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Tricyclic xanthine derivatives containing a basic substituent: adenosine receptor affinity and drug-related properties†

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A library of 27 novel amide derivatives of annelated xanthines was designed and synthesized. The new compounds represent 1,3-dipropyl- and 1,3-dibutyl-pyrimido[2,1-*f*]purinedione-9-ethylphenoxy derivatives including a CH₂CONH linker between the (CH₂)₂-amino group and the phenoxy moiety. A synthetic strategy to obtain the final products was developed involving solvent-free microwave irradiation. The new compounds were evaluated for their adenosine receptor (AR) affinities. The most potent derivatives contained a terminal tertiary amino function. Compounds with nanomolar AR affinities and at the same time high water-solubility were obtained (A₁ (K_i = 24–605 nM), A_{2A} (K_i = 242–1250 nM), A_{2B} (K_i = 66–911 nM) and A₃ (K_i = 155–1000 nM)). 2-(4-(2-(1,3-Dibutyl-2,4-dioxo-1,2,3,4,7,8-hexahydropyrimido[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)-*N*-(3-(diethylamino)propyl)acetamide (**27**) and the corresponding *N*-(2-(pyrrolidin-1-yl)ethyl)acetamide (**36**) were found to be the most potent antagonists of the present series. While **27** showed CYP inhibition and moderate metabolic stability, **36** was found to possess suitable properties for *in vivo* applications. In an attempt to explain the affinity data for the synthesized compounds, molecular modeling and docking studies were performed using homology models of A₁ and A_{2A} adenosine receptors. The potent compound **36** was used as an example for discussion of the possible ligand–protein interactions. Moreover, the compounds showed high water-solubility indicating that the approach of introducing a basic side chain was successful for the class of generally poorly soluble AR antagonists.

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Introduction

Adenosine receptors (ARs) are members of the superfamily of G protein-coupled receptors. They comprise four subtypes designated A₁, A_{2A}, A_{2B}, A₃, which differ in their pharmacological profile, and their distribution in various cells and tissues.^{1,2} A₁ and A₃ ARs couple predominantly to G_i proteins resulting in inhibition of adenylate cyclase, while A_{2A} and A_{2B} ARs stimulate the enzyme thereby increasing the intracellular cyclic AMP concentration *via* the interaction with G_s proteins.^{3–5} Adenosine receptors may interact with other classes of G proteins leading to activation of various second messenger pathways such as phospholipase C, mitogen activated

protein kinase, potassium and calcium channels, arachidonic acid pathways, and phospholipase D.^{6–10}

Adenosine is the endogenous agonist for this class of receptors.¹¹ Considering the effects of adenosine in physiological and pathological processes, the blockade of AR may be beneficial for the treatment of various pathological conditions associated with decreased renal blood flow,¹² inhibition of insulin/glucagon release,¹³ reduced heart rate,¹⁴ and cognitive disorders (A₁ ARs), neurodegeneration^{15,16} and reduced immune response (A_{2A} ARs), pain, inflammatory lung disease and cancer,^{17,18} (A_{2B} ARs), and airway contraction and glaucoma (A₃ ARs).^{3,19,20,28,29}

A₁ ARs are highly expressed in the CNS, especially in the cortex, hippocampus, cerebellum, and also in some peripheral tissues such as the heart and kidneys.² Antagonists of the A₁ AR subtype may improve impaired cognitive dysfunctions *e.g.* in Alzheimer's disease or may be beneficial for treating congestive heart failure by inducing positive inotropic and diuretic effects.^{3,21,22}

Adenosine A_{2A} receptors are widely distributed in peripheral tissues such as immune cells and blood platelets as well

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in the striatum (caudate-putamen) of the CNS.^{2,23,24} Potential therapeutic applications of A_{2A} antagonists include neurodegenerative disorders such as Parkinson's and Alzheimer's disease.^{25,26}

The naturally occurring xanthines, caffeine (1) and theophylline (2) are a well-known class of AR antagonists²⁷ (Fig. 1). They can be classified as weak and non-selective AR antagonists. To date, methylxanthines are therapeutically used as therapeutics for bronchial inflammation associated with asthma (theophylline) and they also constitute widely self-administered psychoactive drugs. Structural modifications of the xanthine core have been intensively investigated in order to synthesize more potent and subtype-selective AR antagonists.²⁸

The most potent A₁-selective antagonists were obtained in the group of 8-cycloalkylxanthine derivatives. Rolofylline (KW-3902) (3) and PSB-36 (4) represent potent and selective antagonists for the A₁ AR. These compounds possess a bulky noradamantyl substituent in position 8 and propyl or butyl residues in the N1 and N3 positions of the xanthine core (Fig. 1).^{2,27–29}

8-Styrylxanthine derivatives are the most potent and selective class of A_{2A} AR antagonists. Istradefylline (5), a 1,3-diethylxanthine derivative with a 3,4-dimethoxystyryl residue in the 8-position, has been approved in Japan for the treatment of Parkinson's disease as an adjunct treatment (Fig. 1).³⁰ Further investigations of the role of ARs in neurodegenerative disorders suggested a potential beneficial effect of dual A₁/A_{2A} antagonists associated with improved motor and neuronal function *via* the blockade of A_{2A} and procognitive effects *via* A₁ antagonism as shown in animal models. Dual target drugs may present higher therapeutic efficacy in Parkinson's disease compared to selective ligands targeting only a single receptor.^{31–34}

Novel AR antagonists are still being intensively explored. Potent and selective antagonists have been obtained in both structural classes, xanthine as well as non-xanthine derivatives.^{27–29} Structure–activity relationship analysis of xanthine derivatives indicated a significant influence of substituents in the 8-position on subtype-selectivity. Introduction

Fig. 1 Structures of selected xanthine derivatives and their K_i values as adenosine receptor antagonists²⁸ (h = human).

of a styryl moiety was preferred by the A_{2A} ARs, while bulky aryl or alicyclic substituents improved the affinity for A₁ and A_{2B} ARs. Considering the impact of alkyl chains in the N1 and N3 positions, propyl and butyl substituents showed preference towards A₁ but also towards A_{2B} ARs.^{35–38}

Research in the group of xanthine derivatives as A₁/A_{2A} AR antagonists has been promoted by our group as well as by others.^{4,39,40} In our previous studies, novel A_{2A} AR antagonists in the class of annelated xanthines with a third heterocyclic ring fused to the f-bond were intensively investigated as constrained bioisosteric analogues of 8-styrylxanthines with a fixed, stable *E*-configuration.^{41,42} Some compounds in the series of N9-phenethylpyrimido[2,1-*f*]purinedione derivatives (6–8) displayed an increased affinity for A₁ upon elongation of alkyl chains in the 1,3-positions while maintaining affinity for A_{2A} in the submicromolar range at the same time (Fig. 2). Previously published structures with alkoxyamine attached in the *para*-position to the benzene ring which showed affinity for A₁ and A_{2A} ARs, displayed rather low water-solubility, which is a serious drawback with regard to bioavailability.⁴³

Based on the above considerations, the primary goal of our efforts was the development of novel A₁/A_{2A} antagonists in the series of tricyclic xanthines that showed enhanced affinity and water-solubility. Compared to the previous series in 8 we incorporated a CH₂CONH linker between (CH₂)₂NR₂ and the phenoxy moiety. Based on this planned modification, various modifications of the (CH₂)₂NR₂ were examined by lengthening the CH₂ chain and introducing groups other than NR₂ like heterocyclic amines, and aromatic amines in the two series of 1,3-dialkyl-substituted xanthine derivatives. Introduction of an amide linker might have an additional effect increasing the AR affinity due to additional ligand–protein interactions. Introducing basic side chains was expected to increase water-solubility.

All synthesized tricyclic xanthines were tested for their affinity to all four AR subtypes of rat (A₁, A_{2A}) and/or human (A_{2B}, A₃). In order to support the observed results of binding assays, the possible binding modes of the compounds at the rat A₁ and A_{2A} adenosine receptors were compared and discussed on the basis of molecular docking calculations. The solubility (QPlogS) of the synthesized compounds was estimated by *in silico* calculations, and for selected compounds it was also experimentally determined. Moreover, two selected compounds were tested in *in silico* and *in vitro* studies to evaluate pharmacokinetic and toxicological parameters. These comprehensive biological studies were

Fig. 2 Structures of selected pyrimido[2,1-*f*]purinediones and K_i values as adenosine receptor antagonists⁴³ (h = human, r = rat).

performed to analyze the properties of this new class of tricyclic compounds based on the xanthine scaffold.

Results and discussion

Chemistry

The structures of all synthesized compounds were confirmed by elemental and spectral analyses including ^1H NMR, ^{13}C NMR, IR, and MS. The purity of all final products was determined to be at least 95% using UPLC coupled to MS.

The designed new series of amido-substituted *N*-9-phenylethyl-1,3-dipropyl- and -1,3-dibutyl-pyrimido[2,1-*f*]purinediones were synthesized according to the synthetic procedure depicted in Schemes 1 and 2. The compounds showed diversity regarding the attached end moiety with nitrogen of the CH_2CONH fragment *i.e.* a bulky aliphatic amine, imidazole, pyridine or substituted phenyl groups linked through nitrogen of the CH_2CONH fragment through one to three methylenes. The amide fragment was introduced to the *N*-9-phenylethylpyrimido[2,1-*f*]purinedione core structure by replacement of the ester group attached in the *para*-position of the phenyl ring using a solvent-free microwave-assisted synthetic protocol. Previously described methods were used to obtain the *N*-9-phenylethylpyrimido[2,1-*f*]purinedione derivatives (9, 10) used as starting compounds.^{42,43}

The xanthine core was prepared by a modified Traube method for dipropylxanthine. In the next step, dipropylxanthine and commercially available dibutylxanthine were oxidatively brominated in position 8 and N7-alkylated by 1-bromo-3-chloropropane using phase transfer catalysis reaction. To synthesize the tricyclic structure of pyrimido[2,1-*f*]purinedione, a ring-closure reaction was performed with tyramine and 8-bromo-7- γ -chloropropyl-1,3-dialkylxanthine (Scheme 1).^{42,43}

Subsequently, *N*-9-(*p*-hydroxy)phenylethyltetrahydropyrimido[2,1-*f*]purinedione derivatives 6 and 7 were *O*-alkylated by methyl bromoacetate using a phase transfer catalyzed (PTC) reaction method.⁴⁴ In the last step, the ester derivatives 10 and 11 were condensed with a variety of commercially available amines using microwave irradiation under different conditions and time protocols in solvent-free reaction medium (see ESI[†]). Two series of amide derivatives were obtained: 1,3-dipropyl- (12–25) and 1,3-dibutylpyrimido[2,1-*f*]purinediones (26–38) (Scheme 2).

Biological activity

Compounds 12–38 were tested in radioligand binding assays to evaluate their affinities for all four AR subtypes. Rat brain

Scheme 1 Synthesis of the tetrahydropyrimido[2,1-*f*]purinedione scaffold.

Scheme 2 Synthesis of the target pyrimido[2,1-*f*]purinedione derivatives. Reagents and conditions: (i) 2-butanone, K_2CO_3 ; (ii) μW .

cortical membranes and rat brain striatal membranes were used for A_1 and $\text{A}_{2\text{A}}$ ARs, respectively. Human $\text{A}_{2\text{B}}$ and A_3 ARs were recombinantly expressed in Chinese hamster ovary (CHO) cells. Selected compounds were tested further for their affinity for human A_1 and $\text{A}_{2\text{A}}$ ARs in recombinant CHO cells using the same radioligands. The affinities to ARs of 1,3-dipropyl- (12–25) and 1,3-dibutyl-pyrimido[2,1-*f*]purinediones (26–38) are presented in Table 1.

Our previous studies on pyrimido[2,1-*f*]purinediones had revealed that compounds belonging to this group all displayed antagonistic properties for adenosine receptors.⁴³ The new compounds may bind to the orthosteric binding area of ARs based on their antagonistic activity using established radiolabeled ligand binding.

1,3-Dipropyltetrahydropyrimido[2,1-*f*]purinediones. The reference compound 6 from the series of 1,3-dipropylpyrimido[2,1-*f*]purinedione derivatives displayed moderate affinity for the A_1 ($K_i = 370$ nM), and $\text{A}_{2\text{A}}$ ($K_i = 464$ nM) and weak affinity for $\text{A}_{2\text{B}}$ ($K_i = 663$ nM) and A_3 ARs ($K_i > 1000$ nM).¹⁴ Introduction of a diethylaminoethyl group connected with the oxygen of the phenoxy moiety (8) resulted in only slightly changed affinity for the A_1 ($K_i = 347$ nM) and $\text{A}_{2\text{A}}$ ($K_i = 654$ nM), but an increased affinity for the $\text{A}_{2\text{B}}$ ($K_i = 338$ nM) and A_3 ($K_i = 633$ nM) ARs.¹⁵ Comparing compound 15 to compound 8, it can be observed that modification of the substituent by addition of a CH_2CONH linker between $(\text{CH}_2)_2\text{NEt}_2$ and the phenoxy moiety increased the affinity not only for the A_1 ($K_i = 120$ nM) and $\text{A}_{2\text{A}}$ ($K_i = 271$ nM) but also for the $\text{A}_{2\text{B}}$ ($K_i = 179$ nM) and A_3 ($K_i = 508$ nM) AR subtypes. However, elongation of the methylene chain between the amide bond and the *N,N*-diethylamino group (in 16) resulted in a drop in affinity for the A_3 subtype ($K_i > 1000$ nM).

Changes in the part of the tertiary amine moiety by replacement of the *N,N*-diethylamino group by a five-membered heterocyclic pyrrolidine ring were investigated. Compounds 12 and 13 with a pyrrolidine ring showed comparable affinities for ARs to their analogues 15 and 16 with an ethyl or propyl linker, respectively.

Replacement of the saturated heterocyclic ring by an imidazole in 25 resulted in significantly lower affinity at all AR subtypes. A drop in affinity was also observed by the enlargement of the saturated heterocyclic ring to a six-membered ring. Compound 14 with a morpholine moiety displayed 2-fold to 3-fold lower affinities for ARs.

Table 1 Affinities of 1,3-dipropyl- and 1,3-dibutyltetrahydropyrimido[2,1-*f*]purinediones for A₁, A_{2A}, A_{2B} and A₃ ARs

Compd	R ²	Rat A ₁ [³ H]CCPA <i>K_i</i> ± SEM (nM) (% inhibition ± SEM at 1 μM)	Rat A _{2A} [³ H]MSX-2	Human A _{2B} [³ H]PSB-603	Human A ₃ [³ H]PSB-11
R¹ – <i>n</i>-propyl					
6 (ref. 43)	–H	370 ± 37	464 ± 66	663 ± 130	>1000
8 (ref. 43)		347 ± 33	658 ± 115	338 ± 14	633 ± 52
12		113 ± 4	551 ± 140	193 ± 39	509 ± 98
13		146 ± 32	320 ± 27	452 ± 7	>1000 (33 ± 6)
14		509 ± 64	1120 ± 204	911 ± 260	>1000 (21 ± 3)
15		120 ± 14	271 ± 53	179 ± 20	508 ± 140
16		136 ± 33	303 ± 58	340 ± 87	>1000 (36 ± 7)
17		>10 000 (22 ± 7)	>10 000 (37 ± 1)	>1000 (39 ± 1)	>1000 (28 ± 5)
18		1370 ± 283	807 ± 161	>1000 (22 ± 2)	572 ± 184
19		145 ± 4	335 ± 101	306 ± 115	458 ± 37
20		563 ± 73	1360 ± 98	>1000 (39 ± 6)	>1000 (31 ± 5)
21		>10 000 (26 ± 2)	3230 ± 326	>1000 (22 ± 0)	>1000 (34 ± 2)
22		>10 000 (37 ± 4)	1360 ± 365	>1000 (31 ± 3)	>1000 (41 ± 4)
23		<i>ca.</i> 10 000 (45 ± 4)	1870 ± 311	>1000 (30 ± 8)	>1000 (28 ± 0)
24		>10 000 (18 ± 3)	>10 000 (24 ± 1)	>1000 (–4 ± 1)	1090 ± 140
25		605 ± 128	637 ± 95	862 ± 227	<i>ca.</i> 1000
R¹ – <i>n</i>-butyl					
7 (ref. 43)	–H	184 ± 57	518 ± 79	131 ± 22	619 ± 110
9 (ref. 43)		80 ± 5	566 ± 43	109 ± 11	310 ± 48
26		50.7 ± 9.4	242 ± 47	113 ± 19	155 ± 37
27		24.8 ± 5.0	318 ± 24	126 ± 23	248 ± 41
28		320 ± 44	1290 ± 25	578 ± 79	602 ± 96

Table 1 (continued)

Compd	R ²	Rat A ₁ [³ H]CCPA K _i ± SEM (nM) (% inhibition ± SEM at 1 μM)	Rat A _{2A} [³ H]MSX-2	Human A _{2B} [³ H]PSB-603	Human A ₃ [³ H]PSB-11
29		181 ± 28	671 ± 146	298 ± 45	>1000 (46 ± 2)
30		684 ± 240	6520 ± 544	>1000 (18 ± 2)	>1000 (42 ± 2)
31		1590 ± 240	1630 ± 333	>1000 (41 ± 3)	>1000 (41 ± 2)
32		1010 ± 201	>10 000 (32 ± 7)	>1000 (43 ± 8)	>1000 (40 ± 8)
33		118 ± 20	1250 ± 180	215 ± 29	353 ± 56
34		382 ± 64	1070 ± 179	>1000 (39 ± 3)	>1000 (44 ± 0)
35		194 ± 41	569 ± 65	275 ± 65	868 ± 159
36		36.6 ± 9.1	455 ± 65	66.3 ± 17.7	204 ± 21
37		>10 000 (27 ± 7)	2270 ± 238	>1000 (31 ± 5)	>1000 (23 ± 4)
38		593 ± 204	7310 ± 2220	>1000 (31 ± 5)	>1000 (23 ± 4)

The replacement of the amine moiety with benzyl groups or CH₂CH₂PhSO₂NH₂ group attached to the amide nitrogen decreased affinity at all AR subtypes with more pronounced effect for A₁, A_{2B} and A₃ affinities. Compounds 18 and 22 exhibited low micromolar A_{2A} affinity and 18, nice affinity against A₃. In the case of the replacement of benzyl with a 2-picolinyl group, the A₁ affinity was retained and the A_{2A} was low micromolar. The final modification was the insertion of a cyclohexylamine moiety connected by a propyl linker to the amide bond. Compound 19 showed affinity for ARs similar to that of the *N,N*-diethylamino-substituted derivative 16 with a slightly higher A₃ affinity (K_i A₃ 458 nM).

1,3-Dibutyltetrahydropyrimido[2,1-*f*]purinediones. Lead compound 7 of the series of 1,3-dibutylpyrimido[2,1-*f*]purinediones displayed high affinity for the A₁ (K_i = 184 nM) and A_{2B} ARs (K_i = 131 nM) with K_i values in the nanomolar range and lower affinity for the A_{2A} (K_i = 518 nM) and A₃ (K_i = 619 nM) ARs. Moreover, compound 9 with a diethylaminoethyl group connected with the oxygen of the phenoxy moiety showed even higher affinity for the A₁ (K_i = 80 nM)

and A₃ (K_i = 310 nM) ARs. Both above-mentioned structures displayed good affinity for all AR subtypes. The modification of the substituent by addition of a CH₂CONH linker between (CH₂)₂NEt₂ and the phenoxy moiety in 26 improved the affinity for A₁, A_{2A} and A₃ ARs (K_i = 50.7 nM for A₁; K_i = 242 nM for A_{2A}; K_i = 113 nM for A_{2B}; K_i = 155 nM for A₃) compared with the reference compounds and the 1,3-dipropyl analogue 15. A two-fold increase in affinity for the A₁ ARs (K_i = 24.8 nM) and a slight improvement in selectivity was achieved by elongation of the linker between the amide bond and the *N*, *N*-diethylamino moiety in compound 27.

Replacement of the *N,N*-diethylamino group by a pyrrolidine ring in 36 resulted in a drop in affinity for the A_{2A} AR, and an increase in affinity for the A_{2B} AR (K_i = 66.3 nM). Subsequently, the influence of other heterocyclic rings was investigated. Compounds with an imidazole (35) or a morpholine ring (29) displayed lower affinity for all AR subtypes. Finally, the introduction of cyclohexylamine instead of a tertiary amine was studied. Compound 33 showed a similar affinity profile as its 1,3-dipropyl analogue 19 except for a lower affinity for the A_{2A} AR (K_i = 1250 nM).

The replacement of the amine moiety with benzyl groups or $\text{CH}_2\text{CH}_2\text{PhSO}_2\text{NH}_2$ group attached to the amide nitrogen resulted in a significant decrease in affinity at all AR subtypes. Some compounds containing a chloro-substituted benzyl group (30, 38) or a 2-picolinyl group moiety (34) still displayed moderate affinity for A_1 ARs.

Molecular modeling and docking

In an attempt to explain the pharmacological data for the synthesized compounds, especially the results of affinity for the rat adenosine receptors A_1 and $\text{A}_{2\text{A}}$, a molecular modeling study was performed. The recently reported homology models of rA_1 and $\text{rA}_{2\text{A}}$ ARs were used for docking of the synthesized compounds.⁴³ Both models were constructed using the X-ray structure of $\text{hA}_{2\text{A}}$ AR (PDB ID: 3EML) and refined by the induced fit docking procedure using known xanthine-based receptor antagonists (tonapofylline for rA_1 AR, istradefylline for $\text{rA}_{2\text{A}}$ AR).

The results of docking experiments, both in the case of rA_1 and $\text{rA}_{2\text{A}}$ AR homology models, clearly point to the similar location of the tricyclic core of compounds inside the binding sites, seen also for available X-ray structures of the human $\text{A}_{2\text{A}}$ AR: 3REY and 3RFM. The tricyclic core of the docked compounds seems to be very well anchored by the H-bond between the conserved Asn^{6.55} and the ligand carbonyl group in the 4-position (Fig. 3), as well as the hydrophobic interactions with surrounding residues (*e.g.* π - π stacking with Phe^{5.29}). However, various conformations can be observed for long flexible substituents at the N9 position directed to the top part of the receptor towards the extracellular loops. This divergence is perfectly illustrated by the superposition of the low-energy conformations for compounds possessing the aliphatic amine function (12–16, 19, 26, 27, 29, 33, 36) when docked to the rA_1 homology model (Fig. 3). The conformation of the “tail” at the N9 position in this se-

ries of compounds is apparently determined by formation of salt bridges between a protonated amine group of ligands and at least one of glutamate residues, located at the extracellular loop EL2 of the rA_1 AR: Glu 170 or Glu 172 (Fig. 3). These interactions probably contribute to better docking scores seen for amine-substituted derivatives compared to other tricyclic analogues in this group.

To compare the docking poses of the tricyclic compounds and their interaction with the rA_1 and the $\text{rA}_{2\text{A}}$ AR binding pockets, an example of the compound 36 was chosen for its high affinity at the rA_1 AR (Fig. 4 and 5). The residues located in the proximity of the tricyclic core of ligands are highly conserved within the binding pocket of the subtypes rA_1 and $\text{rA}_{2\text{A}}$, however, numerous differences in both sequences can be seen for the residues surrounding the N9-substituent.⁴³ Observed differences are located to a large extent in the extracellular region, especially within the second and the third extracellular loop. Unfortunately, due to flexibility of these regions (especially EL2), the prediction of interactions between the ligand and residues placed in extracellular loops can only be estimated.

One of the most significant replacements is undoubtedly the exchange in position 5.28 of EL2. Glutamate Glu 170, present in this position in the rat A_1 AR sequence, corresponds to leucine Leu 162 in the $\text{rA}_{2\text{A}}$ AR sequence (Fig. 4 and 5). The possibility of salt bridge formation between the charged amine group of ligands and Glu 170 is most probably one of the structural factors influencing high rA_1 AR affinity and $\text{A}_1/\text{A}_{2\text{A}}$ subtype selectivity observed in biological assays for the amine-alkyl derivatives, *e.g.* compound 36. Another important structural difference in the proximity of ligands can be observed in the extracellular loop EL3, where Gln 264 in the rA_1 AR corresponds to His 259, present in the $\text{rA}_{2\text{A}}$ AR. The strong interaction between His 259 and Glu 164 changes the conformation of the glutamate side chain in the $\text{rA}_{2\text{A}}$ subtype compared to Glu 172 in the rA_1 AR, which in our

Fig. 3 Superposition of low-energy conformations observed for selected compounds possessing an aliphatic amine group, docked to the rA_1 AR homology model.

Fig. 4 The docking pose of the compound 36 at the binding site of the rA_1 AR. Residue numbering as for the rA_1 AR sequence P25099.

Fig. 5 The docking pose of the compound **36** at the binding site of the rA_{2A} AR. Residue numbering as for the rA_{2A} AR sequence P30543.

studies often interacts with the protonated amine group of ligands (Fig. 4).

The docking of the described series of compounds to the rA_{2A} AR homology model shows various conformations adopted by the long N9-substituent directed towards the extracellular loops (Fig. 5). The H-bonds between the amide group of ligands and the protein can often be observed, however, no position of the “tail” is preferred – all the compounds show in this docking similar values of the docking score function, regardless of the structure of the N9-substituent.

Solubility in water

Four compounds were selected to experimentally determine their water solubility. This was achieved by UV spectroscopy according to previously described methods.^{43,45} The tested compounds **15**, **16**, and **27** displayed high water solubility of approx. 0.5 mg ml⁻¹ (Table 2). As expected, the 1,3-dipropylpyrimido[2,1-*f*]purinediones (**15**, **16**) showed higher solubility than the corresponding more lipophilic 1,3-dibutyl derivatives (**26**, **27**).

Metabolic stability of selected compounds

The metabolic stability of compounds **27** and **36** was initially examined by *in silico* tools. The computational procedure MetaSite 4.1.1 provided by Molecular Discovery Ltd. was used to determine the potential sites of metabolism as well as the most probable structures of potential metabolites.⁴⁶ The plot

Table 2 Water solubility of selected compounds

Compound	Solubility mg ml ⁻¹	Solubility μmol l ⁻¹
15	0.615	1083
16	0.479	823
26	0.113	189
27	0.386	632

of MetaSite predictions for the most probable sites for metabolism of **27** and **36** by a computational liver model, and *in vitro* metabolic stability studies using human (HLMs) or rat (RLMs) liver microsomes are shown in the ESI.†

The model suggested that compound **27** has a moderate metabolic stability since about 50% (in the presence of HLMs) or 70% (in the presence of RLMs) of **27** was metabolized within 2 hours under the applied conditions. However, compound **36** was found to be significantly more stable than **27**. Metabolic pathways of compounds **27** and **36** were predicted to include hydroxylation, dealkylation, dehydrogenation, dealkylation and deamination followed by oxidation. The most probable metabolites were partially identified using UPLC/MS analysis after incubation of tested compounds with HLMs or RLMs. These results will be helpful for the future design of metabolically stable substituted tricyclic xanthine derivatives.

Toxicity of selected compounds

Effect on the recombinant human CYP3A4 P450 cytochrome. Compounds **27** and **36** were also examined to determine their influence on cytochrome P450 3A4 activity. CYP3A4 is involved in the biotransformation of approximately 50% of all marketed drugs, so its potential inhibition or induction may result in drug–drug interactions.⁴⁷ For this purpose, a commercial bioluminescence-based CYP3A4 P450-Glo™ assay was performed. The strong CYP3A4 inhibitor ketoconazole (KE) was used as a reference compound. No inhibitory effect was observed for **36** whereas the structurally related compound **27** showed very strong inhibition, with an IC₅₀ value of 0.23 μM, similar to that obtained for KE: IC₅₀ = 0.14 μM (Fig. 6).

Antiproliferative effects. Next, a preliminary evaluation of potential cytotoxic effects of the selected compounds **27** and **36** was performed using the formazan dye-based EZ4U assay in human embryonic kidney cells (HEK-293) incubated for 48 hours. The antiproliferative drug doxorubicin (DX) for which an IC₅₀ value of 0.46 μM was determined in the same assay was used as a reference. Compounds **27** and **36** showed antiproliferative effects only at high concentrations above 10 μM with IC₅₀ values greater than 20 μM (see Fig. 7). In summary, taking into account the calculated IC₅₀ values for **27** and **36** (Fig. 7), as well as the results obtained for DX, both

Fig. 6 Effect of ketoconazole (KE) and compounds **27** and **36** on CYP3A4 P450 cytochrome activity.

adenosine receptor ligands may be considered as a weakly to moderately cytotoxic compounds. Therefore, the compounds can be useful for further evaluation.

Experimental section

Chemistry

All commercially available reagents and solvents were used without further purification. Melting points (mp.) were determined using a MEL-TEMP II (LD Inc., USA) melting point apparatus and are uncorrected. ^1H NMR spectra were obtained with a Varian Mercury-VX 300 MHz PFG spectrometer or a Bruker AMX 300 (Bruker, Germany) spectrometer in $\text{DMSO}-d_6$ or CDCl_3 with TMS as an internal standard. Chemical shifts were expressed in parts per million (ppm). ^{13}C NMR data were recorded at 75 MHz using a Varian-Mercury-VX 300 MHz PFG or a 400 MHz spectrometer. The purity of the final compounds was determined (%) using a Waters TQD mass spectrometer coupled with a Waters ACQUITY UPLC system. IR spectra were obtained using a Fourier transform infrared spectrometer FT-IR-410 (Jasco) or a FT/IR Nicolet iS5 spectrometer (ThermoScientific) using KBr pellets. Elemental analysis (C, H, N) was accordingly within 0.5% of theoretical values and was performed using an Elementar-Vario-EL III (Hanau, Germany) apparatus. Microwave reactions were performed in a domestic microwave oven Samsung MW71B. Silica gel 60 (0.063–0.20 mm; Merck) was used for column chromatography and a mixture of dichloromethane with methanol was applied as a mobile phase. TLC data were obtained using aluminium sheets coated with silica gel 60 F254 (Merck). Eluent system: cyclohexane/1,4-dioxane 1:1, DCM/MeOH 7:3, DCM/MeOH 9:1, DCM/MeOH 9.5:0.5.

The synthesis and physicochemical properties of the intermediate compounds **10** and **11** were reported in the ESI.[†]

A general procedure for the synthesis of tetrahydropyrimido[2,1-*f*]purinediones **12** and **13**

A mixture of 1 mmol of ester (**10**) and 2 mmol of 1-(2-aminoethylpyrrolidine) was refluxed in propanol for 10 h. Crude products were separated by removing the solvent by rotary evaporation and adding water to the residue. Obtained compounds were recrystallized from EtOH + H_2O .

2-(4-(2-(2,4-Dioxo-1,3-dipropyl-1,2,3,4,7,8-hexahydropyrimido[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)-*N*-(2-(pyrrolidin-1-yl)ethyl)-

acetamide (**12**). Yield: 0.48 g, 86%, mp: 133–136 °C; ^1H NMR (300 MHz, $\text{CHLOROFORM}-d$) δ ppm 0.97 (t, J = 7.95 Hz, 6H, $2 \times \text{CH}_3\text{CH}_2\text{CH}_2$); 1.60–1.86 (m, 8H, $2 \times \text{CH}_3\text{CH}_2\text{CH}_2$, $2 \times \text{NCH}_2\text{CH}_2$, Pyrrolidine); 2.01–2.09 (m, 2H, C7H₂); 2.64 (bs, 4H, $2 \times \text{NCH}_2\text{CH}_2$, Pyrrolidine); 2.73 (t, J = 6.03 Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{N}$); 2.89 (t, J = 7.31 Hz, 2H, $\text{CH}_2\text{C}_6\text{H}_4$); 3.21 (t, J = 5.51 Hz, 2H, N9CH₂); 3.45 (q, J = 6.03 Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{N}$); 3.68 (t, J = 7.31 Hz, 2H, C8H₂); 3.90–3.94 (m, 4H, N1CH₂, N3CH₂); 4.17 (t, J = 5.90 Hz, 2H, N5CH₂); 4.48 (s, 2H, OCH₂-CO); 6.86–6.90 (m, 2H, Ar); 7.12–7.17 (m, 2H, Ar); 7.23 (s, 1H, NHCH₂). ^{13}C NMR ($\text{CHLOROFORM}-d$) δ ppm: 11.30, 11.37, 21.41, 21.47, 21.50, 23.46, 32.98, 37.12, 41.51, 42.43, 44.86, 45.33, 51.57, 53.95, 54.58, 67.48, 102.89, 114.86, 129.97, 132.43, 148.97, 151.33, 153.86, 156.05, 168.55. IR [KBr] 3298 (N-H_r), 1688, 1655 (C=O), 1247 (C-O_{Ar}) [cm^{-1}]. LC-MS: purity 95.14%, t_R = 4.80, (ESI) m/z 567 [$\text{M}^+ + 1$]. Anal. for $\text{C}_{30}\text{H}_{43}\text{N}_7\text{O}_4$: calcd: C, 61.74; H, 7.70; N, 16.79. Found: C, 61.36; H, 7.85; N, 16.55.

2-(4-(2-(2,4-Dioxo-1,3-dipropyl-1,2,3,4,7,8-hexahydropyrimido[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)-*N*-(3-(pyrrolidin-1-yl)propyl)acetamide (**13**). Yield: 0.12 g, 21%; mp: 139 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 0.78–0.89 (m, 6H, $2 \times \text{CH}_3\text{CH}_2\text{CH}_2$); 1.43–1.72 (m, 10H, $2 \times \text{CH}_3\text{CH}_2\text{CH}_2$, $2 \times \text{NCH}_2\text{CH}_2$, Pyrrolidine, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$); 1.92–2.02 (m, 2H, C7H₂); 2.29–2.37 (m, 6H, $2 \times \text{NCH}_2\text{CH}_2$, Pyrrolidine, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$); 2.80 (t, J = 7.44 Hz, 2H, $\text{CH}_2\text{C}_6\text{H}_4$); 3.14 (q, J = 6.67 Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$); 3.24–3.28 (m, 2H, N9CH₂); 3.61 (t, J = 7.44 Hz, 2H, C8H₂); 3.75 (t, J = 7.40 Hz, 2H, N3CH₂); 3.87 (t, J = 7.05 Hz, 2H, N1CH₂); 4.00 (t, J = 5.64 Hz, 2H, N5CH₂); 4.38 (s, 2H, OCH₂CO); 6.85 (d, J = 8.72 Hz, 2H, Ar); 7.14 (d, J = 8.72 Hz, 2H, Ar); 8.10 (t, J = 5.51 Hz, 1H, NHCH₂). ^{13}C NMR ($\text{DMSO}-d_6$) δ ppm: 11.52, 11.63, 21.21, 21.31, 21.44, 23.52, 28.44, 32.52, 37.66, 41.74, 41.88, 44.31, 44.64, 51.14, 53.99, 54.07, 67.67, 102.27, 115.11, 130.12, 132.21, 148.65, 150.98, 151.74, 152.94, 156.68, 167.93. IR [KBr] 3406 (N-H_r); 2962 (C-H); 1651 (C=O); 1616 (N-H_d); 1528 (C=C_{Ar}); 1236 (C-O_{Ar}) [cm^{-1}]. LC-MS: purity 98.85%, t_R = 4.83, (ESI) m/z 580 [$\text{M}^+ + 1$]. Anal. for $\text{C}_{31}\text{H}_{45}\text{N}_7\text{O}_4$: calcd: C, 64.22; H, 7.82; N, 16.91. Found: C, 63.84; H, 7.81; N, 16.58.

A general procedure for the synthesis of tetrahydropyrimido[2,1-*f*]purinediones (**14–25**) and (**26–38**)

A mixture of 1 mmol methyl 2-(4-(2-(2,4-dioxo-1,3-dipropyl-1,2,3,4,7,8-hexahydropyrimido[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)acetate (**10**) or methyl 2-(4-(2-(2,4-dioxo-1,3-dibutyl-1,2,3,4,7,8-hexahydropyrimido[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)acetate (**11**) and 2 mmol of appropriate amine was melted using a domestic microwave oven (power and time in Table S1 in the ESI.[†]). The residue was treated with 10 ml of ethanol. The precipitate was filtered off and the product was purified by crystallization from EtOH in the presence of charcoal or column chromatography (DCM/MeOH 9:1).

Procedures, yields and physical data of the selected compounds are given below for all other obtained xanthine derivatives and the analytical data can be found in the ESI.[†]

Fig. 7 Cytotoxic activity of the reference compound doxorubicin (DX) and xanthines **27** and **36** against HEK-293 cells.

2-(4-(2-(1,3-Dibutyl-2,4-dioxo-1,2,3,4,7,8-hexahydropyrimido-[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)-*N*-(3-(diethylamino)propyl)-acetamide (27). Yield: 0.1 g, 16.4% mp: 79–83 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 0.83–0.94 (m, 12H, 2 × CH₃CH₂CH₂CH₂, 2 × CH₃CH₂N) 1.19–1.33 (m, 4H, 2 × CH₃CH₂CH₂CH₂) 1.41–1.56 (m, 4H, 2 × CH₃CH₂CH₂CH₂) 1.63 (quin, *J* = 7.10 Hz, 2H, NHCH₂CH₂CH₂N) 1.93–2.02 (m, 2H, C7H₂) 2.30–2.43 (m, 6H, CH₂N(CH₂CH₃)₂) 2.80 (t, *J* = 7.31 Hz, 2H, CH₂C₆H₄) 3.14 (q, *J* = 6.50 Hz, 2H, NHCH₂CH₂CH₂N) 3.25–3.27 (m, 2H, N9CH₂) 3.60 (t, *J* = 7.57 Hz, 2H, C8H₂) 3.79 (t, *J* = 7.40 Hz, 2H, N3CH₂) 3.91 (t, *J* = 7.05 Hz, 2H, N1CH₂) 4.01 (t, *J* = 5.90 Hz, 2H, N5CH₂) 4.39 (s, 2H, OCH₂CO) 6.85 (d, *J* = 8.46 Hz, 2H, Ar) 7.14 (d, *J* = 8.72 Hz, 2H, Ar) 8.14 (t, *J* = 5.39 Hz, 1H, NHCH₂). ¹³C NMR (DMSO *d*₆) δ ppm: 12.01, 14.05, 14.18, 19.80, 20.11, 21.23, 26.71, 30.05, 30.34, 32.53, 37.87, 41.73, 42.42, 44.64, 46.75, 50.85, 51.20, 67.59, 102.30, 115.04, 130.10, 132.15, 148.61, 150.90, 151.71, 152.91, 156.64, 167.83. IR [KBr]: 3327 (N–H_r); 2958 (C–H); 1651 (C=O); 1533 (C=C_{Ar}); 1236 (C–O_{Ar}); [cm^{−1}]. LC-MS: purity 99.2%, *t*_R = 5.67, (ESI) *m/z* 610.7 [M⁺ + 1]. Anal. for C₃₃H₅₁N₇O₄: calcd: C, 64.99; H, 8.43; N, 16.08. Found: C, 64.66; H, 8.92; N, 15.96.

2-(4-(2-(1,3-Dibutyl-2,4-dioxo-1,2,3,4,7,8-hexahydropyrimido-[2,1-*f*]purin-9(6*H*)-yl)ethyl)phenoxy)-*N*-(2-(pyrrolidin-1-yl)ethyl)-acetamide (36). Yield: 0.26 g, 43.8% mp: 130–133 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 0.83–0.93 (m, 6H, 2 × CH₃CH₂CH₂CH₂) 1.19–1.34 (m, 4H, 2 × CH₃CH₂CH₂CH₂) 1.46 (quin, *J* = 7.33 Hz, 2H, N3CH₂CH₂) 1.57–1.67 (m, 6H N1CH₂CH₂, 2 × CH₂, Pyrrolidine) 1.93–2.01 (m, 2H, C7H₂) 2.36–2.45 (m, 6H, 2 × NCH₂, Pyrrolidine, NHCH₂CH₂N) 2.80 (t, *J* = 7.33 Hz, 2H, CH₂C₆H₄) 3.16–3.29 (m, 4H, NHCH₂CH₂N, N9CH₂) 3.60 (t, *J* = 7.33 Hz, 2H, C8H₂) 3.78 (t, *J* = 7.33 Hz, 2H, N3CH₂) 3.91 (t, *J* = 7.03 Hz, 2H, N1CH₂) 4.00 (t, *J* = 5.86 Hz, 2H, N5CH₂) 4.40 (s, 2H, OCH₂CO) 6.86 (d, *J* = 8.79 Hz, 2H, Ar) 7.14 (d, *J* = 8.21 Hz, 2H, Ar) 7.92 (t, *J* = 5.57 Hz, 1H NHCH₂). ¹³C NMR (DMSO *d*₆) δ: 14.05, 14.18, 20.09, 23.57, 30.01, 30.32, 32.50, 37.99, 42.42, 53.98, 55.04, 67.52, 102.27, 115.14, 130.06, 132.17, 150.88, 151.70, 152.90, 156.63, 167.97 ppm. IR [KBr]: 3298 (N–H_r); 2955 (C–H); 1658 (C=O); 1619 (N–H_d); 1528 (C=C_{Ar}); 1247 (C–O_{Ar}) [cm^{−1}]. LC-MS: purity 95.72%, *t*_R = 5.71, (ESI) *m/z* 594.67 [M⁺ + 1]. Anal. for C₃₂H₄₇N₇O₄: calcd: C, 64.73; H, 7.98; N, 16.51. Found: C, 64.74 H, 8.06 N, 16.78.

Pharmacology

AR radioligand binding assays were performed as previously described⁵⁰ using rat brain cortical membrane preparations for A₁ and rat brain striatal membrane preparations for A_{2A} AR assays. Frozen rat brains (unstripped) were obtained from Pel Freez, Rogers, Arkansas, USA. For assays of human AR subtypes, cell membranes of CHO cells recombinantly expressing the respective receptor were used as described.⁵⁰ The following compounds were employed as radioligands: A₁: [³H]2-chloro-*N*⁶-cyclopentyladenosine ([³H]CCPA);⁵² A_{2A}: [³H]3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)-1-propargylxanthine ([³H]MSX-2);⁵³ A_{2B}: [³H]8-(4-(4-

chlorophenyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine ([³H]PSB-603);^{50,51} A₃: [³H]phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purine-5-one ([³H]PSB-11).⁵⁴ Initially, a single high concentration of the compound was tested (10 μM or 1 μM, respectively). For potent compounds, full concentration-inhibition curves were determined using six to ten different concentrations of the test compound spanning three orders of magnitude. At least three independent experiments were performed. Data were analyzed using the PRISM program version 4.0 or higher (Graph Pad, San Diego, CA, USA).

Molecular modeling

Both rA₁ and rA_{2A} AR models were constructed by comparative modeling methods and refined by the induced fit docking procedure, as described previously.⁴³ The ligand library was prepared and subjected to the conformational search using the options implemented in the Schrödinger Suite software⁵⁵ (multiple minimization method, OPLS3 force field, Truncated Newton Conjugate Gradient (TNCG)). Docking studies for the low-energy conformations of all compounds to the rigid receptor binding pocket were performed using the Glide module, with the standard precision (SP) mode and the constrained H-bond between the side chain amino group of Asn^{6.55} and the ligand. Five poses obtained after docking for each ligand were post-minimized, and the final poses were kept and analyzed according to the obtained docking score values. For the graphic presentation of the selected structures with the highest docking scores, representing individual clusters of poses, PyMOL software was used.⁵⁶

Water solubility determination

Determination of the water solubility of selected compounds was performed using UV spectroscopy based on previously described methods. Saturated solutions of compounds (basic form) were prepared by suspending each compound (10 mg) in H₂O (2 mL). The suspensions were mixed and boiled for 5 min, then left overnight at 20 °C and filtered off using a filter, Macherey-Nagel MN 619 de. Each filtrate was diluted in MeOH (from 10 to 80-fold) and analyzed by UV spectroscopy as a solution in MeOH/H₂O (90% v/v). Standard curves were determined using known concentrations of each compound. Each stock solution was prepared (2 mg in 2 mL of the 90% MeOH) and further diluted to obtain seven different concentrations ranging from 10^{−3} to 10^{−1} mg mL^{−1}. The concentration of the saturated solutions for the compounds was determined by linear regression of two vicinal points from the standard curves and multiplication by the degree of dilution using MS Excel.

Metabolic stability

Commercial, pooled, human (adult male & female) (HLMs) or rat liver microsomes were obtained from Sigma-Aldrich (St. Louis, USA). The biotransformations were carried out using 1 mg mL^{−1} HLMs or RLMs in 200 μL of a reaction buffer

containing 0.1 M Tris-HCl (pH 7.4), a NADPH regenerating system (Promega, Madison, WI, USA) and an examined compound with a final volume of 50 μ M. The reaction mixture was preincubated at 37 °C for 5 min and then, the reaction was initiated by adding 50 μ l of the regenerating system. To terminate the reaction after 120 min, 200 μ l of cold methanol was added. The mixture was next centrifuged at 14 000 rpm for 15 min and UPLC/MS analysis of the supernatant was performed. Mass spectra were recorded using a UPLC/MS system consisting of a Waters Acquity UPLC (Waters, Milford, USA), coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). The *in silico* study was performed using MetaSite 4.1.1 provided by Molecular Discovery Ltd. The highest metabolism probability sites and metabolites' structures were analyzed during this study using a liver computational model.

Toxicity

Luminescence CYP3A4 P450-Glo™ assay. The luminescence CYP3A4 P450-Glo™ assay was provided by Promega (Madison, WI, USA). The enzymatic reactions were performed in white polystyrene, flat-bottom Nunc™ MicroWell™ 96-well microplates (Thermo Scientific, Waltham, MA USA). The luminescence signal was measured with a microplate reader in a luminescence mode (EnSpire, PerkinElmer, Waltham, MA USA). The CYP3A4 P450-Glo™ protocol was also provided by Promega.⁴⁸ The IC₅₀ value of the reference drug ketoconazole was determined and calculated as reported previously.⁴⁹ The final concentrations of adenosine receptor ligands were 0.025–25 μ M.

Antiproliferative assay. The HEK-293 cells were seeded in 96-well plates at a concentration of 1×10^4 cells per well in 200 μ l culture medium and routinely cultured for 24 h to reach 60% confluence. Next, the stock solutions (25 mM) of the examined compounds in DMSO were diluted into fresh growth medium and added into the microplates at the final concentrations of 0.1–250 μ M (DMSO final concentration did not exceed 1%). After 48 h of incubation, a 20 μ l of EZ4U labeling mixture (Biomedica) was added to each well and the cells were incubated under the same conditions for 5 h. The absorbance of the samples was measured using a microplate reader (PerkinElmer) at 492 nm. The IC₅₀ value of the reference drug doxorubicin was determined and calculated as reported previously.⁴⁹ GraphPad Prism™ software (version 5.01, San Diego, CA, USA) was used to calculate the IC₅₀ values.

Conclusions

A newly designed series of 13 1,3-dipropyl- and 13 1,3-dibutylpyrimido[2,1-*f*]purinedione-9-ethylphenoxy derivatives including a CH₂CONH linker between the (CH₂)₂-amino group and the phenoxy moiety were investigated as A₁/A_{2A} adenosine receptor antagonists. Compounds were synthesized and evaluated in radioligand binding assays for the interaction with ARs. A solvent-free microwave assisted synthetic pathway was developed to link the amide moieties with the pyrimido[2,1-

f]purinedione-9-ethylphenoxy scaffold. Water solubility was determined by experimental assays for selected compounds. The series of 1,3-dipropylpyrimido[2,1-*f*]purinediones showed higher water solubility than the 1,3-dibutylpyrimido[2,1-*f*]purinedione analogues.

In both series, structures containing a tertiary amino group combined with the amide moiety exhibited affinities with K_i values in the range of 24–605 nM for A₁ and 242–1250 nM for A_{2A} ARs. The series of 1,3-dibutylpyrimido[2,1-*f*]purinediones showed higher affinity towards A₁, A_{2B} and A₃ ARs than the corresponding 1,3-dipropylxanthine derivatives. The results of studies have indicated benefits of insertion of CH₂CONH between the tertiary amino group and phenyl ring on affinity for adenosine receptors. The performed modifications of lead structures resulted in the increase of affinity for ARs compared to the reference 1,3-dipropyl- and 13 1,3-dibutyl-pyrimido[2,1-*f*]purinedione-9-ethylphenols 6, 7 and the dialkylaminoethyl derivatives 8, 9.

To support the observed structure–activity relationships, molecular docking studies using the homology models of A₁ and A_{2A} adenosine receptors were performed for the library of the synthesized compounds. The results, illustrated by the example of the potent compound 36, allowed finding of important ligand–protein interactions that may contribute to high affinity and subtype selectivity shown by the analogues containing a tertiary amino group.

Selected ADME-Tox parameters were evaluated for two most potent compounds 27 and 36 in *in silico* and *in vitro* studies. The safety studies showed a moderate anti-proliferative effect of 27 against HEK-293 cells with an IC₅₀ value of 27.26 μ M and a strong inhibition effect on CYP3A4 with an IC₅₀ value of 0.23 μ M. The slight modification in basic residues improved the safety profile of the most potent structure 27 maintaining high affinity for A₁ AR at the same time. Compound 36 in contrast to 27 is more stable in the presence of human or rat liver microsomes and exhibits non-inhibition on human CYP3A4. Therefore, compound 36 may be considered as a potent A₁ AR antagonist with a balanced pharmacological profile.

Conflicts of interest

There are no conflicts to declare.

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