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RECEPTOR AFFINITY AND PHOSPHODIESTERASES 4B AND 10A ACTIVITY OF OCTAHYDRO- AND 6,7-DIMETHOXY-3,4-DIHYDRO-ISOQUINOLIN-2(1*H*)-YL-ALKYL DERIVATIVES OF IMIDAZO- AND PYRIMIDINO[2,1-*f*]PURINES

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Abstract: A series of octahydro- and 6,7-dimethoxy-3,4-dihydro- isoquinolin-2(1*H*)-yl-alkyl derivatives of imidazo- and pyrimidino[2,1-*f*]purines were synthesized and biologically evaluated in *in vitro* competition binding experiments for serotonin 5-HT_{1A}, 5-HT_6, 5-HT_7, and dopamine D_2 receptors and inhibitory potencies for phosphodiesterases - PDE4B1 and PDE10A. The structure-activity relationships allowed to determine the structural features responsible for receptor and enzyme activity. Compound **5** (8-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)butyl)1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-dione) could be regarded as promising structure for further modification and detailed mechanistic study for obtained hybrid ligands.

Keywords: antidepressants; antipsychotic; serotonin receptor ligands; tricyclic theophylline derivatives.

There has been a shift in the theoretical framework addressing the pathophysiology of psychiatric disorders during the last decade. There is growing evidence that, in addition to neurotransmitters and their receptors, various signal transduction pathways may be linked to the pathophysiology of major psychiatric disorders. A complex mechanism of action characterizes drugs used in the pharmacotherapy of anxiety, depression, and schizophrenia; most of them are compounds with affinity for serotoninergic- (5-$HT_{1A,2A,6,7}$) and dopaminergic ($D_{2,3}$)-type receptors (1–4). The transduction of signals from 5-HT/D receptors (5-HTRs, DARs) is associated with the activation of second messenger pathways, which are mainly responsible for the increased level of the cyclic adenosine monophosphate (cAMP).

The involvement of the upstream and downstream components of this system provides a new framework in the treatment of psychiatric disorders (5). Therefore, cyclic nucleotide phosphodiesterases (PDEs), enzymes which degrade cAMP by hydrolysis of phosphodiester bonds, have been an attractive therapeutic target. In animal models, inhibitors of PDE4 and PDE10A by receptor-independent mechanisms reproduced antidepressant-like and antipsychotic effect (6, 7).

In our previous papers, we have described mixed serotonin/dopamine receptor binding profile of long-chain arylpiperazine (LCAP) derivatives with imidazo- and pyrimidino[2,1-*f*]purine moiety as cyclic amide core in terminal fragment (8–10). On the contrary, the early synthetic inhibitors of PDE belong to alkylxanthine or they are closely related to the xanthine ring derivatives. Nowadays, inhibitors of PDE4 and PDE10A are based on RS25344 or papaverine, respectively (Fig. 1) (11).

In this study, our aim was to investigate structure-affinity relationships for the 5-$HT_{1A,6,7}$ and dopaminergic D_2 receptors and inhibition of PDE4B and PDE10A in a group of imidazo- and pyrimidi-

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no[2,1-*f*]purines. Structural modifications comprised introduction of octahydro- and 3,4-dihydroisoquinolin-2(1*H*)-yl-alkyl moieties (instead of LCAP), as structures derived from papaverine, SB-277011 (12), and PZ-376 (13) (Fig. 1), respectively. Further studies focused on the effect of alkylene chain length and the presence of imidazo- and/or exocyclic amide moiety annelated on purine heterocyclic system on the receptor affinity and PDEs' inhibition.

EXPERIMENTAL

Chemistry

Schemes 1 and 2 present the structures of the investigated compounds and their syntheses. As can be seen in Scheme 1, a series of derivatives of 1,3-dimethyl-8-(octahydroisoquinolin-2(1*H*)-yl)alkyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-diones and 8-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)alkyl)-1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-diones (**1**–**7**) were prepared in multistep synthesis. In the first step, the alkylation of decahydroisoquinoline and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**I**, **II**) with 2-(bromoalkyl)-1*H*-isoindoline-1,3(2*H*)-diones **III**–**IV** afforded corresponding derivatives **V**–**VIII**. Next, hydrolysis of the 1*H*-isoindoline-1,3(2*H*)-dione group with 65% hydrazine monohydrate aqueous solution afforded intermediates **IX**–**XII**, which reacted with 7-ketonyl derivatives of 8-bromo-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**XIII**–**XIV**) (8) to provide the final derivatives (**1**–**7**). According to the previously described method, the 9-octahydroisoquinolin-2(1*H*)-yl and 6,7-dimethoxy-3,4-dihydroisoqiunolin-2(1*H*)-yl-alkyl derivatives of 1,3-dimethylpyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones (**8**–**12**) were synthesized by alkylation of the corresponding isoquinolin-2-yl derivatives (**I**, **II**) with 7-bromo-9-(chloroalkyl)-1,3-dimethylpyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones (**XV**–**XVII**) (9). The structures of newly synthesized compounds **1**–**12** were confirmed by ^{1}H NMR and ^{13}C-NMR spectra, LC/MS and elemental analyses. The investigated compounds were pharmacologically tested as free bases. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury VX-300 MHz spectrometer, in $CDCl_3$ solutions, using TMS ($\delta = 0.00$ ppm) as an internal standard. The *J* values are in Hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Liquid chromatography–mass spectrometry (LC/MS) analyses were performed on Waters Acquity TQD apparatus with eλ DAD detector.

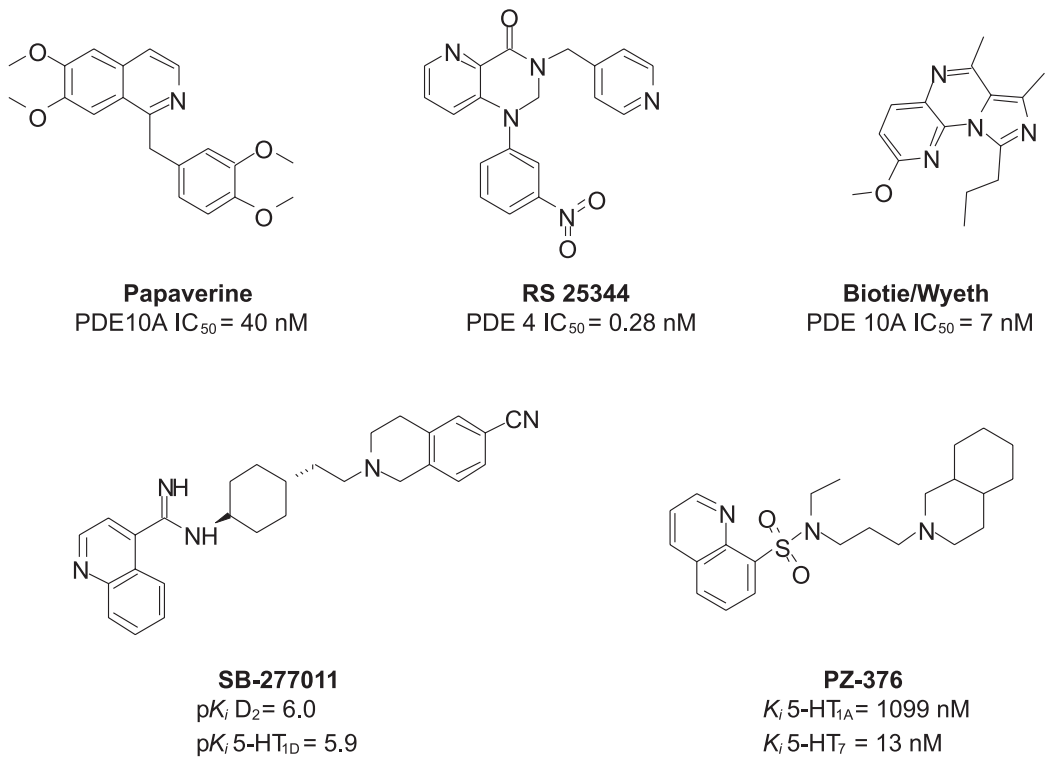
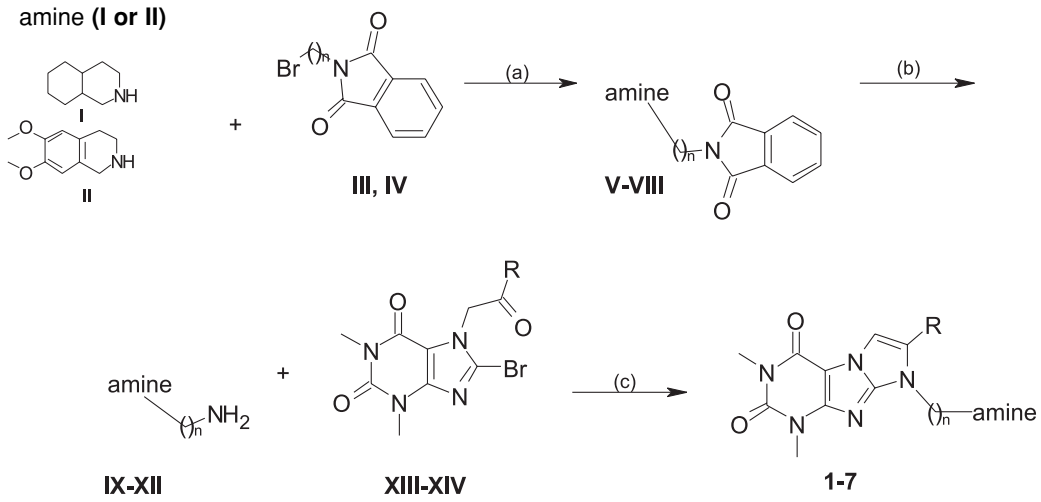
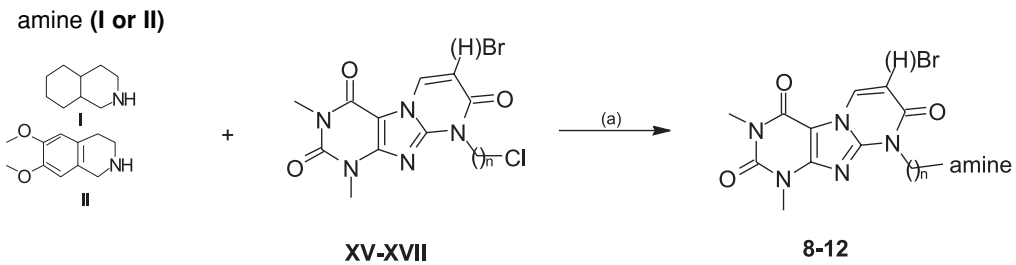


Figure 1. Representatives of the multi-receptor ligands and PDE inhibitors



Compound	n	R	Amine
1	4	-H	octahydroisoquinolin-2(1*H*)-yl
2	5	-H	octahydroisoquinolin-2(1*H*)-yl
3	4	-CH_3	octahydroisoquinolin-2(1*H*)-yl
4	5	-CH_3	octahydroisoquinolin-2(1*H*)-yl
5	4	-H	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl
6	5	-H	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl
7	4	-CH_3	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl

Scheme 1. Synthesis of 1,3-dimethyl-8-(octahydroisoquinolin-2(1*H*)-yl)alkyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-diones and 8-(6,7-dimetoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)alkyl)-1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-diones (**1**-**7**). Reagents and condition (a) K_2CO_3, butanol, reflux; (b) hydrazine, EtOH, reflux; (c) 2-methoxyethanol, reflux



Compound	n		Amine
8	5	-H	octahydroisoquinolin-2(1*H*)-yl
9	4	-Br	octahydroisoquinolin-2(1*H*)-yl
10	4	-H	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl
11	5	-H	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl
12	4	-Br	6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl

Scheme 2. Synthesis of 9-octahydroisoquinolin-2(1*H*)-yl and 6,7-dimethoxy-3,4-dihydroisoqiunolin-2(1*H*)-yl-alkyl derivatives of 1,3-dimethyl-pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones (**8**-**12**). Reagents and condition (a) acetonitrile, microwave, open vessel, 200 W, 1 h

Electrospray ionization in positive mode (ESI^+) was used to acquire spectra. UV spectra were recorded in 200–700 nm range. UV chromatograms were used for establishing the purity of compounds. All investigated final compounds had purity over 95%. Elemental analyses were performed using Elemetar Vario EL III apparatus and were found within ± 0.4% of the theoretical values. Melting points (m.p.) were determined with a Büchi apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminum sheets, and spots were detected by their absorption under UV light ($\lambda = 254$ nm). Column chromatography separations were carried out on Merck Kieselgel 60 column. Microwave-assisted synthesis was performed in laboratory microwave Discover LabMate reactor (CEM Corporation). All chemicals and reagents for the synthesis were obtained from Alfa Aesar (Karlsruhe,Germany), Sigma-Aldrich Co. (St. Louis, United States) and Chempur (Piekary Śląskie, Poland).

General procedure for the synthesis of 1,3-dimethyl-8-(octahydroisoquinolin-2(1H)-yl)alkyl-1H-imidazo[2,1-f]purine-2,4-(3H,8H)-diones and 8-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-1,3-dimethyl-1H-imidazo[2,1-f]purine-2,4-(3H,8H)-diones

A mixture of 7-ketonyl derivatives of 8-bromo-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione **XIII**, **XIV** (5 mmol) with double amount of appropriate isoquinolin-2-yl-alkyl-amine (10 mmol) (**IX**–**XII**) was refluxed in 2-methoxyethanol (20 mL) for 12 h. The solvent was removed *in vacuo*, the obtained residue was purified by flash column chromatography on silica gel using mixture of CH_2Cl_2/MeOH = 9/1 or 9/0.7, v/v as an eluting system.

1,3-Dimethyl-8-(4-(octahydroisoquinolin-2(1*H*)-yl)butyl)1*H*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione (1)

Obtained from **IX** and **XIII** in 60% yield as cream solid; m.p. 96-98°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.40-7.39 (d, 1H, J = 2.3 Hz), 6.82-6.81 (d, 1H, J = 2.3 Hz), 4.17 (t, 2H, J = 7.4 Hz), 3.59 (s, 3H), 3.43-3.41 (m, 4H), 2.64-2.52 (m, 2H), 2.16-1.85 (m, 3H), 1.76-1.62 (m, 8H); ^{13}C NMR ($CDCl_3$, δ, ppm): 153.9, 151.9, 150.2, 147.5, 120.6, 114.9, 100.1, 58.6, 56.9, 55.3, 48.4, 39.5, 36.4, 31.3, 30.3, 30.1, 29.7, 29.3, 28.9, 26.7, 25.6, 24.9; Analysis: calcd. for $C_{22}H_{32}N_6O_4$: C, 64.05; H, 7.82; N, 20.37%; found: C, 65.12; H, 7.58; N, 20.07%. LC/MS *m*/*z* calcd.: 412.52, found: 413.39.

1,3-Dimethyl-8-(5-(octahydroisoquinolin-2(1*H*)-yl)pentyl)1*H*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione (2)

Obtained from **X** and **XIII** in 56% yield as cream solid; m.p. 101-102°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.40-7.39 (d, 1H, J = 2.3 Hz), 6.83-6.82 (d, 1H, J = 2.3 Hz), 4.15 (t, 2H, J = 7.4 Hz), 3.61-3.59 (m, 4H), 3.45-3.39 (m, 6H), 2.64-2.52 (m, 2H), 2.02-0.85 (m, 22H), ^{13}C NMR ($CDCl_3$, δ, ppm): 154.8, 151.3, 150.1, 145.0, 120.6, 114.9, 100.1, 58.6, 57.2, 55.3, 48.4, 39.5, 36.4, 32.1, 30.7, 29.6, 29.0, 28.8, 28.0, 26.1, 25.8, 25.1, 24.5; Analysis: calcd. for $C_{23}H_{34}N_6O_2$: C, 64.76; H, 8.03; N, 19.70%; found: C, 64.67; H, 7.98; N, 19.77%. LC/MS *m*/*z* calcd.: 426.55, found: 427.41.

1,3,7-Trimethyl-8-(4-(octahydroisoquinolin-2(1*H*)-yl)butyl)1*H*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione (3)

Obtained from **IX** and **XIV** in 88% yield as cream solid; m.p. 128-129°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.18 (m, 1H), 4.07 (t, 2H, J = 7.4 Hz), 3.63 (s, 3H), 3.41 (s, 3H), 2.64-2.52 (m, 2H), 2.16-1.92 (m, 5H), 1.85-0.92 (m, 20H); ^{13}C NMR ($CDCl_3$, δ, ppm): 153.9, 151.9, 150.2, 147.5, 132.4, 130.8, 128.7, 58.4, 58.1, 53.7, 48.4, 40.1, 38.7, 38.1, 30.4, 30.2, 28.9, 28.8, 26.7, 26.1, 25.8, 24.9, 14.0; Analysis: calcd. for $C_{23}H_{34}N_6O_2$: C, 64.76; H, 8.03; N, 19.70%; found: C, 64.72; H, 8.08; N, 19.66%. LC/MS *m*/*z* calcd.: 426.55, found: 427.35.

1,3,7-Trimethyl-8-(5-(octahydroisoquinolin-2(1*H*)-yl)pentyl)1*H*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione (4)

Obtained from **X** and **XIV** in 78% yield as cream oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.19 (m, 1H), 4.15 (t, 2H, J = 7.4 Hz), 3.63 (s, 3H), 3.41 (s, 3H), 2.64-2.52 (m, 2H), 2.02-0.85 (m, 25H); ^{13}C NMR ($CDCl_3$, δ, ppm): 154.3, 151.3, 150.1, 149.3, 128.3, 110.3, 100.1, 58.6, 57.2, 53.1, 48.4, 39.5, 36.4, 32.1, 30.7, 30.2, 29.0, 28.8, 26.7, 26.1, 25.8, 25.1, 24.9, 13.9; Analysis: calcd. for $C_{24}H_{36}N_6O_2$: C, 65.43; H, 8.24; N, 19.07%; found: C, 65.48; H, 8.44; N, 19.17%. LC/MS *m*/*z* calcd.: 440.58, found: 441.37.

8-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)butyl)1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione (5)

Obtained from **XI** and **XIII** in 83% yield as cream solid; m.p. 167-169°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.0-7.99 (d, 1H, J = 2.3 Hz), 6.92 (s, 1H), 6.64 (s, 2H), 6.55 (s,2H), 4.18 (t, 2H, J = 7.4 Hz), 3.86-3.81 (m, 6H), 3.59 (s, 3H), 3.43 (m, 3H), 3.20-

2.84 (m, 6H), 2.15-1.85 (m, 6H); ^{13}C NMR ($CDCl_3$, δ, ppm): 154.8, 151.3, 149.1, 148.2, 146.7, 145.0, 126.8, 125.5, 120.6, 114.9, 111.4, 108.3, 100.1, 59.7, 56.5, 56.3, 56.2, 56.1, 55.6, 30.7, 29.6, 29.0, 27.9, 25.0; Analysis: calcd. for $C_{24}H_{30}N_6O_4$: C, 61.79; H, 6.48; N, 18.01%; found: C, 61.87; H, 6.59; N, 18.07%. LC/MS *m/z* calcd.: 466.53, found: 467.37.

8-(5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)pentyl)1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-dione (6)

Obtained from **XII** and **XIII** in 64% yield as cream solid; m.p. 217-219°C; ^{1}H NMR: (300 MHz, $CDCl_3$, δ, ppm): 7.09 (d, 1H, *J* = 2.3 Hz), 6.92 (m, 1H), 6.64 (s, 1H), 6.55 (s, 1H), 4.18 (m, 2H), 3.86-3.81 (m, 6H), 3.60 (s, 3H), 3.41 (m, 3H), 3.28-3.20 (m, 2H), 3.09-3.16 (m, 2H), 2.96-2.89 (m, 2H), 2.15-1.85 (m, 4H), 1.56-1.42 (m, 2H), 1.30-1.22 (m, 2H); ^{13}C NMR ($CDCl_3$, δ, ppm): 154.0, 152.1, 151.9, 148.8, 146.7, 145.2, 126.8, 125.0, 119.6, 114.8, 111.1, 109.2, 100.0, 60.1, 56.5, 56.3, 56.1, 56.0, 55.6, 30.2, 30.0, 39.9, 28.1, 27.3, 25.1; Analysis: calcd. for $C_{25}H_{32}N_6O_4$: C, 62.48; H, 6.71; N, 17.49%; found: C, 62.87; H, 6.59; N, 78.47%. LC/MS *m/z* calcd.: 480.55, found: 481.39.

8-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)butyl)1,3,7-trimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-dione (7)

Obtained from **XI** and **XIV** in 70% yield as cream solid; m.p. 145-147°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.08-7.07 (m, 1H), 6.64 (s, 2H), 6.55 (s, 2H), 4.08 (m, 4H), 3.86-3.81 (m, 6H), 3.60 (s, 3H), 3.41 (m, 3H), 2.88-2.83 (m, 2H), 2.80-2.76 (m, 2H), 2.60-2.57 (m, 2H), 2.38 (s, 3H), 2.06-1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, δ, ppm): 154.1, 151.9, 150.3, 147.66, 146.7, 145.0, 128.3, 127.1, 126.5, 111.4, 110.9, 109.3, 99.8, 60.1, 56.5, 56,4, 56.3, 56.2, 53.1, 30.7, 29.9, 29.0, 28.0, 27.3, 25.1, 13.1; Analysis: calcd. for $C_{25}H_{32}N_6O_4$: C, 62.48; H, 6.71; N, 17.49%; found: C, 62.77; H, 6.66; N, 17.47%. LC/MS *m/z* calcd.: 480.55, found: 481.33.

General procedure for preparation of 9-octahydroisoquinolin-2(1H)-yl and 6,7-dimethoxy-3,4-dihydroisoqiunolin-2(1H)-yl-alkyl derivatives of 1,3-dimethyl-pyrimido[2,1-f]purine-2,4,8(1H,3H,9H)-triones

The starting 9-bromoalkyl-1,3-dimethyl-pirymido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones (**XV**–**XVIII**) were obtained according to the previously described procedure (9). A mixtures of **XV**–**XVII** (0.5 mmol) with a twofold excess of appropriate isoquinolin-2-yl derivatives in acetonitrile (4 mL) were exposed to microwave irradiation for 1 h and power of MW oven (200 W). After evaporation of the solvent products **8**–**12** were purified by flash column chromatography on silica gel using mixture of CH_2Cl_2/MeOH = 9/1 or 9/1.2, v/v as an eluting system.

1,3-Dimethyl-9-(5-(octahydroisoquin-2(1*H*)-yl)pentyl)pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (8)

Obtained fom **I** and **XV** in 50% yield as cream oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.51-8.49 (d, 1H, *J* = 8.1 Hz), 6.23-6.33 (d, 1H, *J* = 7.1 Hz), 4.34-4.32 (m, 2H), 3.60 (s, 3H), 3.41 (m, 3H), 2.51-2.15 (m, 6H), 2.06-1.86 (m, 6H); 1.70-1.38 (m, 18H); ^{13}C NMR ($CDCl_3$, δ, ppm): 162.2, 154.8, 153.2, 151.3, 150.1, 130.8, 127.6, 109.3, 58.6, 57.2, 48.4, 42.2, 39.5, 36.4, 32.1, 30.7, 29.1, 29.0, 28.8, 28.0, 27.1, 26.1, 25.8, 24.5, 24.3; Analysis: calcd. for $C_{24}H_{34}N_6O_3$: C, 63.41; H, 7.54; N, 17.49%; found: C, 62.77; H, 6.66; N, 17.47%. LC/MS *m/z* calcd.: 480.55, found: 481.33.

7-Bromo-1,3-dimethyl-9-(4-(octahydroisoquin-2(1*H*)-yl)butyl)pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (9)

Obtained from **I** and **XVI** in 45% yield as cream oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 6.23 (s, 1H), 4.34-4.32 (m, 2H), 3.60 (s, 3H), 3.41 (m, 3H), 2.51-2.41 (m, 2H), 2.40-2.15 (m, 4H); 1.70-1.38 (m, 16H); ^{13}C NMR ($CDCl_3$, δ, ppm): 155.8, 154.0, 151.3, 149.5, 146.2, 133.7, 104.2, 100.4, 57.9, 57.4, 53.5, 44.2, 40.0, 38.1, 31.4, 29.7, 29.4, 27.9, 26.5, 25.6, 24.9, 23.9, 23.1; Analysis: calcd. for $C_{23}H_{31}BrN_6O_3$: C, 53.18; H, 6.02; N, 16.18%; found: C, 53.27; H, 6.36; N, 16.07%. LC/MS *m/z* calcd.: 519.43, found: 521.21.

9-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-1,3-dimethyl-pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (10)

Obtained from **I** and **XVII** in 46% yield as cream oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.51-8.49 (d, 1H, *J* = 8.1 Hz), 6.53-6.60 (m, 2H), 6.32-6.31 (d, 1H, *J* = 7.1 Hz), 4.40 (t, 2H, *J* = 3.1 Hz), 3.86-3.81 (m, 6H), 3.60 (s, 3H), 3.41 (m, 3H), 2.88-2.83 (m, 4H), 2.79-2.76 (m, 2H), 2.60-2.57 (m, 2H), 2.06-1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, δ, ppm): 162.2, 154.8, 153.2, 151.3, 150.1, 130.8, 127.6, 108.5, 58.6, 56.9, 48.4, 42.1, 39.5, 36.4, 32.1, 30.7, 29.1, 29.0, 28.8, 26.1, 25.8, 25.5, 24.9, 24.5, 24.3; Analysis: calcd. for $C_{25}H_{30}N_6O_5$: C, 60.72; H, 6.11; N, 16.99%; found: C, 60.77; H, 6.27; N, 17.07. LC/MS *m/z* calcd.: 494.54, found: 495.29.

9-(5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl)-1,3-dimethyl-pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (11)

Obtained from **II** and **XV** in 46% yield as cream oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.51-8.49 (d, 1H, *J* = 8.1 Hz), 6.53-6.60 (m, 2H), 6.33-6.23 (m, 1H), 4.37-4.20 (m, 2H), 3.86-3.81 (m, 6H), 3.60-3.39 (m, 8H), 2.88-2.76 (m, 2H), 2.79-2.76 (m, 4H), 2.60-2.57 (m, 2H), 2.06-1.86 (m, 6H); ^{13}C NMR ($CDCl_3$, δ, ppm): 161.8, 153.8, 152.8, 150.3, 150.1, 148.7, 147.8, 145.7, 127.6, 126.5, 109.4, 108.5, 110.3, 60.4, 57.1, 56.9, 56.6, 56.0, 45.1, 30.7, 29.1, 29.0, 28.8, 26.1, 25.8, 24.9. Analysis: calcd. for $C_{26}H_{32}N_6O_5$: C, 61.40; H, 6.34; N, 16.52%; found: C, 61.17; H, 6.27; N, 16.57%. LC/MS *m*/*z* calcd.: 508.56, found: 509.31.

7-Bromo-9-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-1,3-dimethyl-pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (12)

Obtained from **II** and **XVI** in 38% yield as cream oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 6.53-6.60 (m, 2H), 6.32-6.31 (d, 1H, *J* = 7.1 Hz), 4.40 (t, 2H, *J* = 3.1 Hz), 3.86-3.81 (m, 6H), 3.60 (s, 3H), 3.41 (m, 3H), 2.88-2.83 (m, 4H), 2.79-2.76 (m, 2H), 2.60-2.57 (m, 2H), 2.06-1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, δ, ppm): 162.2, 154.8, 153.2, 151.3, 150.1, 149.1, 146.4, 134.8, 127.6, 126.8, 111.2, 109.5, 59.7, 57.1, 56.8, 56.4, 56.0, 55.4, 40.1, 30.7, 29.1, 29.0, 28.8, 27.1, 25.8, 25.5, Analysis: calcd. for $C_{25}H_{29}BrN_6O_5$: C, 53.36; H, 5.10; N, 14.66%; found: C, 53.25; H, 5.27; N, 14.77%. LC/MS *m*/*z* calcd.: 573.43, found: 575.26.

***In vitro* radioligand binding assays**

Radioligand binding assays were employed for determining the affinity and the selectivity profile of the synthesized compounds for cloned 5-HT_{1A}, 5-HT_6, 5-HT_7 and dopamine D_2 receptors. This was accomplished by displacement of respective radioligands from cloned human receptors, all stably expressed in HEK293 cells: [^{3}H]-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) for 5-HT_{1A}, [^{3}H]-LSD for 5-HT_6, [^{3}H]-5-carboxamidotryptamine (5-CT) for 5-HT_7, and [^{3}H]-raclopride for D_2 receptor. All the *in vitro* radioligand assays were carried out using methods published by Zajdel et al. (13).

Prediction of logP values

The octanol-water partitioning coefficients (logP) were predicted from structures, using fragment-based algorithm of ACD/Labs ChemSketch free software. Calculated values were based on an experimental data set of over 18,000 reliable logP measurements.

The phosphodiesterase activity tests

Activity of newly synthesised compounds towards PDE4A and PDE10A was measured using the bioluminescent detection system, based on the activity of PDEs which utilized cAMP as their pref-

Table 1. Binding affinities and calculated log *P* values of the synthesized tricyclic theophylline derivatives with octahydro- and 6,7-dimethoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl)alkyl moieties (**1**-**12**).

Comp.	K_i (nM)				Log P_{ACD}
	5-HT_{1A}	5-HT_6	5-HT_7	D_2	
1	9	3338	413	370	4.10
2	90	9532	4642	1701	4.38
3	340	3767	2465	1966	4.56
4	281	4134	2665	1125	4.84
5	22	925	6011	2880	3.03
6	147	1900	4020	865	3.32
7	120	8666	4892	1189	3.49
8	1399	3948	4434	1460	3.77
9	139	5364	3729	1399	3.90
10	115	2788	5040	2815	2.48
11	88	1904	4209	2215	2.71
12	83	2038	4490	1422	2.83

Table 2. Inhibition of PDE (%) of tricyclic theophylline derivatives with 6,7-dimethoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl)alkyl moieties (comp. **5** and **11**).

Compound	PDE 4B1[a]			PDE 10A[a]		
	10^{-4}	10^{-5}	$10^{-5.5}$	10^{-4}	10^{-5}	$10^{-5.5}$
5	15	0	-	34	20	-
11	14	7	4	52	21	-
Theophylline	-	3	8	-	2	-5
Papaverine	51	13	-	97	78	57
IBMX	-	5	-	-	98	-
Rolipram	95	84	75	-	3	8

[a]percentage of inhibition of PDE was calculated in relation to vehicle control (DMSO).

erential second messenger. Inhibition of PDE4B1 and PDE10A was measured using PDElight HTS cAMP phosphodiesterase assay kit (PDELight™, Lonza) according to manufacturer's recommendations (14, 15). cAMP measurements were performed with homogeneous TR-FRET immunoassay using the LANCE Ultra cAMP kit (PerkinElmer, USA). Luminescence was measured in a multifunctional microplate reader (POLARstar Omega, BMG Labtech, Germany). The percentage of inhibition was calculated to vehicle control (DMSO).

RESULTS AND DISCUSSION

The final derivatives of 1,3-dimethyl-(1*H)*-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-diones **1**–**7** were obtained in a reaction of cyclocondensation of 7-ketonyl derivatives of 8-bromotheophylline (**XIII**–**XIV**) with appropriate amine (**IX**–**XII**) according to the previously reported method (8). The target compounds **8**–**12** were synthesized by the substitution reaction of 1,3-dimethylpyrimido[2,1-*f*]purine-2,4,8-(1*H,*3*H,*9*H*)-triones (9) (**XV**–**XVII**) with appropriate amine (**I, II**) using microwave radiation. The obtained compound structures were confirmed with ^{1}H-NMR and ^{13}C-NMR spectra and their masses were proved with LC/MS analysis.

All the newly synthesized compounds were tested in competition binding experiments for 5-HT_{1A}, 5-HT_6, 5-HT_7, and D_2 receptors. The observed activity of the compounds was variable depending on the receptor subtype (Table 1). Compounds displayed high-to-low affinity for the tested 5-HT_{1A}Rs (K_o from 9 to 1399 nM), lack of activity for 5-HT_6Rs (exception comp. **5** K_o = 925) and for 5-HT_7Rs (exception comp. **1** K_o = 413) and for D_2Rs (exceptions comp. **1** and **6** with K_o = 370 and 865, respectively).

The activity of newly synthesized compounds toward PDEB1A and PDE10A was measured using the bioluminescent detection system, based on the activity of PDEs which utilized second messenger cAMP. The AMP produced from the hydrolysis of cAMP was quantified using the AMP detection reagent that converts AMP directly to ATP. The bioluminescent assay uses luciferase, which catalyzes the formation of light from the newly formed ATP and luciferin. The percentage of inhibition was calculated using DMSO as vehicle control. Rolipram, papaverine, and 3-isobutyl-1-methylxanthine (IBMX) were used as standards for inhibition potency for PDE4B1A and PDE10A. Compounds and standards were tested in screening assays in three concentrations from 10^{-4} to $10^{-5.5}$ M (Table 2). Investigated compounds had no inhibitory potencies for PDE4B1 and PDE10A with two exceptions, compounds **5** and **11**. In concentration of 10^{-4} M compound **5** inhibits PDE4B1 in 15% and PDE10A in 34% whereas compound **11** in 14% and 52%, respectively. It is noteworthy that compounds **5** and **11** were more potent inhibitors than theophylline, which was inactive under test condition. Unfortunately, in lower concentrations their activity significantly decreased in comparison to standards. The results obtained for compounds **5** and **11** suggested some impact of introduction of 6,7-dimetoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl)alkyl moiety on inhibitory activity, especially for PDE10A.

An impact of introduction of octahydroisoquin-2(1*H*)-yl or 6,7-dimetoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl)alkyl (instead of LCAP) moiety on receptors affinity was evident after analyzing structure-activity relationships. Such a substitution causes a significant decrease in receptor activity in comparison with analogues with LCAP moiety. This effect is more pronounced for derivatives of 1,3-

dimethylpyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione. The affinity for 5-HT_{1A}Rs depended mostly on the type of the purine core and the length of the alkylene spacer between purine core and nitrogen atom of amine. Derivatives of 1,3-dimethyl-(1*H*)-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-diones displayed, in general, higher affinity, especially for 5-HT_{1A}, than their counterparts, e.g., compounds **2** *vs.* **8**. Compounds **1** and **5** being the most active ones belong to the group of 1,3-dimethyl-(1*H*)-imidazo[2,1-*f*]purine-2,4-(3*H*,8*H*)-dione lacking a substituent at 7-position. Moreover, for derivatives of 1,3-dimethylpyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione, the presence of high lipophilic bromine substituent at 7 position seems to be more essential for 5-HT_{1A} activity (**8** *vs.* **9** and **10** *vs.* **12**). Interestingly, affinity for other receptors (5-HT_6, 5-HT_7, and D_2) was not significantly influenced by a type of the xanthine core and the length of alkylene spacer.

For further explanation of the structure-activity relationships and as a continuation of our previous studies on physicochemical properties of tricyclic theophylline derivatives, logP parameters were predicted (16). The observed values of logP were between 2.48 and 4.84 (Table 1). In general, the highest logP values were observed for octahydroisoquin-2(1*H*)-yl derivatives of imidazo- and pyrimidino[2,1-*f*]purines (compounds **1**-**4** and **8**, **9**). Introduction of 6,7-dimetoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl moiety caused a significant decrease of logP values, especially pronounced for pyrimidino[2,1-*f*]purine core (comp. **8** *vs.* **11**). Analyzing the impact of lipophilicity on receptor affinity, no relationship between logP values and K_o was observed. Compounds with the lowest and highest logP value were inactive in radioligand receptor binding studies. However, the most potent compound (**1**) showed quite high logP value (4.10). Referring obtained logP values to the rule of five (17), it seems that all compounds could easily penetrate blood-brain barrier (BBB) and other membrane in the body (logP < 5).

CONCLUSION

In summary, a series of 12 new octahydro- and 6,7-dimetoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl-alkyl derivatives of imidazo- and pyrimidino[2,1-*f*]purines were synthetized. An introduction of octahydroisoquin-2(1*H*)-yl or 6,7-dimetoxy-3,4-dihydro-isoquinolin-2(1*H*)-yl)alkyl moiety to the tricyclic theophylline derivatives was to check the possibility of obtaining compounds with dual mechanism of action: receptor-dependent and receptor-independent.

The study allowed the identification of two potent 5-HT_{1A}R ligands (comp. **1** and **5**) and two weak inhibitors of PDE10A (comp. **5** and **11**). This preliminary study showed that compound **5** (8-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)butyl)1,3-dimethyl-1*H*-imidazo[2,1-*f*]purine-2,4(3*H*,8*H*)-dione) could be regarded as promising structure for further modification and detailed mechanistic study for obtaining hybrid ligands.

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