, R = H
3: m = 1, n = 2, R = H
4: m = 1, n = 1, R = 2-OCH_3

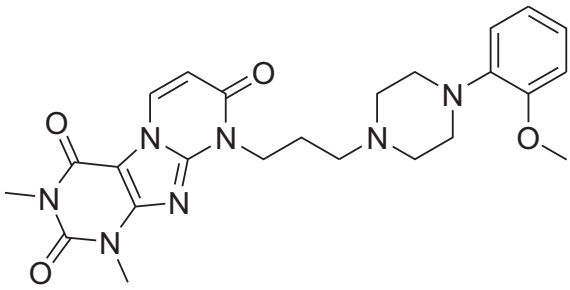


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Fig. 1. Chemical structure of the initial model compounds **1**–**5**

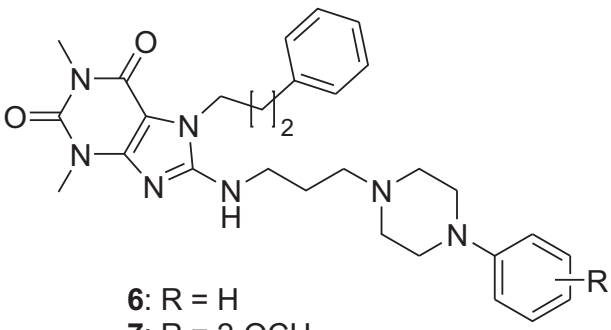
Introduction

A diversity of psychiatric disorders have been associated with dysfunction of serotonin-containing neurons, including depression, anxiety, schizophrenia, bipolar disorder, and Parkinson's disease. Modulation of these serotonin (5-HT) pathways, ranging from activation to blockade of 5-HT receptor subtypes, gave basis for development of several classes of psychotropic drugs or designing compounds being under clinical trials.

Among 5-HT receptor subtypes, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors have focused our interest as suitable targets for treatment of depression and anxiety [3, 15, 21, 22]. 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 towards 5-HT_{1A} and 5-HT_{2A} receptors, and recently 5-HT_7 [5, 7, 8, 19, 20, 28, 29]. 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 annelated heterocyclic ring of lactam or non-lactam structure, i.e., pyrimido[2,1-f]purine and diazepino[2,1-*f*]purine [8, 16, 20].

It was found that 1,3-dimethyl-10-[3-(4-phenylpiperazin-1-yl)-propyl]-2,4-dioxo-1,3,6,7,8,9-hexahydro-10H-1,3-diazepino-[2,1-*f*]-purine (**1**) and its analog **2** behaved as 5-HT_{1A} postsynaptic antagonists, whereas 1,3-dimethyl-9-[3-(4-phenylpiperazin-1-yl)-butyl]-2,4-dioxo-1,3,6,7,8,9-hexahydropyrimido-[2,1-*f*]-purine (**3**) behaved as partial agonist of 5-HT_{1A} receptors [8] (Fig. 1). We successively examined the influence of structural modifications of pyrimido- or diazepino-[2,1-*f*]-purines on the 5-HT_{1A} receptors affinity and 5-HT_{1A}/5-HT_{2A} selectivity; in consequence, pharmacological assays showed that compound **4** behaved as presynaptic agonist and postsynaptic partial agonist of 5-HT_{1A} receptors, while **5** as pre- and post-synaptic agonist of 5-HT_{1A} receptors, respectively [20]. It has been shown that different functional activity of these compounds can be attributed to the enlargement of the π system in **5** *vs.* **4** and/or to the diminished flexibility of the heterocyclic fragment [20].

To evaluate the influence of the 1,3-diazepine or pyrimido-purine rings on the binding affinity, we developed a series of 8-arylpiperazinylpropylamine derivatives of 7-alkyl, 7-arylalkyl substituted theophylline (1,3-dimethyl-3,7-dihydropurine-2,6-dione) [5, 7, 27]. The structural modification involved the opening



6: R = H
7: R = 2-OCH_3
8: R = 3-Cl-PP

Fig. 2. General structure of 8-arylpiperazinylpropylamino derivatives of 7-substituted purine-2,6-diones

of the terminal 1,3-diazepine or pyrimido-purine cores in compounds **1**–**4** to give 8-arylpiperazinylpropylamine derivatives. Among them, the most interesting 7-arylalkyl-8-alkylaminopurine-2,6-dione derivatives displayed high-to-moderate affinity for 5-HT_{1A} receptors and moderate-to-low affinity for 5-HT_{2A} sites (Fig. 2, Tab. 1). Those compounds, examined in functional *in vivo* models, behaved as postsynaptic 5-HT_{1A} receptor antagonists [5].

To continue our research with 7-arylalkyl-8-alkylaminopurine-2,6-dione derivatives we designed and synthesized some analogs of the previously evaluated series. Since 7-arylpiperazinylbutyl derivatives of the previously reported [7] 8-alkoxy-purine-2,6-diones behaved as highly active 5-HT_{1A} receptor ligands (K_i = 11–19 nM) with distinct affinity for 5-HT_7 receptors (K_i = 51–83 nM) the first planned structural modifications consisted in elongation of the linker length between 8-aminopurine-2,6-dione core and arylpiperazine fragment from three to four carbon unit.

Further, by replacing an arylalkyl group with allyl and propynyl substituents, we obtained a series of 8-arylpiperazinylalkyl amino analogs with preserved π electron system and similar low conformational flexibility.

To extend structure-activity relationships, we replaced the arylpiperazinylalkylamino fragment in the 8 position of purine 2,6-dione with arylpiperazinylalkoxy moiety in the series of previously reported propylene derivatives **6**–**8**. This modification aimed at investigating the influence of electron-donor properties of the nitrogen-to-oxygen switch on the receptor binding profile.

The influence of the structural modification on the activity for serotonin receptors was investigated in classic arylpiperazine (R = H, 2-OCH_3, 3-Cl, 4-F) or 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives.

Herein, we report on the synthesis of the new designed compounds **15**–**42**, their biological evaluation related with 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7 receptors, and determination of their *in vivo* properties in animal models of anxiety and depression.

Materials and Methods

Chemical methodology

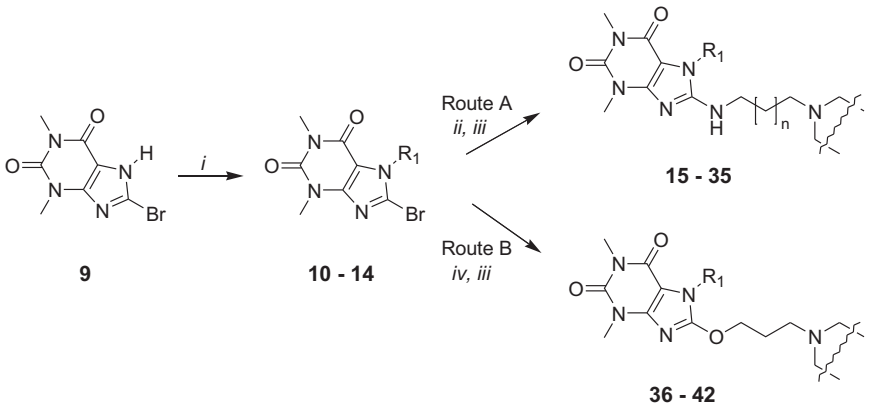
Melting points (m.p.) were determined in open glass capillaries with Büchi 353 melting point apparatus and are uncorrected. ^{1}H-NMR spectra were taken with a Varian BB 200 (300 MHz) spectrophotometer in DMSO solution. Chemical shifts are expressed in δ (ppm), and the coupling constants, *J*, are given in hertz (Hz). The purity of the compounds were routinely checked by thin layer chromatography (TLC) using Kieselgel 60 F_{254} sheets and the following eluents: S_1: benzene/acetone = 7:3, v/v, S_2: benzene/acetone/methanol = 1:1:1, v/v/v. Spots were detected under UV light. Elemental analyses were determined with an Elementar Vario EL III apparatus and were within ± 0.4% of the theoretical values.

The 8-bromo-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (8-BrTh) **9** [11] and its 7-benzyl, 7-phenylethyl, 7-phenylpropyl, 7-allyl, and 7-propyn-2-yn-1-yl (propargyl) derivatives **10**–**14** were synthesized according to the procedure published in the literature [10] and were used for the synthesis of new compounds **15**–**35** and **36**–**42**. 7-Arylalkyl- and 7-allyl-purine-2,6-dione derivatives **15**–**33** were synthesized according to the published procedure by nucleophilic substitution of **10**–**14** with 10% molar excess of the appropriate primary amines (arylpiperazinylalkylamine or tetrahydroisoquinolinylalkylamine) in boiling *n*-butanol in the presence of K_2CO_3, (Scheme 1, Route A; Tab. 1) [5]. In the same conditions, 7-propargyl-8-bromo-1,3-dimethyl-3,7-dihydropurine-2,6-dione (**14**) underwent the sigmatropic rearrangement yielding respective propyn-1-yn-1-yl derivatives **34** and **35**.

8-Arylpiperazin-1-ylpropoxy- derivatives of 7-arylalkyl-purine-2,6-diones (**36**–**42**) were obtained from **10**–**12** by heating them with 10% molar excess of respective 3-[(4-aryl)-piperazin-1-yl]-propan-1-ols [12] in toluene in the presence of finely powdered KOH (Scheme 1, Route B; Tab. 2). Compounds **15**–**42** were isolated as water-soluble hydrochloride salts and were purified by recrystallization from anhydrous ethanol (compounds **15**–**33**) or methanol (compounds **34** and **35**).

7-Benzyl-1,3-dimethyl-8-((4-(4-phenylpiperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (15)

The title compound was obtained in 65% yield, starting from **10**; m.p. 228–230°C; R_f = 0.77 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.56–1.66 (m, 2H), 1.70–1.71 (m, 2H), 2.98–3.18 (m, 9H), 3.35–3.40 (m, 5H), 3.46 (d, 2H, *J* = 5.8 Hz), 3.74 (d, 2H, *J* = 5.8 Hz), 5.34 (s, 2H), 6.81–6.99 (m, 3H), 7.21–7.35 (m, 7H), 7.39–7.43 (t, 1H, *J* = 5.5 Hz), 10.87 (s, 1H). Analysis: for $C_{28}H_{36}ClN_7O_2 \times ½ H_2O$ (547.06): C, H, N.



Scheme 1. The synthesis of 8-aminoalkyl derivatives of 7-substituted-purine-2,6-dione derivatives **15**–**35** and 8-arylpiperazinylalkoxy derivatives of 7-substituted-purine-2,6-dione derivatives **36**–**42**: (*i*) R_1-Cl or R_1-Br, K_2CO_3, TEBA, Me_2CO, 12 h; (*ii*) arylpiperazinylalkylamine or tetrahydroisoquinolinylalkylamine, K_2CO_3, *n*-butanol, 40 h; (*iii*) conc. HCl in anh. ethanol; (*iv*) arylpiperazinylpropanol, KOH, *n*-butanol, 40 h

1,3-Dimethyl-7-phenethyl-8-((4-(4-phenylpiperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (16)

The title compound was obtained in 54% yield, starting from **11**; m.p. 240–242°C; R_f = 0.84 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.50–1.60 (m, 2H), 1.70–1.71 (m, 2H), 2.85–3.10 (m, 9H), 3.35–3.40 (m, 5H), 3.07 (t, 2H, *J* = 6.7 Hz), 3.46 (d, 2H, *J* = 5.5 Hz), 3.75 (d, 2H, *J* = 5.5 Hz), 4.25 (t, 2H, *J* = 6.7 Hz), 6.81–6.99 (m, 3H), 7.21–7.35 (m, 7H), 7.39–7.43 (t, 1H, *J* = 5 Hz), 10.51 (s, 1H). Analysis: for $C_{29}H_{38}ClN_7O_2 \times ½ H_2O$ (561.10): C, H, N.

1,3-Dimethyl-8-((4-(4-phenylpiperazin-1-yl)butyl)amino)-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (17)

The title compound was obtained in 31% yield, starting from **12**; m.p. 244–246°C; R_f = 0.95 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.52–1.65 (m, 2H), 1.75–1.88 (m, 2H), 1.87–1.97 (m, 2H), 2.56 (t, 2H, *J* = 8.1 Hz), 3.01–3.18 (m, 9H), 3.30–3.39 (m, 5H), 3.47 (d, 2H, *J* = 5.4 Hz), 3.71 (d, 2H, *J* = 5.5 Hz), 4.12 (t, 2H, *J* = 7.1 Hz), 6.85 (t, 1H, *J* = 7.2 Hz), 7.11–7.30 (m, 10H), 11.04 (s, 1H). Analysis: for $C_{30}H_{40}ClN_7O_2 \times 1½ H_2O$ (593.12): C, H, N.

7-Benzyl-8-((4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)amino)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (18)

The title compound was obtained in 33% yield, starting from **10**; m.p. 240–242°C; R_f = 0.60 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.58–1.62 (m, 2H), 1.67–1.69 (m, 2H), 2.99–3.19 (m, 9H), 3.21–3.53 (m, 9H), 3.77 (s, 3H), 5.21 (s, 2H), 6.90–7.04 (m, 5H), 7.20–7.37 (m, 6H), 10.48 (s, 1H). Analysis: for $C_{29}H_{38}ClN_7O_3 \times 2 H_2O$ (603.10): C, H, N.

1,3-Dimethyl-7-phenethyl-8-((4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (19)

The title compound was obtained in 32% yield, starting from **11**; m.p. 264–265°C; R_f = 0.65 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.53–1.57 (m, 2H), 1.70–1.75 (m, 2H), 2.91 (t, 2H, *J* = 7.7 Hz), 2.98–3.19 (m, 9H), 3.31–3.39 (m, 5H), 3.41–3.52 (m, 4H), 3.77 (s, 3H), 4.23 (t, 2H, *J* = 7.6 Hz), 6.88–7.03 (m, 4H), 7.17–7.28 (m, 6H), 10.48 (s, 1H). Analysis: for $C_{30}H_{40}ClN_7O_3 \times 2½ H_2O$ (626.12): C, H, N.

1,3-Dimethyl-8-((4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)amino)-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (20)

The title compound was obtained in 30% yield, starting from **12**; m.p. 267–269°C; R_f = 0.75 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.52–1.65 (m, 2H), 1.75–1.88 (m, 2H), 1.87–1.97 (m, 2H), 2.55 (t, 2H, *J* = 8.0 Hz), 3.01–3.20 (m, 9H), 3.31–3.38 (m, 5H), 3.47–3.53 (m, 4H), 3.78 (s, 3H), 4.12 (t, 2H, *J* = 7.1 Hz), 6.82 (t, 1H, *J* = 7.2 Hz), 7.15–7.30 (m, 9H), 11.04 (s, 1H). Analysis: for $C_{31}H_{42}ClN_7O_3 \times H_2O$ (613.31): C, H, N.

7-Benzyl-8-((4-(4-(3-chlorophenyl)piperazin-1-yl)butyl)amino)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (21)

The title compound was obtained in 45% yield, starting from **10**; m.p. 245–247°C; R_f = 0.78 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.55–1.60 (m, 2H), 1.66–1.71 (m, 2H), 2.99–3.20 (m, 9H), 3.33–3.41 (m, 5H), 3.44 (d, 2H, *J* = 7.1 Hz), 3.84 (d, 2H, *J* = 6.4 Hz), 5.33 (s, 2H), 6.84–7.39 (m, 9H), 10.48 (s, 1H). Analysis: for $C_{28}H_{34}Cl_2N_7O_2 \times ½ H_2O$ (580.52): C, H, N.

1,3-Dimethyl-7-phenethyl-8-((4-(4-(3-chlorophenyl)piperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (22)

The title compound was obtained in 39% yield, starting from **11**; m.p. 239–241°C; R_f = 0.79 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.52–1.57 (m, 2H), 1.72–1.75 (m, 2H), 2.89 (t, 2H, *J* = 7.6 Hz), 3.11–3.19 (m, 9H), 3.29–3.39 (m, 5H), 3.41–3.48 (m, 2H), 3.84–3.94 (m, 2H), 3.98 (t, 2H, *J* = 7.6 Hz), 6.84–7.31 (m, 10H), 12.41 (s, 1H). Analysis: for $C_{29}H_{37}Cl_2N_7O_2 \times 1½ H_2O$ (612.55): C, H, N.

1,3-Dimethyl-8-((4-(4-(3-chlorophenyl)piperazin-1-yl)butyl)amino)-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (23)

The title compound was obtained in 37% yield, starting from **12**; m.p. 234–237°C; R_f = 0.82 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.59–1.66 (m, 2H), 1.75–1.79 (m, 2H), 1.90–1.97 (m, 2H), 2.57 (t, 2H, *J* = 8.1 Hz), 3.02–3.22 (m, 9H), 3.32–3.40 (m, 5H), 3.43–3.50 (d, 2H, *J* = 5.0 Hz), 3.79–3.84 (d, 2H, *J* = 5.8 Hz), 4.12 (t, 2H, *J* = 7.1 Hz), 6.84–7.27 (m, 10H), 10.57 (s, 1H). Analysis: for $C_{30}H_{39}Cl_2N_7O_2 \times H_2O$ (617.57): C, H, N.

7-Benzyl-8-((3-(3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)amino)-1,3-dimethyl-1H-purine-2,6(3*H*,7*H*)-dione hydrochloride (24)

The title compound was obtained in 72% yield, starting from **10**; m.p. 227–230°C; R_f = 0.71 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.88–1.95 (m, 2H), 2.71 (t, 2H), 2.75–2.81 (m, 4H), 3.33 (s, 3H), 3.52 (s, 3H), 3.61–3.67 (m, 4H), 4.95 (s, 2H), 6.84 (t, 1H, *J* = 4.6 Hz), 6.91–7.17 (m, 4H), 7.18–7.21 (m, 5H), 10.82 (s, 1H). Analysis: for $C_{26}H_{31}ClN_6O_2$ (496.02): C, H, N.

8-((3-(3,4-Dihydroisoquinolin-2(1*H*)-yl)propyl)amino)-1,3-dimethyl-7-phenethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (25)

The title compound was obtained in 64% yield, starting from **11**; m.p. 213–217°C; R_f = 0.63 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.02–2.09 (m, 2H), 2.87–2.91 (m, 3H), 3.10–3.24 (m, 6H), 3.30–3.42 (m, 6H), 3.58–3.65 (m, 1H), 4.05 (t, 2H, *J* = 7.5 Hz), 4.20–4.25 (m, 1H), 4.51 (d, 1H, *J* = 7.3 Hz), 7.19–7.29 (m, 10H), 10.99 (s, 1H). Analysis: for $C_{27}H_{33}ClN_6O_2 \times 2H_2O$ (546.55): C, H, N.

8-((3-(3,4-Dihydroisoquinolin-2(1*H*)-yl)propyl)-amino)-1,3-dimethyl-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (26)

The title compound was obtained in 47% yield, starting from **12**; m.p. 223–225°C; R_f = 0.79 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.87–1.97 (m, 2H), 2.12–2.17 (m, 2H), 2.61 (t, 2H, *J* = 8.0 Hz), 2.92–3.10 (m, 2H), 3.10–3.22 (m, 5H), 3.34 (s, 3H), 3.30–3.35 (m, 2H), 4.12 (t, 2H, *J* = 7.2 Hz), 4.20–4.25 (m, 2H), 4.25–4.28 (m, 1H), 4.50 (d, 1H, *J* = 6.9 Hz), 7.09–7.27 (m, 9H), 7.58–7.60 (m, 1H), 10.74 (s, 1H). Analysis: for $C_{28}H_{35}ClN_6O_2 \times 2¼ H_2O$ (564.57): C, H, N.

7-Benzyl-8-((4-(3,4-dihydroisoquinolin- 2(1*H*)-yl)butyl)amino)-1,3-dimethyl-1H-purine-2,6(3*H*,7*H*)-dione hydrochloride (27)

The title compound was obtained in 34% yield, starting from **10**; m.p. 250–252°C; R_f = 0.73 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.59–1.66 (m, 2H), 1.73–1.78 (m, 2H), 2.95–3.01 (m, 2H), 3.11–3.22 (m, 6H), 3.30–3.38 (m, 4H), 3.40–3.43 (m, 2H), 4.26–4.29 (m, 1H), 4.41 (d, 1H, *J* = 7.8 Hz), 5.33 (s, 2H), 7.14–7.34 (m, 9H), 7.39 (t, 1H, *J* = 5.4 Hz), 10.74 (s, 1H). Analysis: for $C_{27}H_{33}ClN_6O_2 \times H_2O$ (527.04): C, H, N.

8-((4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)butyl) amino)-1,3-dimethyl-7-phenethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (28)

The title compound was obtained in 32% yield, starting from **11**; m.p. 235–236°C; R_f = 0.65 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.57–1.59 (m, 2H), 1.80–1.84 (m, 2H), 2.89 (d, 2H, *J* = 7.8 Hz), 3.18–3.34 (m, 13H), 3.60–3.64 (m, 1H), 4.24–4.28 (m, 3H), 4.46–4.50 (d, 1H, *J* = 6.5 Hz), 7.14–7.28 (m, 10H), 10.89 (s, 1H). Analysis: for $C_{28}H_{35}ClN_6O_2 \times 2H_2O$ (559.06): C, H, N.

8-((4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)butyl)-amino)-1,3-dimethyl-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (29)

The title compound was obtained in 31% yield, starting from **12**; m.p. 150–152°C; R_f = 0.67 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.63–1.67 (m, 2H), 1.87–1.95 (m, 4H), 2.58 (t, 2H, *J* = 8.1 Hz), 2.61–2.98 (m, 2H), 3.13–3.22 (m, 7H), 3.32–3.38 (m, 5H), 4.14 (t, 2H, *J* = 7.1 Hz), 4.23–4.29 (m, 1H), 4.44 (d, 1H, *J* = 6.4 Hz), 7.10–7.25 (m, 10H), 10.89 (s, 1H). Analysis: for $C_{29}H_{37}ClN_6O_2 \times 3½H_2O$ (600.09): C, H, N.

7-Allyl-1,3-dimethyl-8-((4-(4-phenylpiperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (30)

The title compound was obtained in 28% yield, starting from **13**; m.p. 245–247°C; R_f = 0.26 (S_1). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.58–1.62 (m, 2H), 1.75–1.79 (m, 2H), 3.05–3.11 (m, 6H), 3.14 (s, 3H); 3.35 (s, 3H), 3.35–3.36 (m, 2H), 3.47–3.53 (m, 4H), 4.69–4.70 (m, 2H), 4.96–5.09 (m, 1H), 5.12–5.13 (m, 1H), 5.84–5.92 (m, 1H), 6.72–6.76 (m, 1H), 6.82–6.87 (m, 1H), 6.96–6.99 (m, 4H), 10.84 (s, 1H). Analysis: for $C_{24}H_{34}ClN_7O_2$ (488.03): C, H, N.

7-Allyl-1,3-dimethyl-8-((3-(4-(2-methoxyphenyl)-piperazin-1-yl)propyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (31)

The title compound was obtained in 21% yield, starting from **13**; m.p. 207–209°C; R_f = 0.25 (S_1), R_f = 0.73 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.02–2.09 (m, 2H), 3.09–3.14 (m, 6H), 3.15 (s, 3H), 3.36 (s, 3H), 3.38–3.53 (m, 4H), 3.77 (s, 3H), 3.81–3.84 (m, 2H), 4.71–4.73 (m, 2H), 5.02–5.09 (m, 1H), 5.12–5.13 (m, 1H), 5.77–5.93 (m, 1H), 6.88–7.01 (m, 4H), 7.35–7.39 (m, 1H), 10.52 (s, 1H). Analysis: for $C_{24}H_{34}ClN_7O_3$ (504.08): C, H, N.

7-Allyl-1,3-dimethyl-8-((4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (32)

The title compound was obtained in 27% yield, starting from **13**; m.p. 240–242°C; R_f = 0.22 (S_1), R_f = 0.70 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.59–1.62 (m, 2H), 1.75–1.82 (m, 2H), 3.15 (s, 3H), 3.16–3.27 (m, 6H), 3.35 (s, 3H), 3.43–3.47 (m, 4H), 3.77 (s, 3H), 3.77–4.15 (m, 2H), 4.71–4.73 (m, 2H), 4.93–5.10 (m, 1H), 5.12–5.13 (m, 1H), 5.76–5.92 (m, 1H), 6.86–7.01 (m, 4H), 7.20–7.28 (m, 1H), 10.63 (s, 1H). Analysis: for $C_{25}H_{36}ClN_7O_3$ (518.10): C, H, N.

7-Allyl-1,3-dimethyl-8-((4-(4-(4-fluorophenyl)-piperazin-1-yl)butyl)amino)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (33)

The title compound was obtained in 26% yield, starting from **13**; m.p. 221–223°C; R_f = 0.25 (S_1). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.57–1.62 (m, 2H), 1.71–1.73 (m, 2H), 3.02–3.02 (m, 6H), 3.15 (s, 3H); 3.35 (s, 3H), 3.46–3.58 (m, 2H), 3.66–3.76 (m, 2H), 4.68–4.70 (m, 2H), 4.88–4,98 (m, 2H), 5.08–5.15 (m, 1H), 5.80–5.94 (m, 2H), 6.96–7.14 (m, 5H), 10.21 (s, 1H). Analysis: for $C_{24}H_{33}ClFN_7O_2$ (506.02): C, H, N.

1,3-Dimethyl-8-((3-(4-phenylpiperazin-1-yl)propyl)amino)-7-(prop-1-yn-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (34)

The title compound was obtained in 40% yield, starting from **14**; m.p. 224–225°C; R_f = 0.36 (S_1), R_f = 0.74 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.39 (s, 3H), 2.64–2.69 (m, 2H), 2.99–3.02 (m, 2H), 3.09–3.15 (m, 2H), 3.42 (s, 3H), 3.43–3.56 (m, 2H), 3.56 (s, 3H), 3.57–3.65 (m, 4H), 4.19–4.23 (m, 2H), 6.84–6.96 (m, 1H), 6.97–7.00 (m, 2H), 7.22–7.26 (m, 3H), 10.23 (s, 1H) . Analysis: for $C_{23}H_{30}ClN_7O_2$ (471.21): C, H, N.

8-((3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)amino)-1,3-dimethyl-7-(prop-1-yn-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (35)

The title compound was obtained in 25% yield, starting from **14**; m.p. 237–239°C; R_f = 0.22 (S_1), R_f = 0.72 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.40 (s, 3H), 2.62–2.67 (m, 2H), 3.18–3.38 (m, 2H), 3.17–3.63 (m, 8H), 3.43 (s, 3H), 3.59 (s, 3H), 3.90 (s, 3H), 4.21–4.26 (m, 2H), 6.92–6.96 (m, 2H), 7.15–7.25 (m, 3H), 10.35 (s, 1H) . Analysis: for $C_{24}H_{32}ClN_7O_3$ (501.23): C, H, N.

7-Benzyl-1,3-dimethyl-8-(3-(4-phenylpiperazin-1-yl)propoxy)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (36)

The title compound was obtained in 57% yield, starting from **10**; m.p. 158–161°C; R_f = 0.16 (S_1), R_f = 0.77 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.07–2.25 (m, 2H), 3.08–3.11 (m, 6H), 3.20 (s, 3H), 3.37 (s, 3H), 3.55 (d, 2H, *J* = 6.2 Hz), 3.81 (d, 2H, *J* = 8.5 Hz),

4.53 (t, 2H, *J* = 5.6 Hz), 5.26 (s, 2H), 6.87 (t, 3H, *J* = 7.2Hz) , 6.97 (d, 2H, *J* = 9.3 Hz), 7.22–7.37 (m, 5H), 10.71 (s, 1H). Analysis: for $C_{27}H_{33}ClN_6O_3 \times H_2O$ (543.05): C, H, N.

1,3-Dimethyl-8-(3-(4-phenylpiperazin-1-yl)propoxy)-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (37)

The title compound was obtained in 53% yield, starting from **12**; m.p. 200–202°C; R_f = 0.35 (S_1), R_f = 0.88 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.98–2.08 (m, 2H), 2.24–2.47 (m, 2H), 2.58 (t, 2H, *J* = 8.2 Hz), 3.03–3.15 (m, 4H), 3.10–21 (m, 4H), 3.31 (s, 3H), 3.35 (s, 3H), 3.81 (d, 2H, *J* = 10.5 Hz), 4.07 (t, 2H, *J* = 6.9 Hz), 4.52 (t, 2H, *J* = 5.9 Hz), 6.85 (t, 1H, *J* = 7.2 Hz), 7.11 (d, 2H, *J* = 9.2 Hz), 7.14–7.21 (m, 2H), 7.22–7.27 (m, 5H), 10.50 (s, 1H). Analysis: for $C_{29}H_{37}ClN_6O_3 \times H_2O$ (553.11): C, H, N.

7-Benzyl-8-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propoxy)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (38)

The title compound was obtained in 22% yield, starting from **10**; m.p. 145–147°C; R_f = 0.30 (S_1), R_f = 0.86 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.18–2.28 (m, 2H), 2.99 (t, 2H, *J* = 11.5 Hz), 3.09–3.12 (m, 4H), 3.20 (s, 3H), 3.37 (s, 3H), 3.46–3.57 (m, 4H), 3.78 (s, 3H), 4.53 (t, 2H, *J* = 5.4 Hz), 5.26 (s, 2H), 6.89–7.02 (m, 4H), 7.28–7.38 (m, 5H), 10.55 (s, 1H). Analysis: for $C_{28}H_{35}ClN_6O_4 \times 2H_2O$ (590.26): C, H, N.

7-Benzyl-8-(3-(4-(3-chlorophenyl)piperazin-1-yl)propoxy)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (39)

The title compound was obtained in 57% yield, starting from **10**; m.p. 129–132°C; R_f = 0.27 (S_1), R_f = 0.88 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.22–2.78 (m, 2H), 3.03–3.14 (m, 6H), 3.19 (s, 3H), 3.37 (s, 3H), 3.78–3.98 (m, 4H), 4.53 (t, 2H, *J* = 5.9 Hz), 5.25 (s, 2H), 6.84 (d, 1H, *J* = 1.3 Hz), 6.96 (d, 2H, *J* = 1.8 Hz), 7.04 (t, 1H, *J* = 2.3 Hz), 7.22–7.37 (m, 5H), 11.22 (s, 1H). Analysis: for $C_{27}H_{32}Cl_2N_6O_3$ (559.50): C, H, N.

1,3-Dimethyl-8-(3-(4-(3-chlorophenyl)piperazin-1-yl)propoxy)-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (40)

The title compound was obtained in 50% yield, starting from **12**; m.p. 214–218°C; R_f = 0.37 (S_1), R_f = 0.88 (S_2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.87–1.97 (m, 4H), 2.02–2.30 (m, 2H), 2.55 (t, 2H, *J* = 7.2 Hz), 3.04–3.11 (m, 4H), 3.15 (s, 3H), 3.28 (s, 3H), 3.42–3.58 (m, 4H), 3.74 (t, 2H, *J* = 6.4 Hz), 3.82 (t, 2H, *J* = 2.3 Hz), 6.85 (d, 1H, *J* = 1.3 Hz), 6.94 (d, 2H, *J* = 2.6 Hz), 7.02 (s, 1H), 7.09–7.25 (m, 5H), 11.54 (s, 1H). Analysis: for $C_{29}H_{36}Cl_2N_6O_3 \times 3½H_2O$ (650.55): C, H, N.

8-(3-(4-(4-Fluorophenyl)piperazin-1-yl)propoxy)-1,3-dimethyl-7-phenethyl-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (41)

The title compound was obtained in 20% yield, starting from **11**; m.p. 178–179°C; R_f = 0.42 (S_1). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.06–2.12 (m, 2H), 2.96–2.99 (m, 2H), 3.01–3.16 (m, 6H), 3.23 (s, 3H), 3.36 (s, 3H), 3.40–3.50 (m, 2H), 3.54–3.58 (m, 2H), 3.72–3.76 (m, 2H), 4.21–4.26 (m, 2H), 6.99–7.20 (m 5H), 7.22–7.30 (m, 4H), 10.65 (s, 1H). Analysis: for $C_{28}H_{34}ClFN_6O_3$ (557.06): C, H, N.

8-(3-(4-(4-Fluorophenyl)piperazin-1-yl)propoxy)-1,3-dimethyl-7-(3-phenylpropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (42)

The title compound was obtained in 17% yield, starting from **11**; m.p. 151–152°C; R_f = 0.46 (S_1). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.99–2.10 (m, 4H), 2.95–2.97 (m, 2H), 3.00–3.13 (m, 6H), 3.23 (s, 3H), 3.35 (s, 3H), 3.43–3.50 (m, 2H), 3.55–3.60 (m, 2H), 3.71–3.74 (m, 2H), 4.21–4.26 (m, 2H), 7.05–7.20 (m 5H), 7.22–7.30 (m, 4H), 10.42 (s, 1H). Analysis: for $C_{29}H_{36}ClFN_6O_3$ (571.09): C, H, N.

PHARMACOLOGY

In vitro evaluation

Investigated compounds (7–9 concentrations) were tested in competition binding experiments for native 5-HT_{1A}, 5-HT_{2A} receptors, as well as for cloned human 5-HT_6 and 5-HT_7 receptors, according to the previously published procedures [2, 18, 28].

For 5-HT_{1A} and 5-HT_{2A} receptors, experiments were carried out using membranes from rat hippocampus and [^{3}H]-8-OH-DPAT or rat cortex and [^{3}H]-ketanserin, respectively. Following incubation, the receptor preparations were rapidly filtered under vacuum through GF/B glass fiber filters which were washed extensively with an ice cold 50 mM Tris buffer (pH 7.4) using a Brandel harvester. Radioactivity was determined by liquid scintillation counting in a Beckman LS 6500 apparatus [2].

Binding assays on membranes from HEK 293 cells stably expressing human 5-$HT_{7(b)}$ or 5-HT_6 receptors [28] were performed with the use of [^{3}H]-LSD, and [^{3}H]-5-CT as radioligands, respectively. The experiments were performed in a 96-well plate using a MultiPROBE II Liquid Handling System. After 1-h incubation at 37°C, the assay samples were rapidly filtered using a Unifilter harvester and plates were subsequently washed with ice-cold 50 mM Tris buffer (pH 7.4). Radioactivity retained on the filters was quantified on a Microbeta plate reader.

The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation [4]. Results are expressed as the means of at least three separate experiments.

Animals and drugs used

The experiments were performed on male Albino Swiss mice (24–28 g) obtained from a licensed breeder (Staniszewska, Ilkowice, Poland) and male Wistar rats (accredited facility at Faculty of Pharmacy, Jagiellonian University, Kraków, Poland). The animals were housed in groups of 15 (mice) or 4 (rats) for 3–4 days in polycarbonate Makrolon type 3 cages, in an environmentally controlled experimental room (ambient temperature 22 ± 1°C; relative humidity 50–60%; 12:12 light:dark cycle, lights on at 8 a.m.). Standard laboratory pellets and filtered water were freely available. Each experimental group consisted of 5–10 animals/dose, and all the animals were used only once. Compounds **21**, **42** and diazepam (Polfa, Poznań) were suspended in a 1% aqueous solution of Tween 80 while imipramine (ADAMED Ltd,, Pieńków, Poland), 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT hydrobromide, RBI), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)DOI hydrochloride, RBI) were dissolved in distilled water. All compounds, except 8-OH-DPAT that was given subscutaneously (*sc*), were injected intraperitoneally (*ip*) in a volume of 10 ml/kg (mice) or 2 ml/kg (rats) 30 min before the test, excluding diazepam and 8-OH-DPAT which were administered 60 and 15 min, respectively, before testing and (±)DOI that was given immediately before scoring. The experimental procedures used were approved by the I Local Ethics Commission at the Jagiellonian University in Kraków.

Lower lip retraction (LLR) in rats

LLR was assessed according to the method described by Berendsen et al. [1]. The rats were individually placed in cages (30 × 25 × 25 cm) and were scored three times (at 15, 30 and 45 min after administration of the tested compounds) as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The total maximum score amounted to 3/rat. In a separate experiment, the effect of the studied compounds on LLR induced by 8-OH-DPAT (1 mg/kg) was tested.

Head-twitch responses in mice

In order to habituate mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter) and 20 cm (height) glass cage, lined with sawdust 20 min before the treatment. Head twitches in mice were induced by (±)DOI (2.5 mg/kg) [9]. Immediately after treatment, the number of head twitches was counted during 20 min. ID_{50} (the dose inhibiting the head twitches in mice by 50%) was calculated using Graph Pad Prism 5 software.

Forced swim test in mice

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

Four-plate test in mice

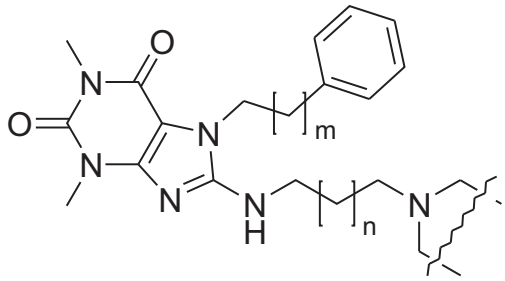
The four-plate test apparatus (BIOSEB, France) consists of a cage (25 × 18 × 16 cm) floored by four identical rectangular metal plates (8 × 11 cm) separated from one another by a gap of 4 mm. The top of the cage is covered by a transparent Perspex lid that prevents escape behavior. The plates are connected to

a device that can generate electric shocks. Following a 15-s habituation period, the animal's motivation to explore a novel environment was suppressed by an electric foot shock (0.8 mA, 0.5 s) every time it moved from one plate to another during a 1-min test session. This action was referred to as a 'punished crossing', and was followed by a 3 s shock interval, during which the animal could move across plates without receiving a shock.

Spontaneous locomotor activity

The locomotor activity was recorded with an Opto M3 multi-channel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). Mice were individually placed in plastic cages (22 × 12 × 13 cm) for 30 min habituation period, and then the crossings of each channel (ambulation) were counted during the first 6 min with data recording every 1 min. Data recorded

Tab. 1. Binding affinities of the 8-aminoalkylamino derivatives of 7-substituted-purine-2,6-dione derivatives **6**–**8** and **15**–**29** for 5-HT receptors



Compound	m	n	Amine[a]	K_i ± SEM [nM]			
				5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
6[b]	2	1	PP	50 ± 2	445 ± 15	> 1 μM	94 ± 3
7[b]	2	1	2-MPP	8 ± 0.3	304 ± 6	> 1 μM	25 ± 1
8[b]	2	1	3-ClPP	10 ± 0.4	346 ± 3	> 1 μM	130 ± 9
15	0	2	PP	39 ± 2	259 ± 13	> 1 μM	90 ± 6
16	1	2	PP	68 ± 4	298 ± 21	> 1 μM	25 ± 2
17	2	2	PP	20 ± 1	116 ± 14	958 ± 17	36 ± 2
18	0	2	2-MPP	4.3 ± 0.5	162 ± 18	> 10 μM	40 ± 4
19	1	2	2-MPP	4.5 ± 0.3	400 ± 25	> 10 μM	10 ± 1
20	2	2	2-MPP	4.5 ± 0.4	179 ± 9	> 10 μM	20 ± 3
21	0	2	3-ClPP	25 ± 3	68 ± 13	> 1 μM	65 ± 5
22	1	2	3-ClPP	66 ± 9	115 ± 17	911 ± 22	113 ± 8
23	2	2	3-ClPP	18 ± 2	114 ± 21	98 ± 22	85 ± 6
24	0	1	THIQ	204 ± 14	428 ± 58	> 1 μM	454 ± 32
25	1	1	THIQ	146 ± 8	3517 ± 654	> 1 μM	3590 ± 220
26	2	1	THIQ	488 ± 22	1789 ± 233	> 1 μM	> 10 μM
27	0	2	THIQ	354 ± 39	574 ± 44	> 1 μM	302 ± 19
28	1	2	THIQ	56 ± 7	2335 ± 308	> 10 μM	99 ± 7
29	2	2	THIQ	104 ± 15	269 ± 23	> 1 μM	239 ± 13

[a] PP – phenylpiperazine; 2-MPP – 2-methoxyphenylpiperazine; 3-ClPP – 3-chlorophenylpiperazine; THIQ – 1,2,3,4-tetrahydroisoquinoline;
[b] compounds reported in ref. [5]; affinities for 5-HT_6 and 5-HT_7 receptors are presented herein for the first time

after 1 min (i.e., the time equal to the observation period in the four-plate test) and recorded between 2–6 min (i.e., the time equal to the observation period in the forced swim test) were calculated. The cages were cleaned up with 70% ethanol after each mouse.

Statistical analysis

The obtained data are presented as the mean ± SEM and were analyzed by a one-way ANOVA followed by Bonferroni's test.

Results

The newly synthesized derivatives of purine-2,6-dione **15**–**42** showed a diversified level of affinity for 5-HT_{1A} receptors, ranging from 4.3 nM to 899 nM, and displayed high-to-low affinity for 5-HT_{2A}R (68–17330 nM) and for 5-HT_7R (10–26000 nM). The compounds practically did not bind to 5-HT_6R, except compound **23** possessing a $K_{i5\text{-}HT6}$ = 98 nM. The receptor binding properties of the new compounds are presented in Tables 1, 2 and 3.

Lower lip retraction in rats

8-OH-DPAT induced LLR with the maximum possible score being 90%. Compound **21** evoked LLR with score being 23% and simultaneously it had no effect on the 8-OH-DPAT-induced LLR. Derivative **42** dose-dependently attenuated LLR evoked by 8-OH- DPAT in rats (Tab. 4); calculated ID_{50} dose is 19.92 (18.80-21.05) mg/kg.

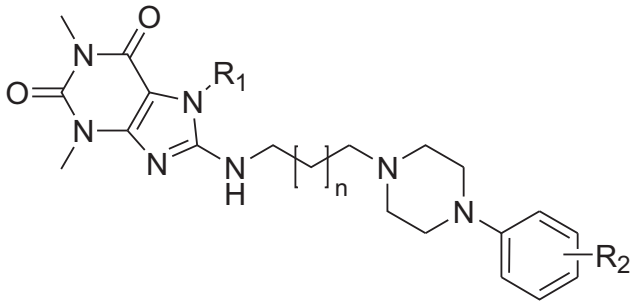
Head twitch responses in mice

Both compounds tested dose-dependently antagonized the effect of (±)DOI in mice. Accordingly to differences in their 5-HT_{2A} receptor affinities, compound **21** produced stronger effect than **42** as compared their ID_{50} doses (Tab. 4).

Forced swim test

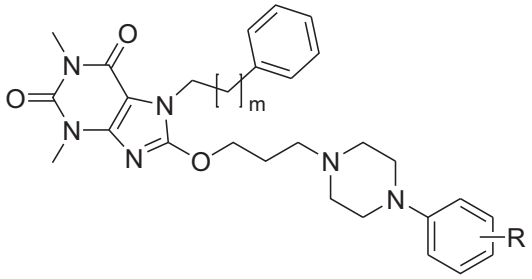
Table 5 shows that **21** at doses of 20 and 30 mg/kg (but not 10 mg/kg) significantly reduced (by 33%) the immobility time of mice in the forced swim test. Compound **42** administered at a dose of 20 mg/kg, slightly but non-significantly, attenuated the immobility time. Imipramine, given as a reference drug, was ineffective at a dose of 5 mg/kg, but at higher ones (10 and 20 mg/kg), it significantly shortened (by 52% and 27%, respectively) the immobility time of mice.

Tab. 2. Binding affinities of the 8-arylpiperazinylalkylamino derivatives of 7-allyl/propynyl substituted-purine-2,6-diones **30**–**35** for 5-HT receptors



Compound	R_1	n	R_2	K_i ± SEM [nM]			
				5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
30	allyl	2	H	70 ± 9	302 ± 15	5706 ± 663	476 ± 38
31	allyl	1	2-OCH_3	15 ± 2	1411 ± 158	> 10 μM	274 ± 15
32	allyl	2	2-OCH_3	20 ± 3	475 ± 69	> 10 μM	622 ± 33
33	allyl	2	4-F	133 ± 17	72 ± 9	> 10 μM	269 ± 18
34	prop-1-yn-1-yl	1	H	899 ± 112	902 ± 54	> 10 μM	1200 ± 6 5
35	prop-1-yn-1-yl	1	2-OCH_3	230 ± 34	> 10 μM	> 10 μM	740 ± 86

Tab. 3. Binding affinities of the 8-arylpiperazinylpropoxy derivatives of 7-substituted purine-2,6-diones **36**–**42** for 5-HT receptors



Compound	m	R	K_i ± SEM [nM]			
			5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
36	0	H	7.6 ±1.0	348 ± 41	> 1 μM	89 ± 5
37	2	H	64 ± 7	154 ± 12	> 1 μM	165 ± 15
38	0	2-OCH_3	48 ± 6	480 ± 62	> 1 μM	75 ± 6
39	0	3-Cl	477 ± 66	388 ± 21	> 1 μM	295 ± 17
40	2	3-Cl	151 ± 20	398 ± 53	> 1 μM	936 ± 54
41	1	4-F	631 ± 47	80 ± 11	> 1 μM	105 ± 12
42	2	4-F	34 ± 2	210 ± 31	717 ± 15	63 ± 4

Tab. 4. Induction of lower lip retraction (LLR) (A) by the tested compounds, and their effect on the 8-OH-DPAT-induced LLR (B) in rats and (±)DOI-induced head twitches (C) in mice

Treatment	Dose (mg/kg)	LLR mean ± SEM behavioral score		ID_{50}
		A	B	C
Vehicle	–	0.1 ± 0.1	2.7 ± 0.2	
21	10	0.2 ± 0.1	2.5 ± 0.2	19.79 (18.24-21.35)
	20	0.7 ± 0.3	2.5 ± 0.2	
	30	0.7 ± 0.2[a]	2.4 ± 0.3	
42	10	0.0 ± 0.0	2.3 ± 0.4	30.22 (29.29-31.16)
	20	0.3 ± 0.2	2.0 ± 0.2	
	30	0.0 ± 0.0	1.6 ± 0.1[a]	

All compounds were administrated *ip* 30 min before the test, except 8-OH-DPAT (*sc*) and (±)DOI that were given 15 min and immediately, respectively, before scoring; n = 5-6 animals per group. [a] $p < 0.05$ *vs.* the respective vehicle group (one way ANOVA followed by Bonferroni's *post-hoc* test); ID_{50} (the dose inhibiting the head twitches in mice by 50% calculated using Graph Pad Prism 5 software), confidence limits (95%) given in parentheses

Tab. 5. Effects of compounds **21**, **42** and imipramine in the forced swim test in mice

Treatment	Dose (mg/kg)	Immobility time (s) mean ± SEM
Vehicle	–	185.6 ± 8.1
21	10	169.3 ± 9.5
	20	123.9 ± 11.3[a]
	30	124.0 ± 17.6[b]
		F (3, 28) = 6.657 $p < 0.01$
42	10	184.7 ± 9.7
	20	155.0 ± 14.1
	30	210.3 ± 9.1
		F (3, 31) = 4.649 $p < 0.01$
Vehicle	–	162.7 ± 6.8
Imipramine	5	170.4 ± 10.9
	10	77.8 ± 12.2[b]
	20	119.6 ± 13.0[a]
		F (3, 36) = 16.757 $p < 0.0001$

All compounds were administrated *ip* 30 min before the test; n = 8–9 mice per group. [a] $p < 0.05$, [b] $p < 0.001$ *vs.* the respective vehicle group (one way ANOVA followed by Bonferroni's *post-hoc* test)

Tab. 6. Effects of compounds **21**, **42** and diazepam in the four plate test in mice

Treatment	Dose (mg/kg)	Number of punished crossings mean ± SEM
Vehicle	–	2.2 ± 0.2
21	10	3.1 ± 0.4
	20	3.5 ± 0.3[a]
		F (2, 23) = 5.709 p < 0.01
42	10	2.2 ± 0.4
	20	2.1 ± 0.3
	30	1.8 ± 0.3
		F (3, 36) = 0.342 ns
Vehicle	–	4.2 ± 0.4
Diazepam	1.25	5.8 ± 0.3[b]
	2.5	6.4 ± 0.5[b]
	5	6.6 ± 0.4[a]
		F (3, 36) = 6.455 p < 0.01

All compounds were administrated *ip* 30 min, excluding diazepam which was administered 60 min, before the test; n = 9–10 mice per group. [a] p < 0.05, [b] p < 0.001 *vs.* the respective vehicle group (one way ANOVA followed by Bonferroni's *post-hoc* test); ns – non significant

Tab. 7. Effects of compounds **21**, **42**, imipramine and diazepam on the spontaneous locomotor activity in mice

Treatment	Dose (mg/kg)	Number of crossings mean ± SEM during	
		1 min	2–6 min
Vehicle	–	69.0 ± 17.8	211.33 ± 20.2
21	20	15.0 ± 8.9[a]	34.3 ± 18.4[b]
	30	17.7 ± 6.3[a]	84.2 ± 33.7[b]
		F (2, 26) = 6.247 p < 0.05	F (2, 26) = 12.530 p < 0.001
42	30	NT	2.44 ± 1.5[b]
		NT	F (1, 16) = 106.550 p < 0.001
Vehicle	–	NT	116.1 ± 41.9
Imipramine	10	NT	28.7 ± 17.4
	20	NT	18.4 ± 9.4[a]
			F (2, 27) = 4.031 p < 0.05
Vehicle	–	47.6 ± 21.3	NT
Diazepam	1.25	34.0 ± 12.6	NT
	2.5	30.3 ± 16.3[b]	NT
	5	23.9 ± 12.2[b]	NT
		F (3, 34) = 1.314 ns	

All compounds were administrated *ip* 30 min, excluding diazepam which was administered 60 min, before the test; n = 9–10 mice per group. [a] p < 0.05, [b] p < 0.001 *vs.* the respective vehicle group (one way ANOVA followed by Bonferroni's *post-hoc* test); ns – non significant. NT – not tested

Four-plate test

Table 6 shows that **21** at a dose of 20 mg/kg (but not 10 mg/kg) significantly increased (by 59%) the number of punished crossings recorded in the four-plate test in mice. Compound **42** given at doses of 10, 20 and 30 mg/kg was ineffective in that test. Administered as a reference drug, diazepam, at doses of 1.25, 2.5 and 5 mg/kg significantly increased (by 28%, 52% and 57%, respectively) the number of punished crossings in mice.

Spontaneous locomotor activity

Derivative **21** administered at doses of 20 and 30 mg/kg significantly decreased spontaneous locomotor activity of mice during 1-min and 4-min observation sessions (Tab. 7). Compound **42** (30 mg/kg) produced a significant sedative effect during a 4-min observation period (i.e., the period of time identical to the observation period in the forced swim test). Both imipramine (20 mg/kg) and diazepam (2.5 and 5 mg/kg) significantly decreased locomotor activity of mice during observation period of 4- and 1-min, respectively.

Discussion

First of all, we analyzed the impact of elongation of the linker length between purine-2,6-dione core and arylpiperazine fragment from three- to four-carbon units on the selected 5-HT receptors affinity. Generally this modification confirmed the rules describing relation between the length of a spacer and the affinity of LCAP derivatives for 5-HT_{1A}R. All the 2-methoxyphenylpiperazine (2-MPP) derivatives containing four-methylene group spacer (**18**–**20**) displayed high affinity for 5-HT_{1A}R and 5-HT_7R, 4.3–4.5 nM and 10–40 nM, respectively, and behaved as dual 5-HT_{1A}/5-HT_7 receptor ligands. Their unsubstituted phenylpiperazine counterparts (**15**–**17**) displayed slightly lower affinity with K_i ranging from 20 to 68 nM for

5-HT_{1A} and from 25 to 36 nM for 5-HT_7 receptors. Interestingly, one of the unsubstituted phenylpiperazines (**16**) was classified as dual 5-HT_{1A}/ 5-HT_7 ligand that preferentially bind to 5-HT_7R ($S_{5\text{-HT7/5-HT1A}} = 2.6$). Introduction of 3-chloro substituent decreased affinity for both 5-HT_{1A} and 5-HT_7 receptors, and 3-chlorophenylpiperazine (3-Cl-PP) derivatives (**21**–**23**) displayed high-to-moderate affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. At the same time this modification shifted affinity for 5-HT_6R from μM to nM range. Among 3-Cl-PP derivatives the most potent compounds **21** and **23** behaved as mixed 5-HT_{1A}/ 5-HT_{2A}/5-HT_7 and 5-HT_{1A}/5-HT_6/5-HT_7 ligands, respectively.

In contrast to data reported by other authors, [17, 24] replacement of 2-MPP moiety with 1,2,3,4-tetrahydroisoquinoline (THIQ) revealed to be unfavorable for affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors. Among THIQ derivatives **27**–**29** only compound **28**, containing phenylethyl substituent in 7-position, displayed affinity for 5-HT_{1A} and 5-HT_7Rs lower than 100 nM. According to the general rule of the influence of the linker length, propylene analogs (**24**–**26**) were even less active.

Within the evaluated series, we could not found a decisive dependence between the length of alkylene fragment connecting purine-2,6-dione core with additional aryl moiety in 7-position. With respect to the linker length in 7-position all the 2-MPP displayed almost the same affinity for 5-HT_{1A}R (5 nM). The highest affinity for 5-HT_7R within the series of unsubstituted, 2-MPP, and THIQ derivatives was displayed by compounds with an ethylene spacer, while, within compounds containing 3-Cl-PP moiety benzyl group revealed to be the best. Interestingly enough, a three-methylene spacer in 7-position separating purine-2,6-dione and phenyl ring was preferential for binding of PP and 3-Cl-PP derivatives, e.g., **17** and **23**, for 5-HT_6Rs.

In the following step, we studied the replacement of an arylalkyl group in 7-position of purine-2,6-dione with allyl and propynyl substituents. The 8-arylpiperazinylalkylamino analogs obtained (**30**–**35**), preserved π electron system and similar low conformational flexibility, but diverse electronic sp^2 and sp configuration. For direct comparison, we have synthesized allyl analogs of benzyl counterparts containing phenylpiperazine (PP) (**6**, **15**) and 2-MPP (**7**, **18**) – compounds **30**, and **31**, **32**, respectively (Tab. 2). This modification slightly (2–3 folds) decreased affinity for 5-HT_{1A} sites and even more dramatically, from 5- to 80-folds, it decreased affinity for 5-HT_7R (e.g., **17** *vs*, **32**). Thus **31** and **32** behaved as 5-HT_{1A} ligands with 5-HT_7/5-HT_{1A} selectivity ratio 18 and 31, respectively. Propynyl substituent in 7-position revealed to be unfavorable for binding of compounds **34** and **35** for 5-HTRs tested. Interestingly, introduction of a halogen atom in 4-position of phenylpiperazine yielded compound that preferentially binds to 5-HT_{2A}Rs (**32** *vs.* **33**).

Last but not least, through the replacement of a nitrogen atom with an oxygen in the spacer connecting purine-2,6-dione core with phenylpiperazine we obtained a series of arylpiperazinylalkoxy derivatives (**36**–**42**). Direct comparison of the selected analogs revealed that this modification has not dramatically modified the receptor affinity; it only slightly decreased affinity for 5-HT_{1A}R (Tab. 3). Interestingly enough, a shift of halogen atom from 3-position of compound **41** to 4-position yielded compound **42** with significantly increased affinity for both 5-HT_{1A} and 5-HT_7 sites. However, in opposite to other literature data, this modification did not increase 5-HT_7/5-HT_{1A} selectivity.

Regarding different classes of 5-HT receptors described in the central nervous system, much attention has been devoted to the role of the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptor subtypes in such psychiatric disorders as anxiety and depression. In fact, several authors have shown that agonists/partial agonists/antagonists of 5-HT_{1A} receptors, antagonists of 5-HT_{2A} receptors, as well as 5-HT_7 receptor antagonists exert anxiolytic- and/or antidepressant-like effect [3, 14, 26]. Moreover, Volk et al. has recently reported on a series of oxindole derivatives of LCAP substituted in position 3 and 4 of phenylpiperazine with halogen atom with significant antidepressant and anxiolytic properties [25]. Taking into account 5-HT receptor profile of the investigated derivatives, we selected 3- and 4-halogen substituted phenylpiperazine derivatives – compound **21** (mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 ligand) and **42** (mixed 5-HT_{1A}/5-HT_7 ligand), for *in vivo* functional studies.

It is generally accepted that the 8-OH-DPAT-induced LLR in rats is mediated by postsynaptic 5-HT_{1A} receptors, whereas the hypothermia evoked by 8-OH-DPAT in mice is connected with activation of presynaptic 5-HT_{1A} ones [1, 13]. The results of LLR model indicate that compound **42** behaves as a postsynaptic 5-HT_{1A} receptor antagonist. Unexpect-

edly, derivative **21** with high 5-HT_{1A} affinity poorly imitated 8-OH-DPAT-induced LLR, producing activity that did not reach significant level (Tab. 4). Again in the hypothermia model, compound **42** attenuated temperature of mice body; however, its effect was not changed by WAY 100635, a 5-HT_{1A} antagonist. Compound **21** produced a weak and non-significant hypothermic effect (data not showed). Both studied compounds demonstrated 5-HT_{2A} antagonistic properties towards the (±)DOI-induced head twitches in mice; their ID_{50} doses are consistent with their 5-HT_{2A} affinity data. The above results show that compound **21** can be classified as a very weak postsynaptic 5-HT_{1A} agonist and 5-HT_{2A} antagonist while analog **42** is a postsynaptic 5-HT_{1A}/5-HT_{2A} antagonist. At the successive stage, screening experiments were performed in order to establish potential antidepressant and/or anxiolytic activity of both compounds. Our results indicate that compound **21** produced an antidepressant-like effect in the forced swim test in mice. The typical antidepressant imipramine (used as a reference drug) was more active than derivative **21**. Furthermore, compound **21** exerted anxiolytic-like activity detected in the four-plate test in mice. Interestingly, its effect was weaker in terms of potency and active doses than that produced by diazepam (used as a reference anxiolytic drug). At the same time, **21** at doses active in the four-plate and the forced swim tests potently attenuated the spontaneous locomotor activity of mice during the initial 1- and 4-min (i.e., at the time identical to the observation period in the four-plate and the forced swim tests, respectively). In comparison, imipramine and diazepam at doses producing a distinct antidepressant- and anxiolytic-like effect, respectively, slightly reduced the locomotor activity of mice. The sedation induced by compound **21** indicates that its potential antidepressant/anxiolytic activity seems to be specific; however, such strong sedative effect practically excluded this ligand from being regarded as a potential drug. Compound **42** with narrower receptor profile produced no effect in both screening models used in mice. On the basis of obtained results we may hypothesize that the lack of antidepressant/anxiolytic activity for analog **42** can be due to different intrinsic activity of the compound toward 5-HT_{1A} receptors as compared with **21**, since both compounds are classified as 5-HT_{2A} antagonists. As concluded by Carr and Lucki [3], postsynaptic 5-HT_{1A} receptors are essential for producing the antidepressant-like effect of 5-HT_{1A} receptor agonists.

In conclusion, we synthesized a series of arylpiperazines and their THIQ analogs containing new 8-alkylamino- (**15**–**35**) and 8-alkoxy- purine-2,6-diones (**36**–**42**). The structural modifications allowed us to select some mixed 5-HT_{1A}/5-HT_{2A}/5-HT_7 (**21**) and 5-HT_{1A}/5-HT_7 (**16**, **17**, **19**, **20**, **42**) receptor ligands or compounds preferentially binding with 5-HT_{1A}R (**31**, **32**). Replacement of an arylalkyl group in 7-position of purine-2,4-dione with allyl substituent opens the chance to design new 5-HT ligands with preserved π electron system and lower molecular weight. Hence compounds displaying mixed 5-HT receptors' profile are interesting with regard to their potential antidepressant/anxiolytic activity, the most promising ones **21** and **42** were pharmacologically evaluated in animal preclinical models of depression and anxiety. Behavioral studies revealed that compound **21** evoked specific antidepressant/anxiolytic effects in mice, while derivative **42** is devoid such activity presumably due to their different intrinsic activity toward 5-HT_{1A} receptors.

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