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ORIGINAL PAPER

New 2,6,9-trisubstituted adenines as adenosine receptor antagonists: a preliminary SAR profile

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Abstract A new series of 2,6,9-trisubstituted adenines (**5–14**) have been prepared and evaluated in radioligand binding studies for their affinity at the human A_1, A_{2A} and A_3 adenosine receptors and in adenylyl cyclase experiments for their potency at the human A_{2B} subtype. From this preliminary study the conclusion can be drawn that introduction of bulky chains at the N^6 position of 9-propyladenine significantly increased binding affinity at the human A_1 and A_3 adenosine receptors, while the presence of a chlorine atom at the 2 position resulted in a not univocal effect, depending on the receptor subtype and/or on the substituent present in the N^6 position. However, in all cases, the presence in the 2 position of a chlorine atom favoured the interaction with the A_{2A} subtype. These results demonstrated that, although the synthesized compounds were found to be quite inactive at the human A_{2B} subtype, adenine is a useful template for further development of simplified adenosine receptor antagonists with distinct receptor selectivity profiles.

Keywords Adenine derivatives · Adenosine receptors · Adenosine receptor antagonists · Adenosine receptor ligands · G protein-coupled receptors

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Introduction

Adenosine, a naturally occurring nucleoside, is involved in a wide variety of physiological and pathophysiological processes [1]. Adenosine mediates these effects through the activation of at least four human receptor subtypes (P1), belonging to the superfamily of G protein-coupled receptors, which have been recently cloned [2] and classified as A_1, A_{2A}, A_{2B} and A_3 [3]. The subtypes are classified on the bases of coupling to second messengers and pharmacological profiles for agonists and antagonists. In fact, A_1 and A_3 adenosine receptor subtypes are linked to inhibition of adenylyl cyclase and A_{2A} and A_{2B} subtypes are linked to stimulation of the same enzyme [4].

In particular, A_{2B} receptors have been implicated in several physiological functions such as the regulation of mast cell secretion [5, 6], gene expression [5, 7, 8], cell growth [9] and intestinal functions. A_{2B} receptors may also play a role in asthma, since they mediate mast cell degranulation from human mast cells and are present in high density in human blood eosinophils [10, 11]. For this reason A_{2B} antagonists could be considered potential antiasthmatic agents [10–12]. While the A_1, A_{2A} and A_3

adenosine receptors have been pharmacologically characterized through the use of highly potent and selective agonists and/or antagonists, an accurate investigation of the pathophysiological role of A_{2B} receptors is precluded due to the lack of very selective ligands [13]. On the other hand, only recently radiolabelled adenosine antagonists have been used for binding assays at the A_{2B} receptor subtype [1]. Recently, xanthine derivatives, such as compounds **1** and **2** in Fig. 1, have been proposed as potent and selective adenosine receptor antagonists [14, 15]. On the other hand, in the non-xanthine family poor results have been obtained in recent years. However, mention should be made of the pyrazolo-triazolo-pyrimidine derivative **3**, which showed promising binding affinity at the A_{2B} adenosine receptor although the level of selectivity vs the human (h) A_3 subtype was still poor [16]. Very recently, a bipyrimidyl derivative **4** has been proposed as an A_{2B} adenosine receptor antagonist, with affinity in the same range of compound **3,** while the selectivity vs the other receptor subtypes was found to be significantly better [17] (Fig. 1).

A structural analysis of the derivatives **1** and **3** clearly shows the presence of bulky substituents such as arylacetylamino-phenyl groups at the 8 position (compound **1**) and arylacetyl moiety at the N5 position (compound **3**).

On the other hand, in recent years a number of substituted adenines have been synthesized and tested at the four adenosine receptor subtypes, demonstrating that the introduction of different substituents at the 2, 8 and 9 positions of the adenine core resulted in high-affinity antagonists with distinct receptor selectivity profile [18–22]. At the A_{2B} receptor the derivatives bearing an ethyl in the 9 position and linear chains in the 2 position showed potency in the μM range, while the presence of sterically hindered substituents in the same positions was detrimental for the potency. Furthermore, substitution of the 9-ethyl group with a propyl chain seems to favour the interaction with human A_{2B} receptors [21].

Hence, on the basis of the results obtained with compound **3**, introduction of a bulky substituent on the N^6 amino group of the adenine and 2-chloroadenine moiety could increase potency and selectivity for the human A_{2B} adenosine receptor subtypes.

Hence, bulky substituents such as arylacetyl or aryloxyphenylacetyl moieties were introduced on the N^6 amino group of compounds **5** and **6** to obtain derivatives **7**–**14**,

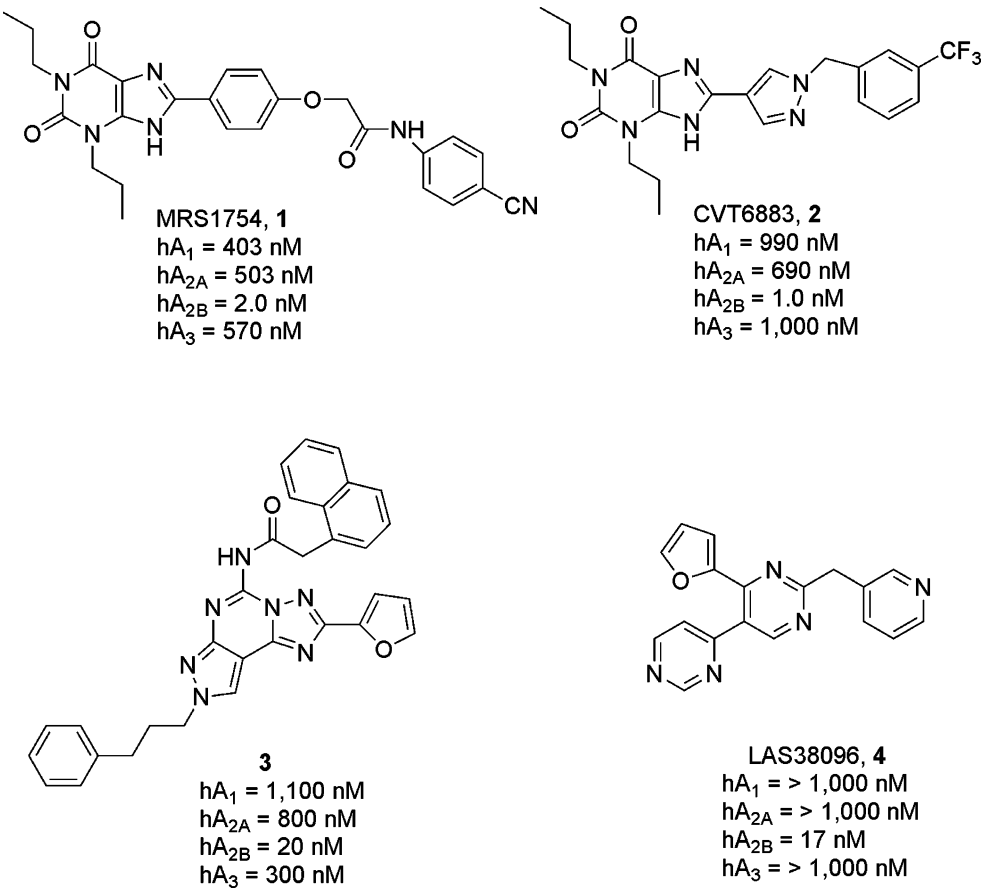


Fig. 1 Structures and binding profile (K_i nM) of some A_{2B} adenosine receptor antagonists

with the aim of finding a new class of A_{2B} antagonists (Fig. 2).

Chemistry

The designed compounds (**5–14**) have been synthesized as summarized in Schemes 1 and 2. The starting 9-propyladenine (**5**) [23] was obtained by alkylation of commercially available adenine (**15**) with propyliodide in the presence of potassium carbonate. Flash chromatography led to the desired 9-substituted isomer **5** as the major product (yield 79%) together with the 7-isomer **5a** (yield 9%). Isomeric structure of compounds **5** and **5a** was assigned on the bases of 1D-^{1}H-NOE difference spectra. In fact, irradiation of both CH_2 groups of the propylic chain in compound **5a** gave a NOE at both H-C(8) and 6-NH_2 groups, demonstrating the 7 position as the alkylation site. On the contrary, a NOE at both H-C(8) and H-C(2), and not at the 6-NH_2 group in compound **5**, upon saturation of CH_2 groups of the propylic chain, confirmed the 9 position as the alkylation site.

The 2-chloro derivative **6** was obtained by alkylation of commercially available 2,6-dichloropurine (**16**) with propyliodide, using the same procedure utilized for compound **5**, to afford the 9-substituted isomer **17** as the major product (yield 75%) along with the 7-isomer **17a** (yield 10%) [24]. [^{1}H]-NMR spectra of **17** and **17a** in $CDCl_3$ are in agreement with those reported in the literature [24]; in the experimental part [^{1}H]-NMR spectra of the same compounds are reported using dimethyl sulfoxide (DMSO) as the solvent. Compound **17** was reacted with liquid ammonia in a sealed tube at room temperature (RT) overnight to give the 2-chloro-9-propyladenine (**6**) [24] (Scheme 1).

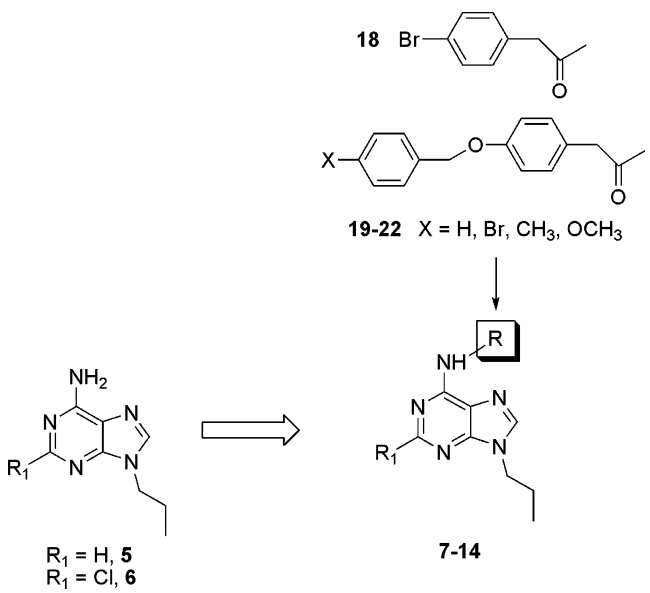


Fig. 2 Structures of designed compounds

Final compounds **7–14** were obtained by condensation of amino compounds **5** or **6** with the appropriate acid **18–22** [25] in the presence of carbonyldiimidazole in tetrahydrofurane (THF) at reflux for 18 h (Scheme 2).

Results and discussion

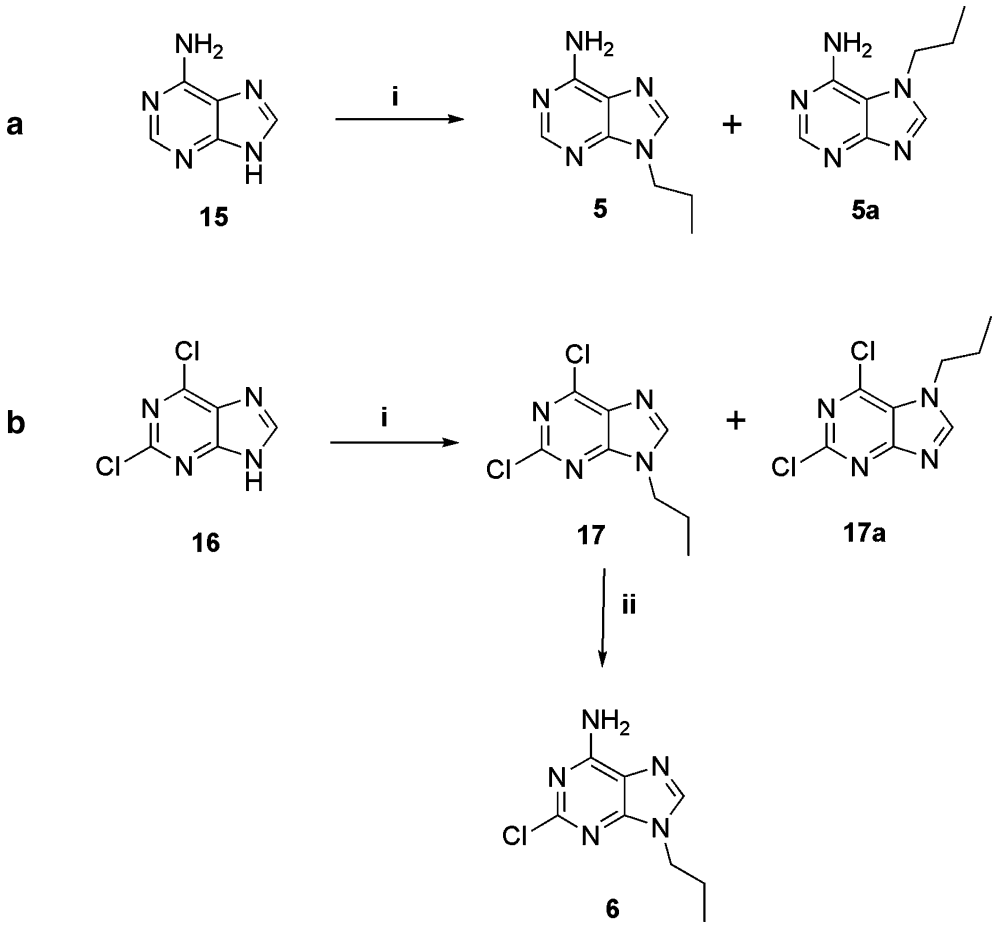
All the compounds were evaluated at the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assay (A_{2B}). Receptor binding affinity was determined using [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) as the radioligand for A_1 receptors, whereas [^{3}H]NECA (5'-*N*-ethylcarboxamidoadenosine) was used for the A_{2A} and A_3 subtypes. In the case of A_{2B} receptors K_i values were calculated from IC_{50} values determined by inhibition of NECA-stimulated adenylyl cyclase activity. K_i values are in μM, with 95% confidence intervals in parentheses [26]. The results of binding and cyclase activity studies are reported in Table 1.

All the tested compounds **5–14** showed affinities at the human A_1, A_{2A} and A_3 adenosine receptors in the μM range without significant levels of selectivity. At A_{2B} receptors most compounds were found to be inactive when tested at a concentration up to 100 μM (K_i values > 30 μM). It is quite evident that the introduction of phenylacetic or aryloxyphenylacetic moieties at the N^6 position of 9-propyladenine (**5**) or of 2-chloro-9-propyladenine (**6**) to give compounds **7–14** modifies the binding profile of the derivatives, although without significantly increasing binding affinity (Table 1). On the other hand, the same substitutions were found to be detrimental for the activity at the A_{2B} receptor subtype. In fact, the N^6-unsubstituted derivative **6** proved to be the most potent of the series with K_i A_{2B}=11 μM.

The effect of the chlorine at the 2 position on binding affinity at the adenosine receptors it is not univocal, depending on the receptor subtype and/or on the substituent in N^6. Analysis of the binding profile of the N^6-unsubstituted derivatives in more detail revealed that the presence of a chlorine atom at the 2 position (compound **6**) increased the affinity (A_1, A_{2A} and A_3) and potency (A_{2B}) at adenosine receptors two- to threefold compared with the unsubstituted analogue **5**.

A quite similar profile could be observed when a 4-bromophenylacetic group was introduced at the N^6 position (compare compound **8** with **7**).

Scheme 1 **a** Synthesis of 9-propyladenine; **b** Synthesis of 2-chloro-9-propyladenine. Reagents: i: DMF, K_2 CO_3, propyliodide, RT; ii: liq. NH_3, sealed tube, RT



An opposite effect of the chlorine atom was detected at A_1, A_{2B} and A_3 receptors when a bulkier substituent, such as the 4-aryloxyphenylacetic chain, was introduced at the N^6 position. In fact, this kind of combination significantly reduced or did not modify the A_1 and A_3 affinity and the A_{2B} potency (**9**: K_i A_1=1.4 μM, K_i A_{2B} > 30 μM, K_i A_3=5.3 μM vs **10**: K_i A_1=26 μM, K_i A_{2B}> 30 μM, K_i A_3=4.9 μM and **12**: K_i A_1=13 μM, K_i A_{2B}= 22 μM, K_i A_3=10 μM vs **13**: K_i A_1=22 μM, K_i A_{2B}> 30 μM, K_i A_3=19 μM).

However, in all cases, the presence of a chlorine atom in the 2 position favoured the interaction with the A_{2A} subtype (compare A_{2A} affinity of **5**, **7**, **9** and **12** with **6**, **8**, **10** and **13**, respectively), while the presence of any substituent on the N^6 position seems to somewhat reduce the affinity. In fact the compound endowed with the highest A_{2A} affinity proved to be the 2-chloro-9-propyladenine (**6**: K_i A_{2A}=2.2 μM). These findings are in agreement with previous observations related to adenosine analogues strongly suggesting that the introduction of substituents in the N^6 position dramatically reduces the A_{2A} affinity [27–32].

Nevertheless, it should be underlined that the presence of a bulky chain at the N^6 position significantly increased (20- to

Scheme 2 Reagents: i: CDI, dry THF, reflux

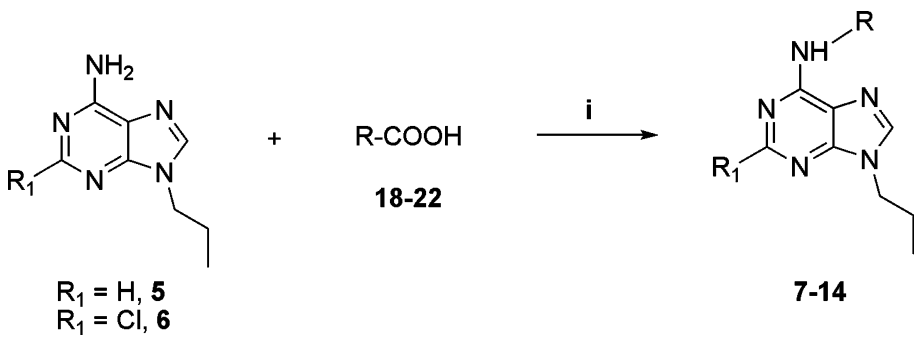
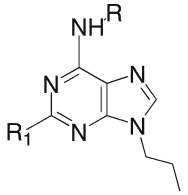
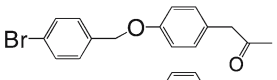
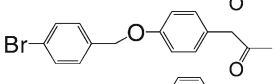
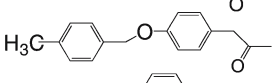
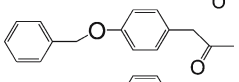
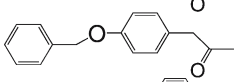
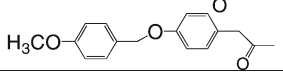


Table 1 Biological profile of synthesized compounds **5–14**



Compd	R_1	R	$hA_1(K_i\ \mu M)$[a]	$hA_{2A}(K_i\ \mu M)$[b]	$hA_{2B}(K_i\ \mu M)$[c]	$hA_3(K_i\ \mu M)$[d]
5	H	H	24 (18-32)	5.1 (4.2-6.2)	22 (13-37)	> 100
6	Cl	H	8.4 (6.1-11)	2.2 (2.0-2.3)	11 (6.6-18)	27 (23-32)
7	H	4-Br-Ph-CH_2-CO	22 (19-24)	10 (8.6-12)	> 30	31 (27-36)
8	Cl	4-Br-Ph-CH_2-CO	5.2 (3.7-7.6)	3.7 (2.9-4.9)	19 (16-22)	10 (7.4-15)
9	H		1.4 (0.96-2.1)	8.9 (7.2 -11)	> 30	5.3 (2.9-9.6)
10	Cl		26 (24-28)	6.2 (3.6-11)	> 30	4.9 (3.8-6.3)
11	H		11 (8.5-14)	12 (10-14)	> 30	1.4 (1.1-2.0)
12	H		13 (10-17)	12 (10-14)	22 (20-24)	10 (8.1-13)
13	Cl		22 (18-28)	6.6 (5.8-7.5)	> 30	19 (14-26)
14	Cl		26 (16-42)	6.5 (3.3-13)	> 30	14 (8.8-22)

[a] Displacement of specific [^{3}H]-CCPA binding at human A_1 receptors expressed in CHO cells. [b] Displacement of specific [^{3}H]-NECA binding at human A_{2A} receptors expressed in CHO cells. [c] K_i values of the inhibition of NECA-stimulated adenylyl cyclase activity in CHO cells expressing human A_{2B} receptors. [d] Displacement of specific [^{3}H]-NECA binding at human A_3 receptors expressed in CHO cells.

70-fold) the affinity at the A_1 and A_3 subtypes in comparison with 9-propyladenine (**9**: K_i A_1=1.4 μM and **11**: K_i A_3= 1.4 μM vs **5**: K_i A_1=24 μM and K_i A_3>100 μM).

This increase of affinity seems also to be modulated by the substituent on the aryloxyphenylacetic group; in fact, substitution with a lipophilic bromine (**9**) or methyl group (**11**) at the *para* position is responsible for the increased A_1 and A_3 receptor affinity, respectively, while the presence of a hydrogen (**12**) or a methoxy group (**14**) did not positively influence the binding profile.

Conclusions

In conclusion the study herein presented, although it did not reach the proposed goal of obtaining A_{2B} adenosine receptor antagonists, increased knowledge of the structure-activity relationships in adenine derivatives.

Moreover, it was demonstrated that the introduction of bulky substituents at the N^6 position of adenine derivatives significantly increased the affinity at the A_1 and A_3 adenosine receptors, while the presence of a chlorine atom in the 2 position favoured the interaction with the A_{2A} subtype. These results demonstrated that, although the synthesized compounds were found to be quite inactive at the human A_{2B} subtype, adenine is a useful template for further development of simplified adenosine receptor antagonists with distinct receptor selectivity profiles, opening up new chances to design structurally simplified A_1 and A_3 adenosine receptor antagonists.

Experimental section

Chemistry

General: melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; δ in ppm, *J* in Hz. All exchangeable protons were confirmed by addition of D_2O. Thin layer chromatography (TLC) was carried out on precoated TLC plates with silica gel 60 F-

254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on Fisons Instruments Model EA 1108 CHNS-O model analyser and are within ± 0.4% of theoretical values.

9-Propyladenine (***5***) *and 7-propyladenine* (***5a***)

To a solution of adenine (**15**) (0.5 g, 3.7 mmol) in dry DMF (10 ml), under nitrogen, K_2CO_3 (0.83 g, 5.97 mmol) and propyliodide (0.433 ml, 4.44 mmol) were added. The mixture was stirred at RT for 16 h, then the solvent was removed under reduced pressure and the crude purified by flash chromatography ($CHCl_3$-MeOH 98:2) to afford **5** [23] and **5a** (yield 79 and 9%, respectively) as white solids, after crystallization from CH_3OH.

5: m.p. 173–175°C. ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, J=7.4, CH_3); 1.82 (m, 2H, CH_2-CH_3); 4.11 (t, 2H, J=7.0, CH_2-N); 7.21 (bs, 2H, NH_2); 8.15 (s, 2H, H-2 and H-8). Anal. Calcd. for $C_5H_5N_5$ (177.2) C, 54.22; H, 6.26; N, 39.52; found: C, 54.54; H, 6.71; N, 39.33.

5a: m.p. >250°C. ^{1}H-NMR (DMSO-d_6) δ 0.86 (t, 3H, J=7.3, CH_3); 1.91 (m, 2H, CH_2-CH_3); 4.26 (t, 2H, J=7.0, CH_2-N); 7.75 (s, 1H, H-2); 7.85 (bs, 2H, NH_2); 8.34 (s, 1H, H-8). Anal. Calcd. for $C_5H_5N_5$ (177.2) C, 54.22; H, 6.26; N, 39.52; found: C, 54.45; H, 6.45; N, 39.45.

2,6-Dichloro-9-propyl-9H-purine (***17***) *and 2,6-dichloro-7-propyl-9H-purine* (***17a***)

To a solution of 2,6-dichloropurine (**16**) (1 g, 5.29 mmol) in dry DMF (14 ml), under nitrogen, K_2CO_3 (1.18 g, 6.61 mmol) and propyliodide (0.59 ml, 6.08 mmol) were added. The mixture was stirred at RT overnight, then the solvent was removed under reduced pressure and the crude purified by flash chromatography (cC_6H_{12}-EtOAc 75:25) to afford **17** and **17a** as white solids (yield 75 and 10%, respectively) [24].

17: m.p. 58–59°C; ^{1}H-NMR (DMSO- d_6) δ 0.86 (t, 3H, J=7.5 Hz, CH_2CH_3), 1.85 (m, 2H, CH_2CH_3), 4.21 (t, 2H, J=7.0 Hz, N-CH_2), 8.76 (s, 1H, H-8). Anal. Calcd. for $C_8H_8Cl_2N_4$ (231.1) C, 41.58; H, 3.49; N, 24.25. Found: C, 41.85; H, 3.70; N, 24.10.

17a: m.p. 103–105°C; ^{1}H-NMR (DMSO-d_6) δ 0.87 (t, 3H, J=7.4 Hz, CH_2CH_3), 1.84 (m, 2H, CH_2CH_3), 4.40 (t, 2H, J=7.2 Hz, N-CH_2), 8.89 (s, 1H, H-8). Anal. Calcd. for $C_8H_8Cl_2N_4$ (231.1) C, 41.58; H, 3.49; N, 24.25. Found: C, 41.75; H, 3.55; N, 24.19.

2-Chloro-9-propyladenine (***6***)

Liquid ammonia (5 ml) and compound **17** (0.46 g, 1.97 mmol) were poured into a sealed tube and the resulting mixture was stirred at RT overnight. Ammonia was evaporated and the crude purified by flash chromatography ($CHCl_3$-MeOH 99:1) to give **6** [24] as a white solid (yield 75%) m.p. 224–226°C. ^{1}H-NMR (DMSO-d_6) δ 0.84 (t, 3H, J=7.3 Hz, CH_2CH_3), 1.79 (m, 2H, CH_2CH_3), 4.05 (t, 2H, J=7.2 Hz, N-CH_2), 7.72 (s, 2H, NH_2), 8.15 (s, 1H, H-8). Anal. Calcd. for $C_8H_{10}ClN_5$ (211.7) C, 45.40; H, 4.76; N, 33.09. Found: C, 45.75; H, 4.80; N, 32.87.

General procedure for the preparation of the N^6-acylaminoadenine (***7–14***)

A solution in dry THF (4 ml) of the appropriate acid (**18–22**) (0.46 mmol) and carbonyldiimidazole (83 mg, 0.51 mmol) was poured at reflux under nitrogen for 1 h. Then the amino compound **5** or **6** (0.46 mmol) was added and the resulting mixture was refluxed overnight. The solvent was removed under reduced pressure and the crude purified by flash chromatography to afford the desired final compounds **7–14**.

6-[(4-Bromophenyl)acetyl]amino-9-propyladenine (***7***)

Eluent for chromatography $CHCl_3$-MeOH 95:5; yield 59%, white solid; m.p. 149–151°C (dec.); ^{1}H-NMR (DMSO-d_6): δ 0.83 (t, 3H, J=7.2 Hz, CH_2CH_3), 1.84 (m, 2H, CH_2CH_3), 3.89 (s, 2H, CH_2-CO), 4.19 (t, 2H, J= 7.1 Hz, N-CH_2), 7.30 (d, 2H, J=8.4 Hz, H-Ph), 7.51 (d, 2H, J=8.4 Hz, H-Ph), 8.47 (s, 1H, H-8), 8.62 (s, 1H, H-2), 10.91 (s, 1H, NH). Anal. Calcd. for $C_{16}H_{16}BrN_5O$ (374.2) C, 51.35; H, 4.31; N, 18.71. Found: C, 51.65; H, 4.80; N, 18.50.

6-[(4-Bromophenyl)acetyl]amino-2-chloro-9-propyladenine (***8***)

Eluent for chromatography $CHCl_3$-cC_6H_{12} 80:20; yield 26%, white solid; m.p. 164–166°C; ^{1}H-NMR (DMSO-d_6): δ 0.84 (t, 3H, J=7.5 Hz, CH_2CH_3), 1.83 (m, 2H, CH_2CH_3), 3.88 (s, 2H, CH_2-CO), 4.15 (t, 2H, J=6.9 Hz, N-CH_2), 7.30 (d, 2H, J=8.4 Hz, H-Ph), 7.53 (d, 2H, J=8.4 Hz, H-Ph), 8.50 (s, 1H, H-8), 11.25 (s, 1H, NH). Anal. Calcd. for $C_{16}H_{15}BrClN_5O$ (408.7) C, 47.02; H, 3.70; N, 17.14. Found: C, 47.49; H, 3.83; N, 17.40.

6-[(4-(4-Bromobenzyloxy)phenyl)acetyl]amino-9-propyladenine (***9***)

Eluent for chromatography $CHCl_3$-MeOH 95:5; yield 58%, white solid; m.p. 154–156°C; ^{1}H-NMR (DMSO-d_6): δ 0.85 (t, 3H, J=7.5 Hz, CH_2CH_3), 1.85 (m, 2H, CH_2CH_3), 3.82 (s, 2H, CH_2-CO), 4.21 (t, 1H, J=7.0 Hz, N-CH_2), 5.07 (s, 2H, CH_2-O), 6.95 (d, 2H, J=8.8 Hz, H-Ph), 7.27 (d, 2H, J= 8.4 Hz, H-Ph), 7.40 (d, 2H, J=8.4 Hz, H-Ph), 7.58 (d, 2H,

J=8.4 Hz, H-Ph), 8.48 (s, 1H, H-8), 8.62 (s, 1H, H-2), 10.81 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{22}BrN_5O_2$ (480.4) C, 57.51; H, 4.62; N, 14.58. Found: C, 57.99; H, 4.66; N, 14.55.

6-[(4-(4-Bromobenzyloxy)phenyl)acetyl]amino-2-chloro-9-propyladenine (***10***)

Eluent for chromatography $CHCl_3$-cC_6H_{12}-MeOH 50:48:2; yield 14%, white solid; m.p. 176–178°C; ^{1}H-NMR (DMSO-d_6): δ 0.81 (t, 3H, *J*=7.5 Hz, CH_2CH_3), 1.79 (m, 2H, CH_2CH_3), 3.76 (s, 2H, CH_2-CO), 4.12 (t, 2H, *J*= 7.4 Hz, N-CH_2), 5.03 (s, 2H, CH_2-O), 6.92 (d, 2H, *J*= 8.6 Hz, H-Ph), 7.22 (d, 2H, *J*=8.4 Hz, H-Ph), 7.36 (d, 2H, *J*=8.4 Hz, H-Ph), 7.54 (d, 2H, *J*=8.4 Hz, H-Ph), 8.46 (s, 1H, H-8), 11.11 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{21}BrClN_5O_2$ (514.8) C, 53.66; H, 4.11; N, 13.60. Found: C, 53.75; H, 4.25; N, 13.29.

6-[(4-(4-Methylbenzyloxy)phenyl)acetyl]amino-9-propyladenine (***11***)

Eluent for chromatography $CHCl_3$-cC_6H_{12}-MeOH 70:28:2; yield 35%, white solid; m.p.131–132°C; ^{1}H-NMR (DMSO-d_6): δ 0.83 (t, 3H, *J*=7.3 Hz, CH_2CH_3), 1.84 (m, 2H, CH_2CH_3), 2.28 (s, 3H, CH_3-Ph), 3.80 (s, 2H, CH_2-CO), 4.20 (t, 2H, *J*=7.1 Hz, N-CH_2), 5.02 (s, 2H, CH_2-O), 6.93 (d, 2H, *J*=8.4 Hz, H-Ph), 7.24 (m, 6H, H-Ph), 8.47 (s, 1H, H-8), 8.62 (s, 1H, H-2), 10.83 (s, 1H, NH). Anal. Calcd. for $C_{24}H_{25}N_5O_2$ (415.5) C, 69.38; H, 6.06; N, 16.86. Found: C, 69.74; H, 6.35; N, 16.54.

6-[(4-Benzyloxyphenyl)acetyl]amino-9-propyladenine (***12***)

Eluent for chromatography $CHCl_3$-MeOH 97:3; yield 52%, white solid; m.p. 121–123°C; ^{1}H-NMR (DMSO-d_6): δ 0.83 (t, 3H, *J*=7.5 Hz, CH_2CH_3), 1.84 (m, 2H, CH_2CH_3), 3.80 (s, 2H, CH_2-CO), 4.19 (t, 2H, *J*=7.1 Hz, N-CH_2), 5.07 (s, 2H, CH_2-O), 6.95 (d, 2H, *J*=8.4 Hz, H-Ph), 7.25 (d, 2H, *J*= 8.8 Hz, H-Ph), 7.37 (m, 5H, H-Ph), 8.47 (s, 1H, H-8), 8.62 (s, 1H, H-2), 10.82 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{23}N_5O_2$ (401.5) C, 68.81; H, 5.77; N, 17.44. Found: C, 68.97; H, 5.89; N, 17.35.

6-[(4-Benzyloxyphenyl)acetyl]amino-2-chloro-9-propyladenine (***13***)

Eluent for chromatography $CHCl_3$-MeOH 99:1; yield 17%, white solid; m.p. 150–152°C; ^{1}H-NMR (DMSO-d_6): δ 0.84 (t, 3H, *J*=7.3 Hz, CH_2CH_3), 1.82 (m, 2H, CH_2CH_3), 3.79 (s, 2H, CH_2-CO), 4.14 (t, 2H, *J*=7.0 Hz, N-CH_2), 5.07 (s, 2H, CH_2-O), 6.95 (d, 2H, *J*=8.6 Hz, H-Ph), 7.25 (d, 2H, *J*= 8.4 Hz, H-Ph), 7.37 (m, 5H, H-Ph), 8.49 (s, 1H, H-8), 11.16 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{22}ClN_5O_2$ (435.9) C, 63.37; H, 5.09; N, 16.07. Found: C, 63.56; H, 5.14; N, 15.76.

6-{[(4-Methoxybenzyloxy)phenyl]acetyl}amino-2-chloro-9-propyladenine (***14***)

Eluent for chromatography $CHCl_3$-MeOH 99:1; yield 29%, white solid; m.p. 174–176°C; ^{1}H-NMR (DMSO-d_6): δ 0.84 (t, 3H, *J*=7.4 Hz, CH_2CH_3), 1.82 (m, 2H, CH_2CH_3), 3.73 (s, 3H, CH_3-O), 3.79 (s, 3H, CH_2-CO), 4.14 (t, 2H, *J*= 7.0 Hz, N-CH_2), 4.98 (s, 2H, CH_2-O), 6.92 (d, 2H, *J*= 8.0 Hz, H-Ph), 6.93 (d, 2H, *J*=8.4 Hz, H-Ph), 7.24 (d, 2H, *J*=8.4 Hz, H-Ph), 7.35 (d, 2H, *J*=8.4 Hz, H-Ph), 8.49 (s, 1H, H-8), 11.15 (s, 1H, NH). Anal. Calcd. for $C_{24}H_{24}ClN_5O_3$ (465.9) C, 61.87; H, 5.19; N, 15.03. Found: C, 61.99; H, 5.33; N, 14.91.

Biology

All pharmacological methods followed the procedures as described earlier [26]. In brief, membranes for radioligand binding were prepared from CHO cells stably transfected with human adenosine receptor subtypes in a two-step procedure. In a first low-speed step (1,000 *g*) cell fragments and nuclei were removed. The crude membrane fraction was sedimented from the supernatant at 100,000 *g*. The membrane pellet was resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored at −80°C. For the measurement of adenylyl cyclase activity only one high speed centrifugation of the homogenate was used. The resulting crude membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4 and immediately used for the cyclase assay.

For radioligand binding at A_1 adenosine receptors 1 nM [^{3}H]CCPA was used, whereas 30 and 10 nM [^{3}H]NECA were used for A_{2A} and A_3 receptors, respectively. Non-specific binding of [^{3}H]CCPA was determined in the presence of 1 mM theophylline; in the case of [^{3}H]NECA 100 pM R-PIA was used. K_i values from competition experiments were calculated with the program SCTFIT [33]. At A_{2B} adenosine receptors inhibition of NECA-stimulated adenylyl cyclase activity was used as a measurement of potency of the new compounds. IC_{50} values from these experiments were converted to K_i values with the Cheng and Prusoff equation [34].

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