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Original article

New N^6- or N(9)-hydroxyalkyl substituted 8-azaadenines or adenines as effective A_1 adenosine receptor ligands☆

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Abstract

In this paper we describe synthesis and biological assays of some A_1 ligands more water-soluble than the effective, but very lipophilic, 8-azaadenines and adenines discovered in the past and obtained introducing on N^6 or N(9) substituent a hydroxy group. Five of the new N^6-hydroxyalkyl- and N^6-hydroxycycloalkyl-2-phenyl-9-benzyl-8-azaadenines showed very high affinity (Ki < 40 nM) and selectivity for A_1 adenosine receptors. Among the 2-phenyl-9-(2-hydroxy-3-alkyl)-8-azaadenines or adenines prepared, the compounds with the higher A_1 affinity and selectivity resulted 2-phenyl-9-(2-hydroxy-3-propyl)-N^6-cyclopentyl- and cyclohexyl-8-azaadenine with Ki 2.2 ± 0.2 nM and 2.8 ± 0.3 nM respectively. From the point of view of water-solubility, 2-phenyl-9-(2-hydroxy-3-propyl)-8-azaadenine was the most interesting compound, having a CLogP of 1.066991 and a water-solubility of 1.2 mg mL^{-1}.

Keywords: N^6-hydroxyalkyl-2-phenyl-9-benzyl-8-azaadenines; 2-phenyl-9-(2-hydroxy-3-alkyl)adenines; 2-phenyl-9-(2-hydroxy-3-alkyl)-8-azaadenines; A_1 adenosine receptor ligands

1. Introduction

A_1 receptors are widely distributed in the central nervous system and in peripheral tissue and mediate diverse biological effects. For example the role of adenosine as neuroprotective agent during hypoxia and ischemic conditions seems to be mediate by the A_1 receptors; these receptors have been implicated in sedative, anticonvulsant, anxiolitic effects. A_1 receptors mediate cardiac depression through negative chronotropic, dromotropic and inotropic effects. In the kidney, activation of A_1 receptors causes vasoconstriction, inhibition of renine secretion, diuresis, natriuresis and others effects [1]. A great number of other physiological effects have been demonstrated so the studies on A_1 adenosine receptor ligands are very interesting; hundreds of agonists and antagonists have been assayed to obtain some compounds useful in therapy. Regarding A_1 antagonists, some of these are studied as novel potassium sparing diuretics with kidney-protection properties, in dementia such as Alzheimer's disease, in cardiac therapy and in kidney disease [2].

In the last years we have synthesised a great number of A_1 adenosine receptor ligands varying the substituent groups in the positions 3, 6 and 9 of 8-azaadenine or adenine nucleus and some of these have been very effective, having a Ki in the range of nanomolar units [3–8]. All these compounds were characterised by a high lipophilicity being the substituents on the bicyclic nucleus, such as alkyl, cycloalkyl or alkyl-aromatic groups, hydrophobic. This can make the compounds nearly water-insoluble, causing the determination of activity in the in vitro binding assays to be uncertain in some cases. Low water-solubility also leads to a low bioavailability of the compounds so they are unsuitable for in vivo studies.

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The design of reasonably water-soluble A_1 adenosine receptor antagonists has become a very important objective in the development of this class of ligands.

In these last years A_1 selective ligands with increased water-solubility have been obtained, including xanthine derivatives, such as 8-(1-aminocyclopentyl)-1,3-dipropylxanthine **I** [9] (Fig. 1), non-xanthine derivatives, like N^6-hydroxy-endonorbornyl-8-(*N*-methyl-*N*-isopropylamino)-9-methyladenine **II** [10] or FR 166124 **III** [11] (Fig. 1), a 2-phenylpyrazolo[1,5-a]pyridine derivative, developed by Kuroda and co-workers. Some authors [12,13] have introduced various polar substituents at C-2 and C-4 position of pyrrolo[2,3-d]pyrimidine or pyrimido[4,5-b]indole structure to improve water-solubility of some interesting A_1 ligands synthesised in the past by Muller and co-workers [14]. Recently other authors have presented the pyrrolo[2,3-d]pirimidinamide **IV** (Fig. 1) as a potent and relatively good water-soluble A_1 antagonist (CLogP = 1.5) [15]. Therefore we planned to obtain some A_1 ligands more water-soluble than the active compounds discovered in the past by introducing on the molecules a polar function. Our first attempt has been reported in a recent paper [16] where we described synthesis of new 2-(2′-phenyl-9′-benzyl-8′-azapurin-6′-ylamino)-carboxylic acid methylesters: the carbonyloxymethyl group could assure the molecules a better water-solubility but the compounds demonstrated only low capability of binding to the receptors.

With the same aim, in this paper we describe synthesis and biological activity as A_1 adenosine receptor ligands of some compounds obtained by introducing polar N^6-hydroxyalkyl- or N^6-hydroxycycloalkyl groups on 2-phenyl-9-benzyl-8-azaadenines. Recently we have described *erythro*-2-phenyl-9-(2-hydroxy-3-nonyl)adenine and its 8-aza analogue which showed high affinity for A_1 adenosine receptors, with Ki 28 and 2.8 nM, respectively [17]; also their N^6-cyclopentyl derivatives were good ligands with Ki 5.5 and 4.3 nM respectively. In these compounds a hydroxy function is bound to a long alkylic chain in the 9 position of adenine or 8-azaadenine nucleus. Given that a shorter chain could decrease the lipophilicity of these compounds, we decided to prepare and assay also the analogues 9-(2-hydroxy-3-heptyl), 9-(2-hydroxy-3-pentyl) and 9-(2-hydroxy-3-propyl) substituted, to verify if these compounds maintained high affinity for A_1 receptors.

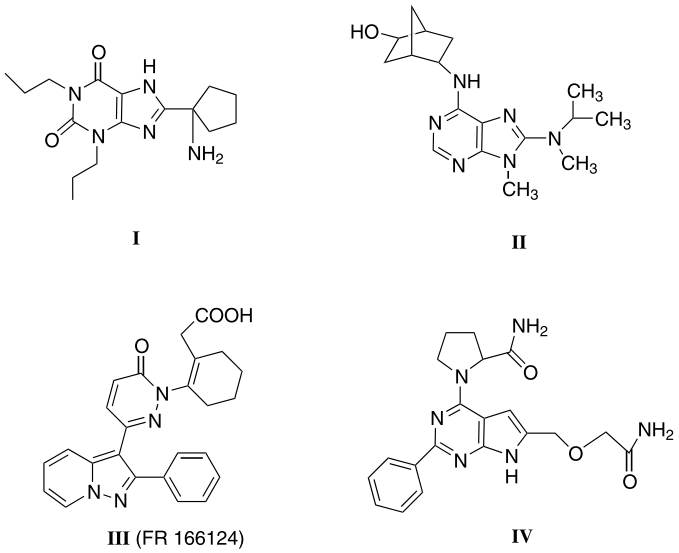


Fig. 1. Some water-soluble A_1 adenosine receptor antagonists reported in the literature.

2. Chemistry

The synthetic route to prepare compounds **3**–**9** (Fig. 2) employed a known two-step reaction in the presence of sodium ethoxide: the first step was the 1,3 dipolar addition reaction of benzylazide and cyanoacetamide to give 1-benzyl-4-carbamoyl-5-amino-1H-1,2,3-triazole which was not isolated; then, in the same flask, ethyl benzoate was added to obtain the 2-phenyl-9-benzyl-8-azahypoxanthine **1** [18] by annulation reaction. The reaction of **1** with phosphorus oxychloride afforded 6-chloro-8-azapurine **2** which, by nucleophilic displacement of chlorine atom operated by suitable amines [4], gave the N^6-substituted-8-azaadenines **3**–**9**.

5-Amino-4,6-dichloro-2-phenylpyrimidine **10** was used for synthesis of compounds **20**–**37** (Fig. 3); it was obtained by a modification of a known method [19] from 5-nitro-4,6-dichloro-2-phenylpyrimidine [20] by reduction with iron powder in a mixture of acetic acid, water and THF.

Substitution of a chlorine atom in compound **10** with the suitable 3-aminoalkan-2-ol gave 5-amino-4-(2-hydroxy-3-alkyl)-6-chloro-2-phenylpyrimidines **11**–**13**. 3-Aminopropan-2-ol is a commercial product (Fluka); 3-aminopentan-2-ol and 3-aminoheptan-2-ol were obtained from (DL)-2-amino-1-butanoic acid and (DL)-2-amino-1-hexanoic acid respectively, by a Daking–West reaction followed by a reduction with $NaBH_4$ as described by Schaeffer and Schwender to obtain 2-amino-nonan-1-ol [21]. Gas-chromatography analysis of the acetyl derivatives showed an erythro–threo ratio of 80:20; the diastereoisomers were not separated. Cyclisation of **11**, **12** and **13** with an acid solution of $NaNO_2$ gave the 8-azaadenines **14**–**16** [22]. Cyclisation with triethyl orthoformate in the presence of catalytic 12N HCl [23] gave the corresponding adenines **17**–**19**. Compounds **14**–**19** were immediately used for the next reaction without purification because they easily decompose. Treatment of **14**–**19** with the suitable amines gave the N^6-substituted 2-phenyl-9-(2-hydroxy-3-propyl)-8-azaadenines **20**–**28** and adenines **29**–**37**.

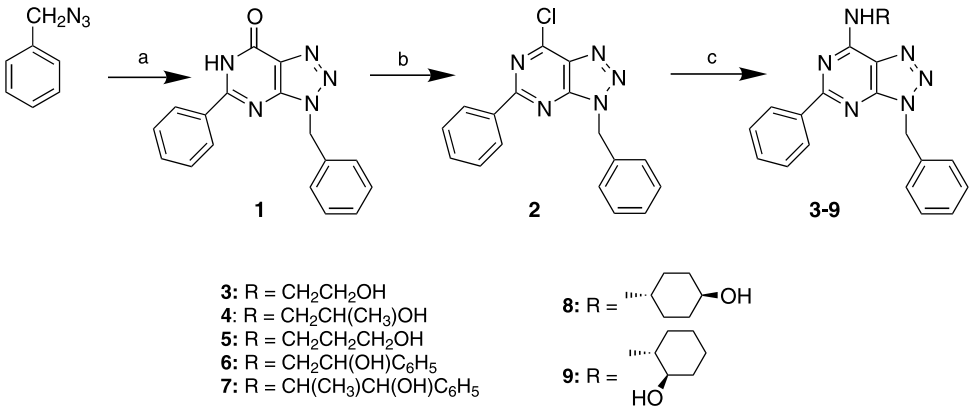
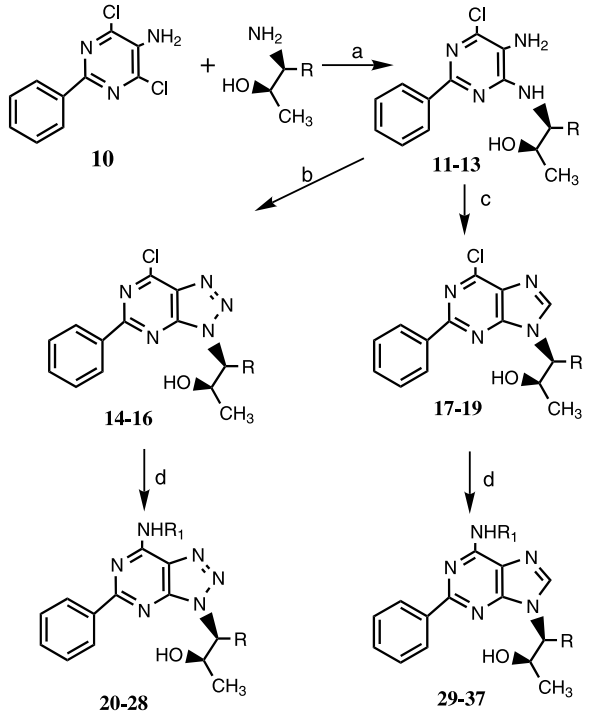


Fig. 2. Synthetic route for the preparation of compounds **3**–**9**.

3. Biological evaluation

Substituted 8-azaadenines **3**–**9** and **20**–**28** and adenines **29**–**37** were tested in radioligand binding assays for affinity at A_1 and A_{2A} adenosine receptors in bovine brain cortical membranes and bovine striatum respectively. In Tables 1 and 2 the results of A_1 adenosine receptor binding are reported; in the A_2 adenosine

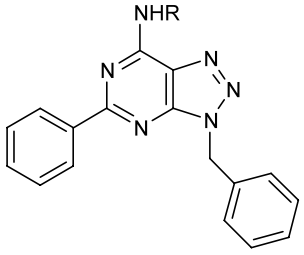


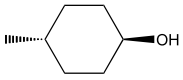
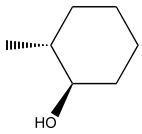
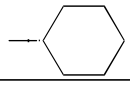
11, 14, 17: R = H; **12, 15, 18:** R = C_2H_5; **13, 16, 19:** R = n-C_4H_9;
20, 29 : R = H R_1 = H; **21, 30 :** R = C_2H_5 R_1 = H; **22, 31 :** R = n-C_4H_9 R_1 = H;
23, 32 : R = H R_1 =cyclopentyl; **24, 33 :** R = C_2H_5 R_1 = cyclopentyl;
25, 34 : R = n-C_4H_9 R_1 = cyclopentyl; **26, 35 :** R = H R_1 =cyclohexyl;
27, 36 : R = C_2H_5 R_1 = cyclohexyl; **28, 37 :** R = n-C_4H_9 R_1 = cyclohexyl.

Reagents: a) N,N-diethylaniline; b) $NaNO_2$, CH_3COOH, THF, H_2O;
c) triethyl ortoformate; d) amine.

Fig. 3. Synthetic route for the preparation of compounds **11**–**37**.

Table 1
Biological results for affinity towards A_1 adenosine receptors and calculated partition coefficients (CLogP) of the compounds **3**–**9** and the compounds **A**–**E** assayed in the past [6,8]



Compound	R	IC_{50} (nM)	Ki (nM)	CLogP
3	CH_2-CH_2OH	85	38	3.18476
4	CH_2-$CHOH$-CH_3	17	7.5	3.49376
A	CH_2-CH_3	170[a]	91[a]	4.47756
5	CH_2-CH_2-CH_2OH	48	21.4	3.55356
B	CH_2-CH_2-CH_3	69[a]	37[a]	5.00656
6	CH_2-$CHOH$-C_6H_5	2970	1600	4.755476
7	$CH(CH_3)$-$CHOH$-C_6H_5	1000	545	5.066376
C	$CH(CH_3)$-C_6H_5	> 5000[a]		5.70556
D	CH_2-C_6H_5	> 5000[a]		5.39656
8		6	2.7	3.89256
9		23	10.3	4.58676
E		4[b]	1.6[b]	5.97956

[a] See ref. [6]. [b] See ref. [8]

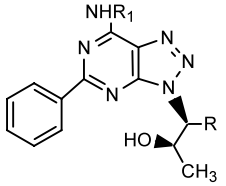
receptor binding assays, all the compounds showed inhibition values $<60\%$ at 1 μM, so were considered inactive.

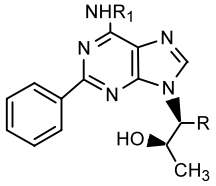
4. Results and discussion

4.1. Structure–activity relationships

Five of the N^6-hydroxyalkyl substituted compounds (**3**, **4**, **5**, **8**, **9**) showed very high affinity (Ki < 40 nM) and selectivity for A_1 adenosine receptors and this fact allowed us to make some evaluations about structure–activity relationships, also comparing the affinity of these compounds with that of the corresponding dehydroxy ones obtained in the past and indicated by capital letters (see Table 1). In our compounds, when the substituent in the 9-position is a benzyl group, an aromatic substituent in N^6 is not allowed. This is suggested by the very low affinity of compounds **6**, **7**, **C** and **D**. On the contrary, it is known that an aliphatic substituent on N^6 increases affinity of the unsubstituted compound; biological results of this work showed that compounds bearing on the N^6-aliphatic substituent a hydroxy function have affinity very similar to the corresponding dehydroxy compounds (see in Table 1: **3** versus **A**; **4** and **5** versus **B**; **6** and **7** versus **C** and **D**; **8** and **9** versus **E**) and, in some cases, better. So we can deduce that a hydroxy function is well tolerated by the receptor in the lipophilic pocket in front of N^6 of adenosine. It is worth noting that the steric relation between the nitrogen atom and the hydroxy group is *trans*-diequatorial in the case of 2′-hydroxycyclohexyl substituted compound **9**. We can also see that, moving OH from positions 2′ to 3′ (compounds **4** and **5**) or from 2′ to 4′ (compounds **8** and **9**), Ki does not vary significantly. It could mean that this pocket is quite large and permits different types of interactions. In a recent paper [24], the study of a model of A_1 adenosine receptor suggested that this protein could modify the side chain orientation of some aminoacids in the binding site so that they give rise to the best interaction with the ligand. The results of the present work can support this

Table 2
Biological results for affinity towards A_1 adenosine receptors and calculated partition coefficients (CLogP) of the compounds **20**–**37** and the compounds **F**–**M** assayed in the past [9]





Compd	R	R_1	Ki	CLogP	Compd	R	R_1	Ki	CLogP
20	H	H	26±2	1.06991	**29**	H	H	263±23	1.17742
21	C_2H_5	H	[a]	1.90791	**30**	C_2H_5	H	89±8	2.01542
22	n-C_4H_9	H	122±10	2.96591	**31**	n-C_4H_9	H	188±15	3.07342
F	n-C_6H_{13}	H	2.8±0.3 [b]	4.13142	**I**	n-C_6H_{13}	H	28±2 [b]	4.02391
23	H	cyclopentyl	2.2±0.2	3.36836	**32**	H	cyclopentyl	22±2	3.48001
24	C_2H_5	cyclopentyl	25±2	4.20636	**33**	C_2H_5	cyclopentyl	53±5	4.31801
25	n-C_4H_9	cyclopentyl	23±3	5.26436	**34**	n-C_4H_9	cyclopentyl	77±6	5.37601
G	n-C_6H_{13}	cyclopentyl	4.3±0.5 [b]	6.43401	**L**	n-C_6H_{13}	cyclopentyl	5.5±0.5	6.32236
26	H	cyclohexyl	2.8±0.3	3.92736	**35**	H	cyclohexyl	25±2 [b]	4.03901
27	C_2H_5	cyclohexyl	38±24	4.76536	**36**	C_2H_5	cyclohexyl	125±13	4.87701
28	n-C_4H_9	cyclohexyl	31±4	5.82336	**37**	n-C_4H_9	cyclohexyl	65±6	5.93501
H	n-C_6H_{13}	cyclohexyl	118±12 [b]	6.9931	**M**	n-C_6H_{13}	cyclohexyl	116±10 [b]	6.88138

[a] Inhibition: 53% 10 μM.
[b] See reference [9].

interesting hypothesis which allowed us to see receptors not as a rigid structure but as a versatile part of the cellular membrane.

Regarding compounds with the hydroxy group on N(9) substituent, the new compounds were compared with the analogues synthesised in the past, indicated by capital letters in Table 2, having a 2-hydroxy-3-nonyl chain. We can see in Table 2 that in the case of 2-phenyl-9-(2-hydroxy-3-alkyl)-8-azaadenines or adenines N^6-cyclopentyl substituted (compounds **23**–**25** versus **G** and **32**–**34** versus **L**) a shorter chain retains a good affinity for the receptors. The compounds with the higher affinity were **23** and **26** with Ki 2.2±0.2 nM and 2.8±0.3 nM respectively; for N^6-cyclohexyl substituted derivatives (compounds **26**–**28** versus **H** and **35**–**37** versus **M**), we can see a general increase in affinity. In the case of N^6-unsubstituted compounds the biological results showed a general decrease in affinity; nevertheless in this series compound **20** retains a good affinity (Ki 26±2 nM).

4.2. Water-solubility

For all compounds octanol–water partition coefficient (CLogP) was calculated (Tables 1 and 2) using ChemDraw Ultra version 6.0.1 program. All the new compounds showed lower values than the compounds of the past cited in this paper. Compound **20** which showed a good affinity for A_1 adenosine receptors can be considered very important from the point of view of water-solubility because its CLogP is 1.066991 ($P \cong 1.68$), indicating that this compound has a fairly good solubility in water. In fact, for this compound water-solubility was assayed and resulted 1.2 mg mL^{-1} (4.44 mM).

5. Conclusion

Introduction of a hydroxy function on N^6 or N(9) substituents of 2-phenyl-9-benzyl-8-azaadenines and adenines improves solubility and effectiveness towards A_1 adenosine receptors of these molecules which could represent a useful tool for pharmacological and biological studies.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra in Nujol mulls were recorded on a Perkin–Elmer Model 1310 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 spectrometer; chemical shifts are expressed in δ units from TMS as an internal standard; the compounds were dissolved in $CDCl_3$ or DMSO-d_6.

Table 3
Reaction and physico-chemical data for compounds **3**–**9**, **11**–**13** and **21**–**37**

Compd.	Yield	Crystallisation solvents	Chromatography eluent	M.p. (°C)	Formula (anal.)
3	51	CH_2Cl_2–petroleum ether		198–200	$C_{19}H_{18}N_6OH_2O$ (C, H, N)
4	61	$CHCl_3$–petroleum ether		195–197	$C_{20}H_{20}N_6O$ (C, H, N)
5	62	ethyl acetate		191–193	$C_{20}H_{20}N_6O$ (C, H, N)
6	35	isopropanol		205–206	$C_{25}H_{22}N_6O$ (C, H, N)
7	36	EtOH–hexane		180–182	$C_{26}H_{24}N_6O$ (C, H, N)
8	30	EtOH–H_2O		185–187	$C_{23}H_{24}N_6O$ (C, H, N)
9	30	EtOH		180–181	$C_{23}H_{24}N_6O$ (C, H, N)
11	65	EtOH–Et_2O		165	$C_{13}H_{15}ClN_4O$ (C, H, N)
12	68		$CHCl_3$ 100%	oil	$C_{15}H_{19}ClN_4O$ (C, H, N)
13	56		$CHCl_3$–MeOH 95:5	oil	$C_{17}H_{23}ClN_4O$ (C, H, N)
20	20	EtOH–Et_2O		188–190	$C_{13}H_{14}N_6O$ (C, H, N)
21	52		CH_2Cl_2–MeOH 96:4	219	$C_{15}H_{18}N_6O$ (C, H, N)
22	88		$CHCl_3$–MeOH 96:4	oil	$C_{17}H_{22}N_6O$ (C, H, N)
23	57	EtOH		141	$C_{18}H_{22}N_6O$ (C, H, N)
24	48		$CHCl_3$ 100%	oil	$C_{20}H_{26}N_6O$ (C, H, N)
25	54		$CHCl_3$ 100%	oil	$C_{22}H_{30}N_6O$ (C, H, N)
26	61	EtOH		155	$C_{19}H_{24}N_6O$ (C, H, N)
27	44		$CHCl_3$ 100%	oil	$C_{21}H_{28}N_6O$ (C, H, N)
28	30		$CHCl_3$ 100%	133	$C_{23}H_{32}N_6O$ (C, H, N)
29	45	EtOH–Et_2O		224	$C_{14}H_{15}N_5O$ (C, H, N)
30	60		CH_2Cl_2–MeOH 96:4	171	$C_{16}H_{19}N_5O$ (C, H, N)
31	73		$CHCl_3$–MeOH 98:2	oil	$C_{18}H_{23}N_5O$ (C, H, N)
32	71	EtOH		207	$C_{19}H_{23}N_5O$ (C, H, N)
33	62		AcOEt–petroleum ether 1:5	oil	$C_{21}H_{27}N_5O$ (C, H, N)
34	67		AcOEt–petroleum ether 1:5	oil	$C_{23}H_{31}N_5O$ (C, H, N)
35	67	EtOH		223	$C_{20}H_{25}N_5O$ (C, H, N)
36	68		AcOEt–petroleum ether 1:5	153	$C_{22}H_{29}N_5O$ (C, H, N)
37	32		AcOEt–petroleum ether 1:5	oil	$C_{24}H_{33}N_5O$ (C, H, N)

Mass spectra were performed on a Hewlett-Packard GC/MS System 5988A. TLC was performed on precoated silica gel F_{254} plates (Merck). Flash-column chromatographies were performed using Merck Kieselgel 60 (230–400 mesh). GLC analyses of compounds 3-aminopentan-2-ol and 3-aminoheptan-2-ol were performed after transformation into the corresponding acetate derivatives prepared as follows: 10 mg of compound in anhydrous pyridine (1 mL) was treated with acetic anhydride (1 mL) at 100 °C for 20 min. An aliquot of the reaction mixture was gas-chromatographed on a Carlo-Erba mod. 4200 apparatus using a glass column (1.5 m × 2.8 mm) packed with 10% neopentyl glycol succinate on 80–100 mesh silanised Chromosorb W and a FID detector.

Microanalyses (C H N) were carried out on a Carlo Erba elemental analyser (Model 1106) and were within ±0.4% of the theoretical values. Petroleum-ether corresponds to fraction boiling at 40–60 °C.

6.1.1. 2-Phenyl-9-benzyl-8-azahypoxanthine (*1*)

This compound was prepared according to a described procedure [18].

6.1.2. 6-Chloro-2-phenyl-9-benzyl-8-azaadenine (*2*)

A mixture of 2-phenyl-9-benzyl-8-azahypoxanthine (1.0 g, 3.3 mmol), *N*,*N*-diethylaniline (0.8 g, 5.36 mmol) and $POCl_3$ (3.0 mL, 32.55 mmol) was heated at 90 °C for 4 h then was evaporated at reduced pressure. The residue was crystallised from chloroform–ether to give the title compound (0.7 g, 2.18 mmol, 67% yield). M.p. 184 °C. ^{1}H-NMR ($CDCl_3$) δ: 5.94 (s, 2H, N-CH_2); 7.38 (m, 3H, arom); 7.57 (m, 5H, arom); 8.59 (m, 2H, arom).

6.1.3. General procedure to prepare 6-aminosubstituted-2-phenyl-9-benzyl-8-azaadenines *3*–*9*

In a well-stoppered flask a mixture of **2** (0.2 g, 0.62 mmol), toluene (2 mL), the suitable amine (1 mmol) and *N*,*N*-diethylaniline (0.09 g, 0.63 mmol) was heated at 130 °C for 16 h (72 h for compound **10**). Then the solution was evaporated at reduced pressure, diluted with chloroform and washed with 10% HCl and water. After evaporation of the organic layer the residue was crystallised or chromatographed (see Tables 3 and 4).

6.1.4. 5-Amino-4,6-dichloro-2-phenylpyrimidine (*10*)

This compound was prepared according to a described procedure [17].

6.1.5. General procedure for 3-aminopentan-2-one or 3-aminoheptan-2-one

A mixture of 125 mmol of D,L-2-amino-1-butanoic acid or D,L-2-amino-1-hexanoic acid, pyridine (66 mL)

Table 4
^{1}H-NMR and mass spectra of synthesised compounds **3**–**9**, **11**–**13** and **21**–**37**

Compound (solvent)	^{1}H-NMR				MS (*m/e*)
	Aromatic H	Benzylic H	Aliphatic H	Exchang. H	
3 ($CDCl_3$)	8.45 (m, 2H); 7.53–7.15 (m, 8H)	5.76 (s, 2H)	3.97 (m, 4H)	6.78 (1H)	346 (M^+, 3.5); 316 (30); 91 (100)
4 ($CDCl_3$)	8.45 (m, 2H); 7.54–7.28 (m, 8H)	5.76 (s, 2H)	1.33 (d, 3H); 4.01 (m, 2H); 4.19 (m, 1H)	6.53 (1H)	360 (M^+, 8.3); 316 (18); 91 (100)
5 ($CDCl_3$)	8.45 (m, 2H); 7.51–7.25 (m, 8H)	5.80 (s, 2H)	1.94 (m, 2H); 3.67 (m, 2H); 4.01 (m, 2H)	3.53 (1H); 6.72 (1H)	360 (M^+, 2.5); 316 (5); 91 (100)
6 ($CDCl_3$)	8.46(m, 2H); 7.49–7.29 (m, 8H)	5.77 (s, 2H); 5.11 (m, 1H)	3.90 (m, 2H)	4.20 (1H); 6.87 (1H)	316 (23); 287 (15); 91 (100)
7 ($CDCl_3$)	8.52 (m, 2H); 7.51–7.33 (m, 8H)	5.81 (s, 2H); 5.15 (m, 1H)	1.28 (d, 3H); 5.0 (m, 1H)	6.35 (1H); 4.29 (1H)	329 (32); 301 (2); 91 (100)
8 ($CDCl_3$)	8.40(m, 2H); 7.42–7.20(m, 8H)	5.69 (s, 2H)	1.40–1.62 (m, 4H); 2.10 (m, 2H); 2.30 (m, 2H); 3.74 (m, 1H); 4.35 (m, 1H)	4.77 (1H); 6.01 (1H)	400 (M^+, 2.1); 302 (9); 273 (19); 91 (100)
9 ($CDCl_3$)	8.45 (m, 2H); 7.52–7.26 (m, 8H)	5.61 (s, 2H)	1.45–1.57 (m, 4H); 1.81 (m, 2H); 2.20 (m, 2H); 3.64 (m, 1H); 4.23 (m, 1H)	4.68 (1H); 6.68 (1H)	400 (M^+, 1); 303 (15); 273 (5); 91 (100)
11 (DMSO-d_6)	8.16 (m, 2H); 7.40 (m, 3H)		1.13 (d, 3H); 3.47 (m, 2H); 3.94 (m, 1H)	4.83 (1H); 5.22 (2H): 6.95 (1H)	278 (M^+, 10); 233 (36); 45(100)
12 ($CDCl_3$)	8.25 (m, 2H); 7.46 (m, 3H)		1.05 (t, 3H); 1.21 (d, 3H); 1.64 (m, 2H); 4.09 (m, 1H); 4.35 (m, 1H)	5.13 (1H)	
13 ($CDCl_3$)	7.94 (m, 2H); 7.52 (m, 3H)		0.89 (t, 3H); 1.20–1.60 (m, 9H); 3.12 (m,1H); 4.34 (m, 1H)		
20 ($CDCl_3$)	8.37 (m, 2H); 7.49 (m, 3H)		1.13 (d, 3H); 3.32 (d, 2H); 4.45 (m, 1H)	5.20 (1H); 6.08 (2H)	270 (M^+, 2); 213 (36); 104 (100)
21 (DMSO-d_6)	8.15 (m, 2H); 7.59 (m, 3H)		0.70 (t, 3H); 0.91 (d, 3H); 2.20 (m, 2H); 4.15 (m, 1H); 4.54(m, 1H)	1.23 (1H)	254 (5);104 (45); 45 (100)
22 ($CDCl_3$)	8.36 (m, 3H); 7.48 (m, 2H)		0.84 (t, 3H); 1.04–2.34 (m, 9H); 4.45 (m, 1H); 4.92 (m, 1H)	5.32 (1H); 6.48 (2H)	326 (M^+, 3); 213 (20); 104 (30); 83 (100)
23 ($CDCl_3$)	8.45 (m, 2H); 7.50 (m, 3H);		1.37 (d, 3H); 1.65–2.23 (m, 8H); 4.45 (m, 1H); 4.62 (m, 1H); 4.84 (m, 2H)	5.36 (1H); 6.35 (1H)	338 (M^+, 20); 270 (60); 225 (97); 104 (100)
24 ($CDCl_3$)	8.49 (m, 2H); 7.40 (m,3H);		0.83 (t, 3H); 1.05–2.30 (m, 13H); 4.44 (m, 2H); 4.81 (m, 1H);	4.80 (1H); 6.43 (1H)	366 (M^+, 2); 149 (27); 41 (100)
25 ($CDCl_3$)	8.42 (m, 2H); 7.49 (m, 3H)		0.84 (t, 3H); 0.95–2.26 (m, 17H); 4.15–4.46 (m, 2H); 4.80 (m, 1H)	5.71 (1H); 6.34 (1H)	394 (M^+, 2); 322 (11); 104 (31); 45 (100)
26 (DMSO-d_6)	8.42 (m, 2H); 7.51 (m, 3H);		1.15 (d, 3H); 1.40–2.00 (m, 10H); 4.28–4.50 (m, 4H)	5.50 (1H); 8.70 (1H)	352 (M^+, 3); 104 (31); 45 (100)
27 ($CDCl_3$)	8.40 (m, 2H); 7.50 (m, 3H);		0.83–2.30 (m, 18H); 4.31 (m, 1H); 4.31 (m, 1H); 4.43 (m,1H); 4.80 (m,1H)	5.72 (1H); 6.29 (1H)	380 (M^+, 2); 295 (30); 149 (57); 43 (100)
28 ($CDCl_3$)	8.42 (m, 2H); 7.48 (m, 3H)		0.84 (t, 3H); 1.22–2.25 (m, 19H); 4.15–4.46 (m, 2H); 4.41 (m, 2H); 4.83 (m, 1H)	6.43 (m, 1H); 5.85 (m, 1H)	408 (M^+, 2.2); 391 (4); 104 (61); 41 (100)
29 (DMSO-d_6)	8.38 (m, 2H); 8.07 (s, 1H); 7.46 (m, 3H)		1.09 (d, 3H); 3.32 (d, 2H); 4.12 (m, 1H)	4.12 (1H); 7.25 (1H)	269 (M^+, 7.6); 104 (47); 45 (100)
30 (DMSO-d_6)	8.33 (m, 2H); 8.16 (s, 1H); 7.43 (m, 3H)		0.67 (t, 3H); 0.94 (d, 3H); 2.12 (m, 2H); 4.16 (m, 2H)	5.21 (1H); 7.26 (2H)	297 (M^+, 2); 212 (20); 45 (100)
31 ($CDCl_3$)	8.31 (m, 2H); 7.72 (s, 1H); 7.44 (m, 3H)		0.84 (t, 3H); 1.18–1.36 (m, 7H); 2.12 (m, 2H); 4.30 (m, 2H)	6.05 (1H); 6.12 (1H)	325 (M^+, 2.4); 212 (40); 45 (100)
32 ($CDCl_3$)	8.42 (m, 2H); 7.71 (s, 1H); 7.48 (m, 3H)		1.32 (d, 3H); 1.60–2.30 (m, 8H); 4.28 (m, 3H); 4.80 (m, 1H)	5.84 (2H)	337 (M^+, 28); 269 (100); 211 (99)
33 ($CDCl_3$)	8.34 (m, 2H); 7.64 (s, 1H); 7.42 (m, 3H)		0.80 (m, 3H); 1.33–2.21 (m, 13H); 4.07 (m, 1H); 4.35 (m, 1H); 4.76 (m, 1H)	5.81 (1H); 6.66 (1H)	365 (M^+, 20); 211(100); 104 (47)
35 ($CDCl_3$)	8.39 (m, 2H); 7.71 (s, 1H); 7.46 (m, 3H)		1.32 (d, 3H); 1.40–2.20 (m, 10H); 4.32 (m, 4H)	5.74 (2H)	351 (M^+, 21); 269 (100); 211 (74)
36 ($CDCl_3$)	8.33 (m, 2H); 7.65 (s, 1H); 7.44 (m, 3H)		0.82 (t, 3H); 1.35–2.31 (m, 15H); 4.04 (m, 1H); 4.36 (m, 2H)	5.73 (1H); 6.68 (1H)	379 (M^+, 7); 211 (29); 41 (100)
37 ($CDCl_3$)	8.36 (m, 2H); 7.67 (s, 1H); 7.46 (m, 3H)		0.86 (t, 3H); 1.10–2.30 (m, 19H); 4.15 (m, 3H)	5.85 (1H); 6.75 (1H)	407 (M^+, 19); 211 (58); 45 (100)

and acetic anhydride (100 mL) was heated at 100 °C for 3 h. After evaporation of the mixture, an oil was obtained which was partitioned with ethyl ether and 5% $NaHCO_3$. The organic layer was evaporated in vacuo to give an oil (3-acetamidopentan- or hexan-2-one) which was treated with concentrated HCl (160 mL)

at 120 °C for 2 h. Evaporation of the mixture gave title compounds which were crystallised from ethanol–ethyl ether. 3-Aminopentan-2-one: m.p. 165 °C; 3-aminoheptan-2-one: m.p. 125 °C.

6.1.6. General procedure for 3-aminopentan-2-ol or 3-aminoheptan-2-ol

To an iced solution of 3-aminopentan-2-one or 3-aminoheptan-2-one (18.5 mmol) in MeOH (20 mL), $NaBH_4$ (0.7 g, 37.0 mmol) was slowly added maintaining the pH ≅ 5–6 by addition of glacial acetic acid. The reaction mixture was stirred at room temperature for 20 h, then evaporated. The residue was solved in NaOH (10 mL) and the solution was extracted with $CHCl_3$. Evaporation of the organic layer gave title compounds as an oil (GLC, NMR), which were used without purification for the next reactions.

*6.1.7. 5-Amino-6-chloro-4-(2-hydroxy-3-propyl)-2-phenylpyrimidine (**11**)*

A mixture of **10** (0.15 g, 0.63 mmol), *erythro*-3-aminopropan-2-ol (0.28 g, 3.73 mmol), *N*,*N*-diethylaniline (0.09 g, 0.63 mmol) in absolute ethanol (5 mL) was heated at 110 °C for 12 h in a steel bomb. Evaporation of the mixture gave an oil which was diluted with ethyl acetate. The solution was washed with 10% HCl, 5% $NaHCO_3$ and water, then evaporated. The residue was flash-chromatographed using CH_2Cl_2–MeOH 97:3 as eluent to give compound **11** (0.115 g, 0.41 mmol) which was crystallised with ethanol–ethyl ether (see Tables 3 and 4).

*6.1.8. 5-Amino-6-chloro-4-(2-hydroxy-3-pentyl)-2-phenylpyrimidine (**12**)*

A mixture of **10** (0.80 g, 3.35 mmol), 3-aminopentan-2-ol (0.517 g, 5.02 mmol), *N*,*N*-diethylaniline (0.50 g, 3.35 mmol) in pentanol (7 mL) was heated at 170 °C for 72 h in a steel bomb. Evaporation of the mixture gave an oil which was flash-chromatographed using $CHCl_3$ as eluent to give compound **12** (0.70 g, 2.29 mmol, 68% yield, see Tables 3 and 4).

*6.1.9. 5-Amino-6-chloro-4-(2-hydroxy-3-heptyl)-2-phenylpyrimidine (**13**)*

A mixture of **10** (0.80 g, 3.35 mmol), 3-aminoheptan-2-ol (0.65 g, 5.02 mmol), *N*,*N*-diethylaniline (0.5 g, 3.35 mmol) in pentanol (7 mL) was heated at 170 °C for 72 h in a steel bomb. Evaporation of the mixture gave an oil which was flash-chromatographed using $CHCl_3$–MeOH 95:5 as eluent to give compound **13** (0.63 g, 1.88 mmol, see Tables 3 and 4).

*6.1.10. 6-Chloro-9-(2-hydroxy-3-propyl)-2-phenyl-8-azapurine (**14**)*

To an ice cold solution of **11** (0.33 g, 1.17 mmol), H_2O (3.6 mL), glacial acetic acid (1.6 mL) and THF (7 mL), a solution of $NaNO_2$ (84 mg, 1.22 mmol) in 3.6 mL of H_2O was added dropwise. The mixture was stirred for 3 h at 0 °C and for 2 h at room temperature, then evaporated in vacuo to give the crude title compound which was used without purification for the next reaction.

*6.1.11. 6-Chloro-9-(2-hydroxy-3-pentyl)-2-phenyl-8-azapurine (**15**)*

Title compound was obtained as described for **14** and used without purification for the next reaction.

*6.1.12. 6-Chloro-9-(2-hydroxy-3-heptyl)-2-phenyl-8-azapurine (**16**)*

Title compound was obtained as described for **14** and used without purification for the next reaction.

*6.1.13. 6-Chloro-9-(2-hydroxy-3-propyl)-2-phenylpurine (**17**)*

A solution of **11** (0.20 g, 0.72 mmol), triethyl orthoformate (3 mL) and 12N HCl (0.14 mL) was stirred at room temperature for 12 h; then the reaction mixture was evaporated and used without purification for the next reaction.

*6.1.14. 6-Chloro-9-(2-hydroxy-3-pentyl)-2-phenylpurine (**18**)*

Title compound was obtained as described for **17** and used without purification for the next reaction.

*6.1.15. 6-Chloro-9-(2-hydroxy-3-hexyl)-2-phenylpurine (**19**)*

Title compound was obtained as described for **17** and used without purification for the next reaction.

*6.1.16. General procedure to prepare 6-aminosubstituted-9-(2-hydroxy-3-alkyl)-2-phenyl-8-azapurines **20**–**28** and 6-aminosubstituted-9-(2-hydroxy-3-alkyl)-2-phenylpurines **29**–**37***

A mixture of 6-chloro-9-(2-hydroxy-3-alkyl)-2-phenyl-8-azapurine or 6-chloro-9-(2-hydroxy-3-alkyl)-2-phenylpurine (0.27 mmol), 5 mL of anhydrous ethanol and the suitable amine (1.5 mmol) was heated in a sealed tube at 120 °C for 24 h. After evaporation, the residue was diluted with chloroform, washed with 10% HCl and evaporated. The residue was chromatographed and/or crystallised to give pure products (see Tables 3 and 4).

6.2. Water solubility

Bidistilled water (3 mL) was saturated with 10 mg of compound **20** by heating. After cooling at room temperature, the undissolved residue was filtered off and 1 mL of solution was lyophilised to give 1.2 mg,

corresponding to a solubility of 4.44 mM at room temperature.

6.3. Biochemical assays

6.3.1. A_1 receptor binding

Bovine cerebral cortex was homogenised in ice-cold 0.32 M sucrose containing protease inhibitors, as previously described [25]. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant again centrifuged at 48 000*g* for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 units mL^{-1}) to remove endogenous adenosine at 37 °C for 60 min, and then recentrifuged at 48000*g* for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^{3}H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris–HCl, pH 7.7, containing 2 mM $MgCl_2$, with approximately 1.2 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 50 μM *R*-PIA. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. Radioactivity was counted in a 4 mL Beckman Ready-Protein scintillation cocktail in a scintillation counter.

6.3.2. A_{2A} receptor binding

Bovine striatum was homogenised in 20 volumes of ice-cold 50 mM Tris–HCl, pH 7.5, containing 10 mM $MgCl_2$ and protease inhibitors. The membrane homogenate was centrifuged at 48 000g for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 units mL^{-1} of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet frozen at −80 °C. Routine assays were performed in triplicate by incubation of an aliquot of striatal membranes (0.2–0.3 mg of protein) in cold 50 mM Tris–HCl, pH 7.5, containing 10 mM $MgCl_2$ with approximately 5 nM [^{3}H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out for 90 min at 25 °C. Non-specific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. Radioactivity was counted in a 4 mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on binding. The concentrations of the tested compounds to produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) were determined from semilog plots of data from experiments of binding inhibition. The Ki values were calculated from the IC_{50} values using the equation $IC_{50}/(L/K_d)$ [26] ([^{3}H]CHA $K_d = 10.5$ nM and $L = 1.2$ nM; [^{3}H]CGS 21680 $K_d = 1$ nM and $L = 5$ nM); protein estimation was based on the method reported [27] using bovine serum albumin as standard.

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