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8-Bromo-9-alkyl adenine derivatives as tools for developing new adenosine A_{2A} and A_{2B} receptors ligands

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ABSTRACT

Importance of making available selective adenosine receptor antagonists is boosted by recent findings of adenosine involvement in many CNS dysfunctions. In the present work a series of 8-bromo-9-alkyl adenines are prepared and fully characterized in radioligand binding assays or functional cyclase experiments in respect to their interaction with all the four adenosine receptor subtypes. Results show that the presence of the bromine atom in 8-position of 9-substituted adenines promotes in general the interaction with the adenosine receptors, in particular at the A_{2A} subtype. The present study also demonstrates that adenine derivatives could be a good starting point to obtain selective adenosine A_{2B} receptor antagonists.

1. Introduction

Adenosine modulates a variety of physiological and pathophysiological processes through the interaction with four subtypes of a family of cell-surface G protein-coupled receptors. The receptors of this nucleoside have been cloned[1] and classified[2] as A_1, A_{2A}, A_{2B}, and A_3 (AA_1R, $AA_{2A}R$, $AA_{2B}R$, and AA_3R, respectively) on the bases of their respective coupling to second messengers, tissue distribution and unique pharmacological profiles. In fact, a variety of physiological actions can be referred to adenosine, including effects on heart rate and atrial contractility, vascular smooth muscle tone, release of neurotransmitters, lipolysis, renal, platelet and white blood cell functions.[3] Hence, selective AR modulators have promise for numerous therapeutic applications, including cardiovascular, inflammatory and neurodegenerative diseases.[4]

The prototypical adenosine receptor antagonists are the naturally occurring methylxanthines which have been extensively modified to achieve increased potency and selectivity for specific adenosine receptor subtypes.[5–7] However, only a few xanthine antagonists as caffeine and theophylline have been approved as drugs for their CNS stimulating, diuretic, and bronchodilating effects.[7,8] Other adenosine receptor antagonists, strictly correlated to the natural ligand adenosine, are the adenine derivatives substituted in the different position of the purine core. In fact, in many papers we have demonstrated that the introduction of different substituents in 2-, 8-, and 9-position of adenine resulted in high-affinity antagonists with distinct receptor selectivity profile.[9–15]

Recently, we reported that some 8-substituted-9-ethyladenine derivatives ameliorate motor deficits in rat models of Parkinson's disease, suggesting potential therapeutic application for these compounds.[16] Furthermore, substituted adenine derivatives proved to possess affinity and selectivity for all the AR subtypes.[17–20]

Starting from the observation that introduction of a bromine atom in 8-position of 9-ethyladenine (**3**) led to compound **22** endowed with $AA_{2A}R$ affinity in the nanomolar range but with low selectivity versus AA_1R subtype, the synthesis of a number of adenine derivatives bearing different substituents in 9-position was undertaken, aimed at improving $AA_{2A}R$ affinity and selectivity.

2. Results and discussion

2.1. Chemistry

The synthesis of 9-alkyladenines **2–20** was carried out by reacting the commercially available adenine (**1**) with the suitable alkyl halide in dry DMF, employing potassium carbonate as base. In the case of compounds **2–7** and **15–20**, the corresponding N-7 derivatives were obtained in a yield ranging from 9% to 27% (Scheme 1).

The site of alkylation, in addition to the fact that compounds **2a–7a** and **15a–20a** were obtained in much lower yield than that

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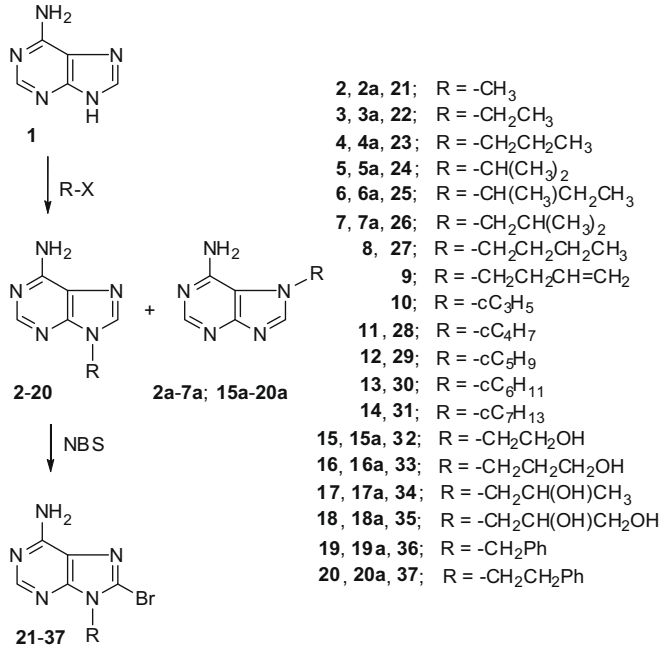


Scheme 1.

of the corresponding N-9 isomers, was unambiguously assigned by ^{1}H NMR. In fact, in DMSO-d_6, pronounced downfield shifts were observed for the signals resulting from the H-8, NH_2, and the protons on the carbon atom linked to the imidazol nitrogen (N–CH_n) of all N-7 when compared with those of N-9 isomers. These data are in agreement with previous reports by several authors relative to similar N-7 and N-9 purine isomers.[21–23] On the other hand, an opposite trend is highlighted in the case of H-2 signals for N-7 isomers, which were found to be shifted upfield relative to those of the corresponding N-9 isomers (see Table 1). The assignment (N-7 or N-9) of the alkylation site can be confirmed applying NOE difference spectroscopy. As an example, experiments were performed with **3** and **3a**. Saturation of CH_2 protons of **3** resulted in NOEs of the H-2 and H-8 signals (5.3% and 7.3%, respectively) and of CH_3 (2.7%). On the other hand, saturation of the same protons in compound **3a** resulted in stronger NOE effects of the H-8 and CH_3 signals (13.7% and 4.4%, respectively) while there was none at H-2 signal, establishing N-7 configuration. Moreover, saturation of CH_3 protons of **3** gave NOEs of H-2 and H-8 signals (3.6% and 2.4%, respectively) and of CH_2 (2.4%), while the same experiments performed with the N-7 isomer **3a** showed NOEs only of H-8 and CH_2 signal (2.3% and 2.9%, respectively).

Table 1
Comparison of ^{1}H chemical shifts (ppm) in DMSO-d_6 of H-2, H-8, and N–CH_n signals of N-9 and N-7 isomers (compounds **2–7** and **15–20** and the corresponding **2a–7a** and **15a–20a**, Scheme 1)

Compound	R	H-2	H-8	NH_2	N–CH_n
2	CH_3	8.10	8.15	7.20	3.72
2a	CH_3	8.48	8.50	8.17	3.94
3	CH_2CH_3	8.16	8.18	7.19	4.29
3a	CH_2CH_3	7.77	8.37	7.88	4.35
4	$CH_2CH_2CH_3$	8.15	8.15	7.21	4.11
4a	$CH_2CH_2CH_3$	7.75	8.34	7.85	4.26
5	$CH(CH_3)_2$	8.14	8.24	7.19	4.73
5a	$CH(CH_3)_2$	7.77	8.43	7.86	4.96
6	$CH(CH_3)CH_2CH_3$	8.13	8.22	7.20	4.50
6a	$CH(CH_3)CH_2CH_3$	7.76	8.40	7.85	4.68
7	$CH_2CH(CH_3)_2$	8.14	8.14	7.22	3.97
7a	$CH_2CH(CH_3)_2$	7.76	8.33	7.94	4.12
15	CH_2CH_2OH	8.14	8.03	7.19	4.19
15a	CH_2CH_2OH	7.76	8.23	7.86	4.35
16	$CH_2CH_2CH_2OH$	8.10	8.09	7.18	4.17
16a	$CH_2CH_2CH_2OH$	7.76	8.31	7.87	4.37
17	$CH_2CH(OH)CH_3$	8.13	8.05	7.21	4.06
17a	$CH_2CH(OH)CH_3$	7.74	8.20	7.85	4.33 4.05
18	$CH_2CH(OH)CH_2OH$	8.10	8.01	7.17	4.27 3.97
18a	$CH_2CH(OH)CH_2OH$	7.75	8.19	7.87	4.50 4.12
19	CH_2Ph	8.15	8.26	7.25	5.37
19a	CH_2Ph	7.75	8.55	7.92	5.51
20	CH_2CH_2Ph	8.12	7.90	7.14	4.36
20a	CH_2CH_2Ph	7.81	8.07	7.87	4.54

For compounds structure, see Scheme 1.

Furthermore, compound **3** and **13** were also synthesized through a cyclization reaction pathway (Scheme 2) to further demonstrate the alkylation site in the case of compound **3**, and to obtain a better yield in the case of compound **13**.[24,25] In fact, reaction of commercially available 5-ammino-4,6-dichloropyrimidine (**38**) with the suitable amine furnished the corresponding 4-alkylamino derivatives **39** and **40**.

Reaction of the latter compounds with diethoxymethyl acetate gave the 6-chloro-9-alkylpurines **41** and **42** which after treatment with liquid ammonia gave compounds **3** and **13** as unambiguously N-9 isomers.

An additional indirect evidence of the alkylation site came from the very weak or absent pharmacological activity of the N-7 isomers (Table 4; see biological evaluation section).

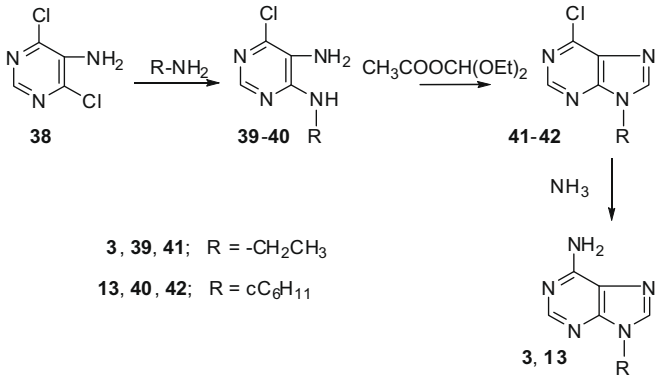
Reaction of the 9-alkyladenines **2–20** with NBS at room temperature gave the corresponding 8-bromo-9-alkyladenine **21–37** (Scheme 1).

The bromination reaction of compound **2** was carried out in CH_3CN instead of DMF, used for the other compounds, since in the latter solvent a complex mixture of side products was obtained.

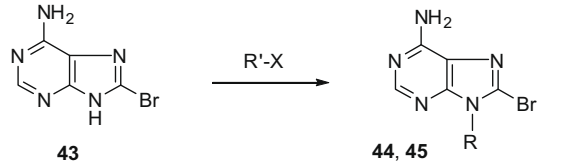
In the case of 9-cyclopropyladenine **10**, the compound was obtained in a very low yield, hence the preparation of the corresponding 8-bromo-9-cyclopropyladenine was attempted by coupling the commercially available 8-bromoadenine **43** with cyclopropylbromide. However, this reaction, carried out in dry DMF and potassium carbonate, yielded the 9-allyl-8-bromoadenine **44** instead of the desired compound (Scheme 3).

The same procedure was utilized to prepare 8-bromo-9-(3-buten-1-yl)adenine **45**, since bromination of compound **9** with NBS led to the obvious addition of a bromine atom also to the double bond. In Table 2 we report the reaction conditions of alkylations described in Schemes 1 and 3, and in Table 3 the reaction time and yield of brominations described in Scheme 1.

Some of the prepared compounds have been already synthesised (the references being reported in the tables), but in some cases they lack full characterization. Hence, this work is intended to provide an overview of chemical and receptor binding affinity properties of various 9-substituted adenine derivatives.



Scheme 2.



Scheme 3.

2.2. Biological evaluation

All the compounds were evaluated at the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (AA_1R, $AA_{2A}R$, AA_3R) or adenylyl cyclase activity assay ($AA_{2B}R$). Receptor binding affinity was determined using [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) as the radioligand for AA_1R receptors, whereas [^{3}H]NECA (5′-*N*-ethylcarboxamidoadenosine) was used for the $AA_{2A}R$ and AA_3R subtypes.[42] In the case of $AA_{2B}R$ K_i values were calculated from IC_{50}-values determined by inhibition of NECA-stimulated adenylyl cyclase activity.[42] DPCPX, a potent antagonist, and 8-bromo-9-ethyladenine (**22**), have been used as reference compounds. K_i values are in nM, with 95% confidence intervals in parentheses. The results of binding studies and adenylyl cyclase activity are summarized in Table 4.

Our goal was to evaluate the effect of the introduction in 9-position of alkyl and cycloalkyl chains and to combine the presence of different sized chains in N-9 with the presence of a bromine atom in 8 position of the purine moiety. During the alkylation of purine nucleobases, some N-7 analogues have been obtained in a significant amount. Hence, they were evaluated in the above mentioned assays to gain more insights into spatial interactions of purine moiety with adenosine receptor subtypes (biological evaluation results are reported in Table 4).

2.3. Structure–activity relationships

In our previous investigation on a series of purine derivatives we found that 8-bromo-9-ethyladenine (**22**) is endowed with affinity in the nanomolar range at AA_1R and $AA_{2A}R$ subtypes (K_i AA_1R = 280 nM and K_i $AA_{2A}R$ = 52 nM) and with good activity at $AA_{2B}R$ (K_i $AA_{2B}R$ = 840 nM).[10] Moreover, these data demonstrated that compound **22** is only moderately $AA_{2A}R$ selective whereas the potential application of $AA_{2A}R$ antagonists in CNS diseases would require more selective compounds. With this in mind, we carried out the synthesis of a number of adenine derivatives by introducing in 9-position linear and branched alkyl chains, cycloalkyl groups, and hydroxyalkyl chains. Furthermore, a bromine atom was introduced in 8-position of all compounds since from the preliminary study by Camaioni et al. an about 40-fold increase in $AA_{2A}R$ affinity was obtained when 9-ethyladenine (**3**) was brominated (**22**: K_i $AA_{2A}R$ = 0.052 μM vs **3**: K_i $AA_{2A}R$ = 2.2 μM).[10]

However, also the affinity and activity at the other three subtypes were improved by bromination of **3** (**22**: K_i AA_1R = 0.28 μM, K_i $AA_{2B}R$ 0.84 μM, K_i AA_3R = 28 μM vs **3**: K_i AA_1R = 7.4 μM, K_i $AA_{2B}R$ >30 μM, K_i AA_3R = >100 μM), hence introduction of different chains in 9-position was undertaken in the attempt to find a more discriminating group. From the results reported in Table 4, it is possible to conclude that in all cases the introduction of the 8-bromine atom led to an increase of the affinity at all adenosine receptor subtypes, with the exception of compound **31** (having a bulky cycloheptyl group in 9-position). Furthermore, none of the substituents introduced at the 9-position of adenine and 8-bromoadenine brought about an increase in both AA_1R and $AA_{2A}R$ affinity. On the other hand, $AA_{2A}R$ versus AA_1R selectivity was slightly improved when an isopropyl (**24**: K_i $AA_{2A}R$ = 0.074 μM vs K_i AA_1R = 0.83 μM) or a 1-hydroxy-3-propyl group (**33**: K_i $AA_{2A}R$ = 0.085 μM vs K_i AA_1R = 1.0 μM) was introduced at the 9-position. In general, going from methyl to butyl N-9 substituent, the optimal length for affinity at both AA_1R and $AA_{2A}R$ subtype seems to be a two carbon chain (**21**: K_i AA_1R = 0.57 μM, **22**: K_i AA_1R = 0.28 μM, **23**: K_i AA_1R = 1.1 μM, and **27**: K_i AA_1R = 3.2 μM; **21**: K_i $AA_{2A}R$ = 0.12 μM, **22**: K_i $AA_{2A}R$ = 0.052 μM, **23**: K_i $AA_{2A}R$ = 0.30 μM, and **27**: K_i $AA_{2A}R$ = 0.73 μM). Moreover, in the branched chain series, the isopropyl and the

Table 2
Preparation of compounds **2**–**20**, **2a**–**7a**, **15a**–**20a** and **44**, **45** reported in Schemes 1 and 3

Compound	X	*t* (h)	*T* (°C)	Chromatography solvent	Yield% (cryst. solv.)
2[26,27], **2a**[26,28,29]	I	2	25	$CHCl_3$–CH_3OH (98:2–97:3)[a]	69[b], 23[c] (CH_3OH)[b,c]
3[24,26,27,29], **3a**	I	6	25	$CHCl_3$–CH_3OH (98:2)[a]	59[b], 10[c] (CH_3OH)[b,c]
4[15,26], **4a**[15]	I	16	25	$CHCl_3$–CH_3OH (98:2)[a]	79[b], 9[c] (CH_3OH[b], CH_2Cl_2[c])
5[31,33], **5a**[34]	I	24	25	$CHCl_3$–cC_6H_{12}–CH_3OH (80:10:10)	54[b], 16[c] (CH_3OH)[b]
6, **6a**	I	96	25	$CHCl_3$–cC_6H_{12}–CH_3OH (80:10:10)	59[b], 16[c] (AcOEt)[b]
7, **7a**	I	96	25	$CHCl_3$–cC_6H_{12}–CH_3OH (80:10:10)	68[b], 12[c] (CH_3OH)[b]
8[25]	Br	96	25	$CHCl_3$–CH_3OH (98:2)[a]	81 (AcOEt)
9[40]	Br	16	50	$CHCl_3$–CH_3OH (90:10)	70 (CH_3CN)
10[31]	Br	72	125	AcOEt–CH_3CN–CH_3OH–NH_3/CH_3OH (79:10:10:1)	13 (CH_3CN)
11[41]	Br	168	60	$CHCl_3$–cC_6H_{12}–CH_3OH (85:10:5)[a]	23 (CH_3OH)
12[25]	Br	72	25	$CHCl_3$–CH_3OH (98:2)[a]	44 (Et_2O)
13[25,30]	I	8 days	80	$CHCl_3$–CH_3OH (95:5)[a]	3 ($CHCl_3$/nC_6H_{14})
14[31]	Br	72	50	$CHCl_3$–CH_3OH (95:5)	41 (CH_3CN)
15[32], **15a**[22,25]	I	144	25	$CHCl_3$–CH_3OH (95:5–85:15)	31[b], 20[c] (CH_3OH)[b,c]
16[35], **16a**[35]	I	96	25	$CHCl_3$–CH_3OH–NH_3/CH_3OH (94:4:2)	28[b], 27[c] (CH_3OH)[b,c]
17[32,36], **17a**[37]	Br	72	60	$CHCl_3$–CH_3OH–NH_3/CH_3OH (90:6:4)	38[b], 10[c] (CH_3OH)[b,c]
18[38], **18a**[37,39]	Br	72	60	$CHCl_3$–CH_3OH–NH_3/CH_3OH (94:4:2)[a]	40[b], 5[c] (CH_3OH)[b]
19[27], **19a**[28]	Br	16	25	$CHCl_3$–cC_6H_{12}–CH_3OH–NH_3/MeOH (60:35:4:1)	60[b], 21[c] (CH_3OH)[b]
20[29,33], **20a**	Br	72	50	$CHCl_3$–CH_3OH (95:5)	82[b], 2[c] (CH_3CN)
44	Br	96	150	$CHCl_3$–CH_3OH (90:10)	11
45	Br	24	50	$CHCl_3$–CH_3OH (97:3)	50

[a] Flash chromatography.
[b] N-9 isomer.
[c] N-7 isomer.

Table 3
Preparation of the 9-alkyl-8-bromoadenines (**21–37**) reported in Scheme 1

Compound	*t* (h)	Chromatography solvent	Yield % (cryst. solv.)
21[43]	1.5	$CHCl_3$–CH_3OH (98:2–97:3)[a]	22 (CH_3OH)
22[10]	20	$CHCl_3$ to $CHCl_3$–CH_3OH (100%–98:2)[a]	60 (EtOAc)
23[44]	48	$CHCl_3$–CH_3OH (99.7:0.3)[a]	44 (C_2H_5OH)
24	103	cC_6H_{12}–AcOEt–CH_3OH (70:28:2)[a]	40 (CH_3OH)
25	102	cC_6H_{12}–$CHCl_3$–CH_3OH (70:28:2)[a]	31 (CH_3OH)
26	144	$CHCl_3$ grad. $CHCl_3$–CH_3OH (98:2)[a]	45 (CH_3OH)
27[44]	40	$CHCl_3$–CH_3OH (99.5:0.5)	41 (CH_3OH)
28	52	$CHCl_3$–cC_6H_{12}–CH_3OH (83:15:2)	51 (CH_3CN)
29	20	$CHCl_3$–cC_6H_{12}–CH_3OH (87:10:3)	35 (CH_3OH)
30	72	$CHCl_3$–cC_6H_{12}–CH_3OH (83:15:2)	53 (CH_3CN)
31	12	$CHCl_3$–CH_3OH (97:3)	44
32[45,46]	120	$CHCl_3$–CH_3OH (85:15)	30 (CH_3OH)
33[45]	46	$CHCl_3$–CH_3OH (97:3)[a]	65 (CH_3OH)
34[45]	30	$CHCl_3$–CH_3OH (93:7)	35 (C_2H_5OH)
35[45,47]	20	$CHCl_3$–CH_3OH (95:5–94:6)[a]	16 (CH_3OH)
36	12	$CHCl_3$–CH_3CN–CH_3OH (88:10:2)	23 (CH_3OH)
37	12	$CHCl_3$–CH_3OH (99:1)[a]	45 (CH_3OH)

[a] Flash chromatography.

Table 4
Affinity (K_i, μM) of compounds in radioligand binding assays at human AA_1R, $AA_{2A}R$, and AA_3R subtypes

Compound	R	X	AA_1R	$AA_{2A}R$	$AA_{2B}R$	AA_3R
DPCPX			0.004	0.13	1.0	4.0
8-BrAdenine	H	Br	33 (13–82)	3.2 (1.3–7.9)	>30	>10
2	CH_3	H	12 (8.1–18)	6.9 (3.7–13)	>30	>100
2a	CH_3		>100	>100	>30	>100
21	CH_3	Br	0.57 (0.56–0.59)	0.12 (0.076–0.20)	0.72 (0.52–0.98)	>100
3	CH_2CH_3	H	7.4 (4.2–13)	2.2 (1.4–3.5)	>30	>100
3a	CH_2CH_3		>100	30 (24–37)	>30	>100
22	CH_2CH_3	Br	0.28 (0.25–0.32)	0.052 (0.024–0.11)	0.84 (0.63–1.1)	28 (22–35)
4	$CH_2CH_2CH_3$	H	9.4 (7.9–11)	9.6 (5.8–16)	1.7 (0.79–3.8)	⩾100
4a	$CH_2CH_2CH_3$		>100	18 (9.3–34)	>30	>100
23	$CH_2CH_2CH_3$	Br	1.1 (1.0–1.3)	0.30 (0. 26–0.35)	0.20 (0.11–0.38)	⩾100
5	$CH(CH_3)_2$	H	4.3 (3.3–5.6)	4.1 (2.2–7.8)	16 (13–19)	>100
5a	$CH(CH_3)_2$		20 (17–24)	12 (5.9–23)	>30	>100
24	$CH(CH_3)_2$	Br	0.83 (0.53–1.3)	0.074 (0.028–0.19)	1.1 (0.59–2.1)	15 (11–20)
6	$CH(CH_3)CH_2CH_3$	H	34 (28–40)	16 (10–24)	19 (13–27)	>100
6a	$CH(CH_3)CH_2CH_3$		>100	>100	>30	>100
25	$CH(CH_3)CH_2CH_3$	Br	3.1 (2.3–4.2)	0.39 (0.24–0.65)	1.2 (0.59–2.5)	15 (11–20)
7	$CH_2CH(CH_3)_2$	H	>100	>100	>30	>100
7a	$CH_2CH(CH_3)_2$		>100	>100	>30	>100
26	$CH_2CH(CH_3)_2$	Br	4.9 (3.7–6.5)	6.0 (3.7–9.8)	4.2 (3.4–5.3)	15 (11–20)
8	$CH_2CH_2CH_2CH_3$	H	>100	7.8 (6.6–9.3)	>30	>100
27	$CH_2CH_2CH_2CH_3$	Br	3.2 (2.7–3.9)	0.73 (0.062–0.86)	5.6 (2.7–12)	>100
9	$CH_2CH_2CH{=}CH_2$	H	>100	8.5 (5.0–15)	>30	>100
45	$CH_2CH_2CH{=}CH_2$	Br	4.9 (4.4–5.4)	1.6 (1.4–1.9)	>30	>100
10	cC_3H_5	H	>100	2.3 (1.2–4.6)	>30	>100
44	$CH_2CH{=}CH_2$	Br	8.4 (5.4–13)	0.73 (0.61–0.87)	4.6 (1.9–11)	20 (11–36)
11	cC_4H_7	H	8.7 (8.3–9.2)	4.8 (3.8–5.9)	17 (11–28)	33 (24–47)
28	cC_4H_7	Br	2.2 (1.6–3.0)	0.66 (0.44–0.99)	4.1 (3.0–5.6)	6.2 (5.1–7.4)
12	cC_5H_9	H	10 (3.7–16)	1.8 (0.68–4.6)	>30	13 (8.5–19)
29	cC_5H_9	Br	1.0 (0.73–1.5)	1.9 (1.6–2.3)	0.86 (0.46–1.6)	4.2 (2.4–7.3)
13	cC_6H_{11}	H	>100	>100	>30	>100
30	cC_6H_{11}	Br	4.6 (3.2–6.6)	15 (13–17)	>30	11 (5.1–23)
14	cC_7H_{13}	H	>100	11 (6.6–19)	>30	>100
31	cC_7H_{13}	Br	>100	>100	>30	>100
15	CH_2CH_2OH	H	29 (18–45)	11 (6.5–18)	>30	>100
15a	CH_2CH_2OH		>100	>100	>30	>100
32	CH_2CH_2OH	Br	1.5 (1.1–2.2)	0.62 (0.54–0.71)	2.8 (1.8–4.5)	>100
16	$CH_2CH_2CH_2OH$	H	>100	3.9 (3.4–4.5)	>30	>100
16a	$CH_2CH_2CH_2OH$		>100	>100	>30	>100
33	$CH_2CH_2CH_2OH$	Br	1.0 (0.82–1.3)	0.085 (0.030–0.24)	5.1 (3.7–6.9)	3.2 (2.0–5.4)
17	$CH_2CH(OH)CH_3$	H	29 (20–44)	3.1 (1.6–5.9)	>30	>100
17a	$CH_2CH(OH)CH_3$		>100	>100	>30	>100
34	$CH_2CH(OH)CH_3$	Br	2.9 (1.8–4.8)	1.1 (0.83–1.4)	0.64 (0.32–1.3)	⩾100
18	$CH_2CH(OH)CH_2OH$	H	>100	8.5 (6.3–12)	>30	>100
18a	$CH_2CH(OH)CH_2OH$		>100	>100	>30	>100
35	$CH_2CH(OH)CH_2OH$	Br	16 (10–25)	4.0 (2.9–5.4)	7.1 (6.0–8.4)	⩾100
19	CH_2Ph	H	>100	8.5 (5.8–12)	>30	>100
36	CH_2Ph	Br	6.7 (4.8–9.3)	6.1 (5.1–7.2)	>30	⩾100
20	CH_2CH_2Ph	H	>100	9.6 (5.5–17)	>30	>100
37	CH_2CH_2Ph	Br	12 (11–14)	4.9 (2.1–11)	>30	>30

NECA-stimulated adenylyl cyclase assays at membranes from stably transfected CHO cells expressing human $AA_{2B}R$ subtype.

1-methylpropyl derivatives proved to have affinity slightly higher than that of the corresponding compounds bearing linear chains (**24**: K_i AA_1R = 0.83 μM and K_i $AA_{2A}R$ = 0.074 μM vs **23**: K_i AA_1R = 1.1 μM and K_i $AA_{2A}R$ = 0.30 μM; **25**: K_i AA_1R = 3.1 μM and K_i $AA_{2A}R$ = 0.39 μM vs **27**: K_i AA_1R = 3.2 μM and K_i $AA_{2A}R$ = 0.73 μM, respectively). However, in the four-carbon chains the 9-*n*-butyl derivative **27** showed higher affinity when compared to 9-isobutyl and to 9-(1-buten-4-yl) analogues (**27**: K_i AA_1R = 3.2 μM and K_i $AA_{2A}R$ = 0.73 μM vs **26**: K_i AA_1R = 4.9 μM and K_i $AA_{2A}R$ = 6.0 μM and **45**: K_i AA_1R = 4.9 μM and K_i $AA_{2A}R$ = 1.6 μM, respectively). In the case in which 9-cycloalkyl groups are present in the 8-bromoadenine analogues, the optimal size for affinity at AA_1R AR subtype seems to be a five-ring substituent (**29**: K_i AA_1R = 1.0 μM vs **28**: K_i AA_1R = 2.2 μM, **30**: K_i AA_1R = 4.6 μM, and **31**: K_i AA_1R >100 μM). On the other hand, the optimal size for affinity at $AA_{2A}R$ subtype seems to be a four-ring substituent (**28**: K_i $AA_{2A}R$ = 0.66 μM vs **29**: K_i $AA_{2A}R$ = 1.9 μM, **30**: K_i $AA_{2A}R$ = 15 μM, and **31**: K_i $AA_{2A}R$ >100 μM).

It is worth to note that comparison with 8-bromo-9-cyclopropyladenine is lacking since attempts to obtain this compound from the corresponding 9-cyclopropyladenine (**10**) failed, leading to the allylic derivative **44**, which showed $AA_{2A}R$ affinity similar to that of the corresponding 9-butyl analogue **27** and slightly higher than that of the higher homologue **45** (**44**: K_i $AA_{2A}R$ = 0.73 μM vs **27**: K_i $AA_{2A}R$ = 0.73 μM, and **45**: K_i $AA_{2A}R$ = 1.6 μM). Considering the hydroxyalkyl chains, it is possible to underline that the best result, both for AA_1R and $AA_{2A}R$ subtypes, has been obtained with the 9-(1-hydroxypropyl) substituent, suggesting that the hydroxy group should be spaced from the N-9 nitrogen atom (**33**: K_i AA_1R = 1.0 μM and K_i $AA_{2A}R$ = 0.085 μM vs **32**: K_i AA_1R = 1.5 μM and K_i $AA_{2A}R$ = 0.62 μM and **34**: K_i AA_1R = 2.9 μM and K_i $AA_{2A}R$ = 1.1 μM, respectively). When two hydroxy groups are present in the molecule, as in compound **35**, the drop in AA_1R and $AA_{2A}R$ affinity is even more marked (**35**: K_i AA_1R = 16 μM and K_i $AA_{2A}R$ = 4.0 μM vs **33**: K_i AA_1R = 1.0 μM and K_i $AA_{2A}R$ = 0.085 μM and **34**: K_i AA_1R = 2.9 μM and K_i $AA_{2A}R$ = 1.1 μM, respectively). The presence of a benzyl or a phenylethyl group in 9-position of 8-bromoadenine is detrimental for all subtypes.

At the $AA_{2B}R$ the reference compounds DPCPX and 8-bromo-9-ethyladenine (**22**) showed functional activity in the high nanomolar/micromolar range (DPCPX; K_i $AA_{2B}R$ = 1 μM, **22**; K_i $AA_{2B}R$ = 0.84 μM). In the case of linear and branched N-9 alkyl substituents, the optimal length for affinity at $AA_{2B}R$ subtype seems to be a three-carbon chain. In fact, 8-bromo-9-propyladenine (**23**) proved to be the most potent $AA_{2B}R$ antagonist in the series with K_i $AA_{2B}R$ = 0.20 μM. This trend is confirmed in the hydroxyalkyl derivatives since the 9-(2-hydroxy)propyl analogue **34** showed a sub-micromolar $AA_{2B}R$ activity and a moderate $AA_{2B}R$ versus $AA_{2A}R$ selectivity. In the cycloalkyl series the optimal ring-size for $AA_{2B}R$ activity resulted a five-carbon ring. Also in this case, compound **29** proved to be slightly $AA_{2B}R$ versus $AA_{2A}R$ selective. All the compounds showed from weak to no affinity at AA_3R, confirming our previous finding that for this subtype is important the size of the substituent in 8-position.[11,13]

The N-7 derivatives were also evaluated and they resulted to be in general inactive at all subtypes with the exception of the 7-propyl and 7-isopropyladenine (**4a** and **5a**) in which an affinity at $AA_{2A}R$ subtype, comparable to that of the corresponding 9-isomers, was detected (**4a**: K_i $AA_{2A}R$ = 18 μM vs **4**: K_i $AA_{2A}R$ = 9.6 μM, **5a**: K_i $AA_{2A}R$ = 12 μM, and **5**: K_i $AA_{2A}R$ = 4.1 μM).

2.4. Molecular modeling

A molecular docking analysis of the adenine derivatives was performed using the recently solved human $AA_{2A}R$ crystal structure[48] as target, with the aim at getting a possible rationalization of the different binding affinities of the molecules for this receptor. The human $AA_{2A}R$ crystal structure was solved in complex with the antagonist ZM241385, and the cavity occupied by the ligand was selected as binding site for the docking study. The presence of a cavity suitable for docking studies is one of the most remarkable differences respect to the previously used templates, in particular the bovine rhodopsin crystal structures. Another important feature is the different fold of the extracellular loop 2 (EL2) region, which presents a short α-helix segment in the case of human $AA_{2A}R$ and two β-strands in the case of bovine rhodopsin. Furthermore, human $AA_{2A}R$ crystal structure presents four disulfide bridges located in the extracellular loops, instead of the unique analogue feature present in bovine rhodopsin crystal structure (see Fig. 1).

To prepare the $AA_{2A}R$ structure for docking stage, the receptor was added of hydrogen atoms and then subjected to energy minimization. Finally, ZM241385 was removed and the receptor was used as target for the docking analysis of adenine derivatives, and the obtained docking conformations were subjected to energy minimization and rescoring. Due to the presence in the template crystal structure of some water molecules playing a relevant role in human $AA_{2A}R$–ZM241385 interaction, it was chosen to conserve these water molecules in the binding site for post-docking energy minimization stage, to verify possible roles of solvent molecules in stabilizing adenines binding and to compare these roles to the one played for human $AA_{2A}R$–ZM241385 interaction. Top poses for each ligand were then selected and subjected to rescoring within MOE, by using *London dG* and *Affinity dG* scoring functions and the *dock-pK_i* predictor. The last tool uses the MOE scoring.svl script to estimate for each ligand a pK_i value, which is described by the H-bonds, transition metal interactions, and hydrophobic interactions energy. The *dock-pK_i* predictor tool showed a fairly good ability in predicting compounds pK_i, as showed in Figure 2.

The binding cavity (Fig. 3) is inserted between TM2 (A63, I66) TM3 (A81, V84, L85), EL2 (F168, E169), TM5 (M174, M177) TM6 (W246, L249, N253) and TM7 (I274, S277, H278) domains. Docking simulation results present the adenine derivatives sharing a similar binding motif inside the transmembrane (TM) region of the human $AA_{2A}R$ with the adenine pyrimidine ring located between TM3 and

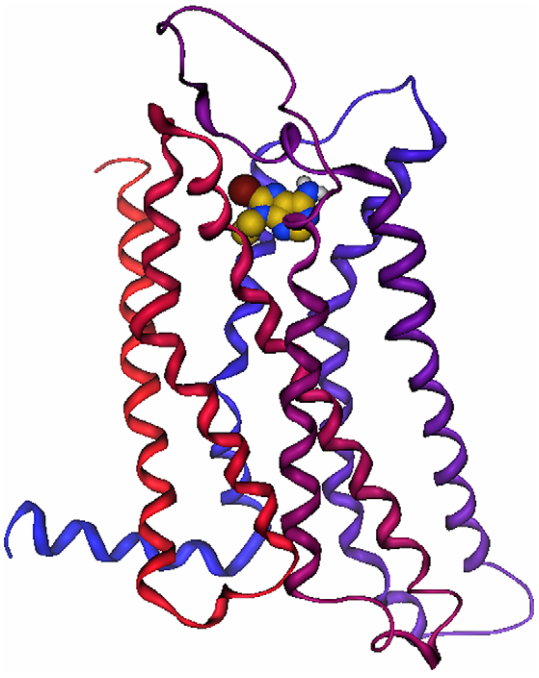


Figure 1. Human $AA_{2A}R$ structure and location of binding site (compound **22** is showed).

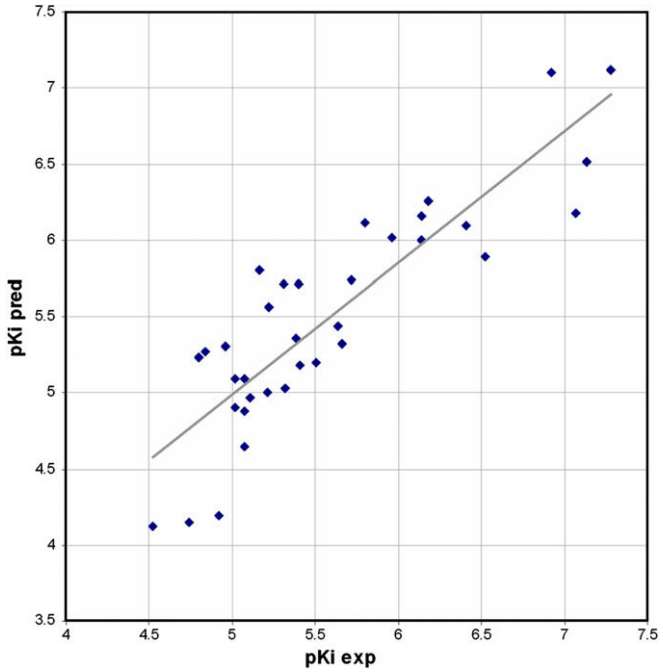


Figure 2. Correlation between predicted and experimental pK_i values (R = 0.8728; $R2$ = 0.7618).

TM6 domains, and the imidazole ring located between TM3 and TM7 helices (Fig. 4A).

This scaffold orientation makes the substituents in 8-position to be pointed towards the top of the receptor, while the region N1–C2–N3 of adenine scaffold is oriented towards the central transmembrane core. The binding region is mainly hydrophobic, even if the presence of even polar residues in the TM3–EL2–TM6 regions provides additional hydrophilic properties.

Interestingly, superimposition of compound **22** (the compound with the highest $AA_{2A}R$ affinity) docking conformation and ZM241385 original position and orientation in human $AA_{2A}R$ crystal structure shows that the two different scaffolds are oriented in opposite directions but the interaction scheme is conserved (Fig. 4B). In particular, N-1 and amino group in 6-position of adenines are oriented towards the amino and the carbonyl groups of N253 amide moiety, respectively, obtaining a stable H-bond interaction with this residue; the same roles are played respectively by N-1 and amino group in 7-position of ZM241385. Further key receptor residues for the interaction with adenine derivatives are F168, whose phenyl ring forms a π-stacking interaction with purine scaffold, and E169 whose carboxy group is involved in H-bonding with adenine N^6 amine (N^7 amine in case of ZM241385). The 8-bromine atom is located in a pocket between TM2 (A63 and I66) and TM7 (I274). This region is slightly polar, with the presence of aromatic residues which could favour an interaction with this atom. These data could explain the role of this substituent in increasing the $AA_{2A}R$ affinity respect to the 8-unsubstituted analogues, as reported in Table 4.

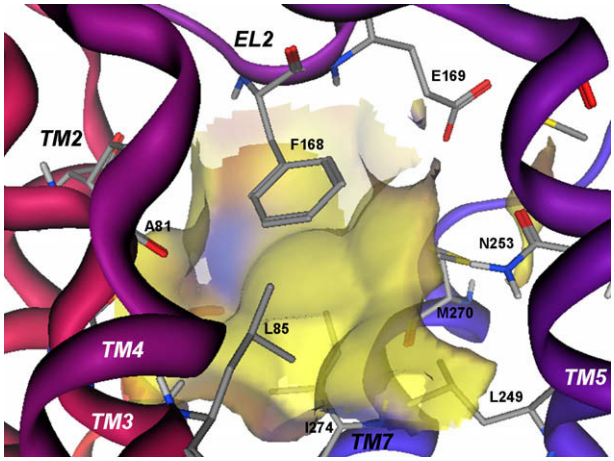


Figure 3. Human $AA_{2A}R$ structure binding site. The cavity surface is represented with 'MOE surface' tool. Most relevant residues for adenine derivatives interaction are indicated.

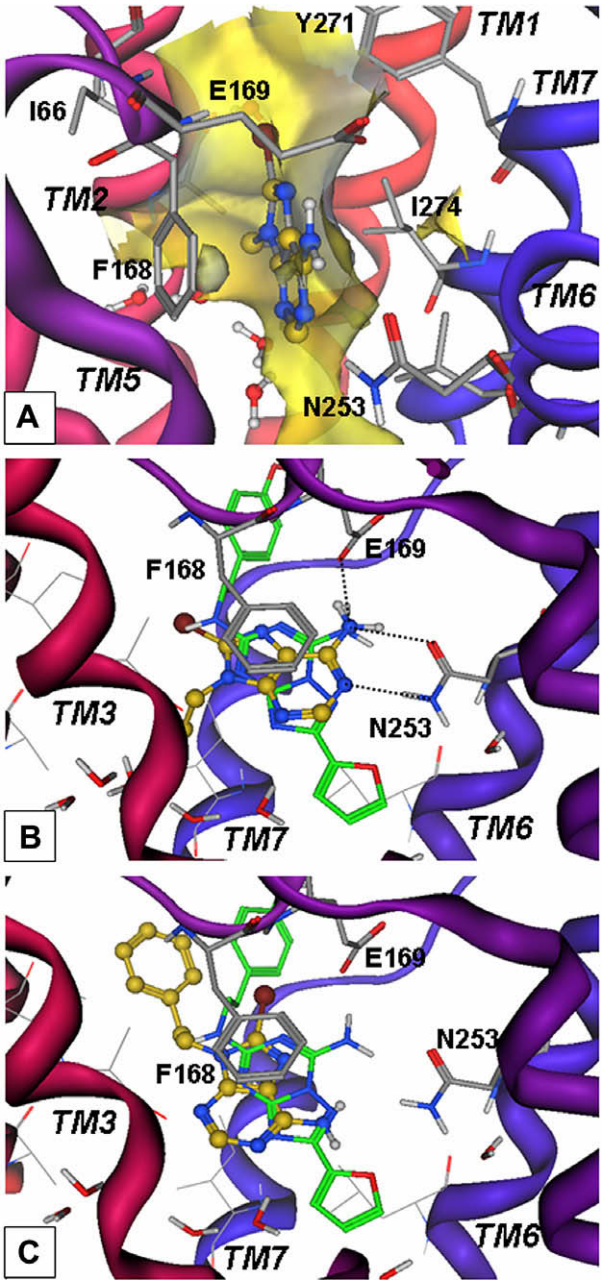


Figure 4. (A) Top-view of compound **22** docking conformation; binding cavity volume is indicated by surface representation; TM2 and TM7 residues involved in definition of N-9 ethyl and C-8 substituent subpocket are indicated. (B) Superimposition of compound **22** and ZM241385 original position and orientation in human $AA_{2A}R$ crystal structure; H-bond interactions between compound **22** and binding site residues are indicated. (C) Superimposition of compound **37** and ZM241385 original position and orientation in human $AA_{2A}R$ crystal structure.

9-Substituents are located in a sub-cavity located between V84, S277 and H278 and presenting hydrophobic-mild polar properties. Just small 9-substituents seem allowed to be located in this subcavity, in fact ethyl or isopropyl groups (like in the case of compounds **22** and **24**, respectively) provide good $AA_{2A}R$ affinity to adenine derivatives. Small 9-substituents with polar groups are allowed, but when more than one hydroxy function is inserted the $AA_{2A}R$ affinity decreases, and this is probably due at the same time to the high hydrophilicity of the substituent and to its size.

When 9-position is substituted with large substituents like benzyl or phenylethyl groups (as in the case of compounds **36** and **37**, respectively), the ligands are not allowed to well fit the binding pocket, and there is a partial re-orientation of adenine scaffold, which turns about 60° with respect to the compounds with small substituents in 9-position (Fig. 4C). This fact leads to a partial lose of the hydrophilic interaction with N253 residue. This could be one of the reasons related to the decrease of affinity of the compounds when small substituents in this position are replaced by bulky groups. Furthermore, the 9-substituent is oriented between TM2–TM7 domains, partially mimicking the orientation of phenylethylamino substituent of ZM241385 antagonist.

It must be noted that 9-substituents position was originally occupied by water molecules in the human $AA_{2A}R$ crystal structure. During docking conformations energy refinements, these water molecules were displaced by their original position and they found a new location in proximity of adenine scaffold N3.

3. Conclusion

From the results reported in Table 4, it is possible to conclude that our goal to obtain purine derivatives with higher $AA_{2A}R$ potency and selectivity compared to 8-bromo-9-ethyladenine (**22**) was only partially achieved. In fact, none of the substituents introduced at the 9-position of adenine and 8-bromoadenine brought about an increase in both AA_1R and $AA_{2A}R$ subtype affinity. On the other hand, $AA_{2A}R$ versus AA_1R selectivity was slightly improved when an isopropyl or a 1-hydroxy-3-propyl group was introduced at the 9-position (see compounds **24** and **33**, respectively). Furthermore, it has been confirmed that the presence of a bromine atom in 8-position favours the interaction with all the four ARs. The present study also demonstrated that adenine derivatives could be a good starting point to obtain selective $AA_{2B}R$ antagonists. In fact, from the results reported in Table 4 it is possible to point out that two compounds bearing a three-carbon chain, namely 8-bromo-9-propyladenine (**23**) and 8-bromo-9-(2-hydroxy)propyl-adenine (**34**), proved to be good $AA_{2B}R$ antagonists with K_i $AA_{2B}R$ = 0.2 μM and 0.64 μM, respectively. They are also endowed with a moderate $AA_{2B}R$ versus $AA_{2A}R$ selectivity. The same trend is showed by the 9-cyclopentyl derivative **29** which, although its $AA_{2B}R$ activity was similar to that of the parent compound 8-bromo-9-ethyladenine (**22**), proved to be slightly $AA_{2B}R$ versus $AA_{2A}R$ selective.

4. Experimental

4.1. Chemistry

Melting points were determined with a Büchi apparatus and are uncorrected. ^1H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; d in ppm, *J* in hertz. All exchangeable protons were confirmed by addition of D_2O. TLCs were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, Silica Gel 60 (Merck) was used. Elemental analyses were determined on Carlo Erba model 1106 analyser and are within ±0.4% of theoretical values.

4.1.1. General procedure for the synthesis of 9-alkyladenines (2–20) and 7-alkyladenines (2a–7a, 15a–20a) and for the synthesis of 9-alkyl-8-bromoadenines (44, 45)

To 1.0 mmol of adenine (**1**) or 8-bromoadenine (**43**), dissolved in 4 mL of dry DMF, dry K_2CO_3 (200 mg) and the suitable alkyl halide (1.2 mmol) were added. The mixture was stirred under a nitrogen atmosphere at the temperature and for the time listed in Table 1. The solvent was evaporated and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give compounds **2–20** and **44**, **45**.

4.1.1.1. 9-Methyl-9*H*-adenine (2). Mp: > 250 °C[49]; ^1H NMR (DMSO-d_6) δ 3.72 (s, 3H, CH_3), 7.20 (br s, 2H, NH_2), 8.06 (s, 1H, H-8), 8.12 (s, 1H, H-2). Anal. Calcd for $C_6H_7N_5$: C, 48.32; H, 4.73; N, 46.95. Found: C, 48.49; H, 4.78; N, 46.66.

4.1.1.2. 7-Methyl-7*H*-adenine (2a). Mp: > 250 °C; ^1H NMR (DMSO-d_6) δ 3.91 (s, 3H, CH_3), 8.15 (br s, 2H, NH_2), 8.48 (s, 1H, H-2), 8.50 (s, 1H, H-8). Anal. Calcd for $C_6H_7N_5$: C, 48.32; H, 4.73; N, 46.95. Found: C, 48.53; H, 4.92; N, 46.77.

4.1.1.3. 7-Ethyl-7*H*-adenine (3a). Mp: 237–240 °C; ^1H NMR (DMSO-d_6) δ 1.46 (t, 3H, *J* = 7.2 Hz, CH_3), 4.35 (q, 2H, *J* = 7.2 Hz, CH_2), 7.77 (s, 1H, H-2), 7.88 (br s, 2H, NH_2), 8.37 (s, 1H, H-8). Anal. Calcd for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found: C, 51.75; H, 5.60; N, 42.59.

4.1.1.4. 9-Propyl-9*H*-adenine (4). Mp: 173–175 °C; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H, *J* = 7.4 Hz, CH_3), 1.82 (m, 2H, CH_2CH_3), 4.11 (t, 2H, *J* = 7.0 Hz, CH_2 N), 7.21 (br s, 2H, NH_2), 8.15 (s, 2H, H-2 and H-8). Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.61; H, 6.45; N, 39.31.

4.1.1.5. 7-Propyl-7*H*-adenine (4a). Mp: > 250 °C; ^1H NMR (DMSO-d_6) δ 0.86 (t, 3H, *J* = 7.3 Hz, CH_3), 1.91 (m, 2H, CH_2CH_3), 4.26 (t, 2H, *J* = 7.0 Hz, CH_2 N), 7.75 (s, 1H, H-2), 7.85 (br s, 2H, NH_2), 8.34 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.58; H, 6.34; N, 39.41.

4.1.1.6. 9-Isopropyl-9*H*-adenine (5). Mp: 114–116 °C[31]; ^1H NMR (DMSO-d_6) δ 1.53 (d, 6H, *J* = 6.7 Hz, $CH(CH_3)_2$), 4.73 (m, 1H, CH), 7.19 (br s, 2H, NH_2), 8.14 (s, 1H, H-2), 8.24 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.60; H, 6.55; N, 39.37.

4.1.1.7. 7-Isopropyl-7*H*-adenine (5a). Mp: > 250 °C; ^1H NMR (DMSO-d_6) δ 1.62 (d, 6H, *J* = 6.9 Hz, $CH(CH_3)_2$), 4.96 (m, 1H, CH), 7.77 (s, 1H, H-2), 7.86 (br s, 2H, NH_2), 8.43 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.46; H, 6.58; N, 39.23.

4.1.1.8. 9-(*sec*-Butyl)-9*H*-adenine (6). Mp: 127–129 °C; ^1H NMR (DMSO-d_6) δ 0.74 (t, 3H, *J* = 7.4 Hz, CH_3), 1.52 (d, 3H, *J* = 6.9 Hz, $CHCH_3$), 1.91 (m, 2H, CH_2), 4.50 (m, 1H, CH), 7.20 (br s, 2H, NH_2), 8.13 (s, 1H, H-2), 8.22 (s, 1H, H-8). Anal. Calcd for $C_9H_{13}N_5$: C, 56.53; H, 6.85; N, 36.62. Found: C, 56.70; H, 7.01; N, 36.48.

4.1.1.9. 7-(*sec*-Butyl)-7*H*-adenine (6a). Mp: 177–179 °C; ^1H NMR (DMSO-d_6) δ 0.74 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 1.62 (d, 3H, *J* = 6.8 Hz, $CHCH_3$), 1.93 (m, 1H, *H*–CH), 2.19 (m, 1H, *H*–CH), 4.68 (m, 1H, CH), 7.76 (s, 1H, H-2), 7.85 (br s, 2H, NH_2), 8.40 (s, 1H, H-8). Anal. Calcd for $C_9H_{13}N_5$: C, 56.53; H, 6.85; N, 36.62. Found: C, 56.65; H, 7.22; N, 36.60.

4.1.1.10. 9-Isobutyl-9*H*-adenine (7). Mp: 165–168 °C; ^1H NMR (DMSO-d_6) δ 0.86 (d, 6H, *J* = 6.7 Hz, $CH(CH_3)_2$), 2.20 (m, 1H, CH),

3.97 (d, 2H, J = 7.3 Hz, CH_2), 7.22 (br s, 2H, NH_2), 8.14 (s, 2H, H-2 and H-8). Anal. Calcd for $C_9H_{13}N_5$: C, 56.53; H, 6.85; N, 36.62. Found: C, 56.83; H, 6.99; N, 36.49.

4.1.1.11. 7-Isobutyl-7*H*-adenine (7a). Mp: 227–230 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 2.39 (m, 1H, CH), 4.12 (d, 2H, J = 7.3 Hz, CH_2), 7.76 (s, 1H, H-2), 7.94 (br s, 2H, NH_2), 8.33 (s, 1H, H-8). Anal. Calcd for $C_9H_{13}N_5$: C, 56.53; H, 6.85; N, 36.62. Found: C, 56.75; H, 7.15; N, 36.45.

4.1.1.12. 9-Butyl-9*H*-adenine (8). Mp: 131–133 °C (lit.[25] mp: 138–139 °C); ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 7.2 Hz, CH_3), 1.22 (m, 2H, CH_2CH_3), 1.76 (m, 2H, $CH_2CH_2CH_2$), 4.11 (t, 2H, J = 7.0 Hz, CH_2 N), 7.15 (br s, 2H, NH_2), 8.11 (s, 1H, H-2 and H-8). Anal. Calcd for $C_9H_{13}N_5$: C, 56.53; H, 6.85; N, 36.62. Found: C, 56.62; H, 6.92; N, 36.55.

4.1.1.13. 9-But-3-enyl-9*H*-adenine (9). Mp: 167–169 °C (lit.[40] mp: 169–171 °C); ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 2H, CH_2CH), 4.22 (t, 2H, J = 7.0 Hz, CH_2N), 5.50 (m, 2H, CH=CH_2), 5.80 (m, 1H, CH=CH_2), 7.20 (br s, 2H, NH_2), 8.13 (s, 1H, H-8), 8.15 (s, 1H, H-2). Anal. Calcd for $C_9H_{11}N_5$: C, 57.13; H, 5.86; N, 37.01. Found: C, 57.50; H, 6.01; N, 36.75.

4.1.1.14. 9-Cyclopropyl-9*H*-adenine (10). Mp: 232–234 °C; ^{1}H NMR (DMSO-d_6) δ 1.06 (m, 4H, H cyclopropyl), 3.45 (m, 1H, H-1 cyclopropyl), 7.36 (br s, 2H, NH_2), 8.15 (s, 1H, H-8), 8.18 (s, 1H, H-2). Anal. Calcd for $C_8H_9N_5$: C, 54.85; H, 5.18; N, 39.98. Found: C, 54.93; H, 5.36; N, 39.73.

4.1.1.15. 9-Cyclobutyl-9*H*-adenine (11). Mp: 163–165 °C (lit.[41] mp: 175–178 °C); ^{1}H NMR (DMSO-d_6) δ 1.86 (m, 2H, H cyclobutyl), 2.43 (m, 2H, H cyclobutyl), 2.67 (m, 2H, H cyclobutyl), 4.97 (m, 1H, H-1 cyclobutyl), 7.21 (br s, 2H, NH_2), 8.13 (s, 1H, H-2), 8.32 (s, 1H, H-8). Anal. Calcd for $C_9H_{11}N_5$: C, 57.13; H, 5.86; N, 37.01. Found: C, 57.42; H, 6.15; N, 36.81.

4.1.1.16. 9-Cyclopentyl-9*H*-adenine (12). Mp: 153–155 °C[25]; ^{1}H NMR (DMSO-d_6) δ 1.68 (m, 2H, H cyclopentyl), 1.88 (m, 2H, H cyclopentyl), 2.00 (m, 2H, H cyclopentyl), 2.13 (m, 2H, H cyclopentyl), 4.82 (m, 1H, H-1 cyclopentyl), 7.19 (br s, 2H, NH_2), 8.12 (s, 1H, H-2), 8.20 (s, 1H, H-8). Anal. Calcd for $C_{10}H_{13}N_5$: C, 59.10; H, 6.45; N, 34.46. Found: C, 59.33; H, 6.55; N, 34.31.

4.1.1.17. 9-Cyclohexyl-9*H*-adenine (13). Mp: 199–201 °C[25]; ^{1}H NMR (DMSO-d_6) δ 1.27 (m, 1H, H cyclohexyl), 1.44 (m, 2H, H cyclohexyl), 1.71 (m, 1H, H cyclohexyl), 1.90 (m, 6H, H cyclohexyl), 4.34 (m, 1H, H-1 cyclohexyl), 7.18 (br s, 2H, NH_2), 8.12 (s, 1H, H-2), 8.22 (s, 1H, H-8). Anal. Calcd for $C_{11}H_{15}N_5$: C, 60.81; H, 6.96; N, 32.23. Found: C, 60.95; H, 7.20; N, 32.01.

4.1.1.18. 9-Cycloheptyl-9*H*-adenine (14). Mp: 182–183 °C (lit.[31] mp: 173–174 °C); ^{1}H NMR (DMSO-d_6) δ 1.53 (m, 6H, H cycloheptyl), 1.73 (m, 2H, H cycloheptyl), 2.00 (m, 4H, H cycloheptyl), 4.50 (m, 1H, H-1 cycloheptyl), 7.14 (br s, 2H, NH_2), 8.10 (s, 1H, H-2), 8.20 (s, 1H, H-8). Anal. Calcd for $C_{12}H_{17}N_5$: C, 62.31; H, 7.41; N, 30.28. Found: C, 62.55; H, 7.62; N, 30.14.

4.1.1.19. 9-(2-Hydroxyethyl)-9*H*-adenine (15). Mp: 237–239 °C[32]; ^{1}H NMR (DMSO-d_6) δ 3.75 (m, 2H, CH_2OH), 4.19 (t, 2H, J = 5.5 Hz, CH_2 N), 5.02 (t, 1H, J = 5.4 Hz, OH), 7.19 (br s, 2H, NH_2), 8.03 (s, 1H, H-8), 8.14 (s, 1H, H-2). Anal. Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.09. Found: C, 47.23; H, 5.41; N, 38.82.

4.1.1.20. 7-(2-Hydroxyethyl)-7*H*-adenine (15a). Mp: > 250 °C (lit.[22] mp: 132–133 °C); ^{1}H NMR (DMSO-d_6) δ 3.82 (m, 2H, CH_2OH), 4.35 (t, 2H, J = 5.1 Hz, CH_2 N), 5.10 (t, 1H, J = 5.5 Hz, OH), 7.76 (s, 1H, H-2), 7.86 (br s, 2H, NH_2), 8.23 (s, 1H, H-8). Anal. Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.09. Found: C, 47.16; H, 5.36; N, 38.96.

4.1.1.21. 9-(3-Hydroxypropyl)-9*H*-adenine (16). Mp: 218–220 °C (lit.[38] mp: 209–210 °C); ^{1}H NMR (DMSO-d_6) δ 1.91 (m, 2H, CH_2CH_2OH), 3.35 (m, 2H, CH_2OH), 4.17 (t, 2H, J = 7.0 Hz, CH_2 N), 4.64 (t, 1H, J = 5.2 Hz, OH), 7.18 (br s, 2H, NH_2), 8.09 (s, 1H, H-8), 8.10 (s, 1H, H-2). Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.85; H, 5.92; N, 36.11.

4.1.1.22. 7-(3-Hydroxypropyl)-7*H*-adenine (16a). Mp: 246–249 °C (lit.[35] mp: 201–203 °C); ^{1}H NMR (DMSO-d_6) δ 2.02 (m, 2H, CH_2CH_2OH), 3.39 (m, 2H, CH_2OH), 4.37 (t, 2H, J = 6.9 Hz, CH_2 N), 4.85 (t, 1H, J = 5.2 Hz, OH), 7.76 (s, 1H, H-2), 7.87 (br s, 2H, NH_2), 8.31 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.99; H, 5.81; N, 36.10.

4.1.1.23. 9-(2-Hydroxypropyl)-9*H*-adenine (17). Mp: 192–193 °C[32]; ^{1}H NMR (DMSO-d_6) δ 1.05 (d, 3H, J = 5.8 Hz, CH_3), 4.06 (m, 3H, CHOH and CH_2N), 5.04 (d, 1H, J = 3.07 Hz, OH), 7.21 (br s, 2H, NH_2), 8.05 (s, 1H, H-8), 8.13 (s, 1H, H-2). Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 50.01; H, 5.89; N, 36.01.

4.1.1.24. 7-(2-Hydroxypropyl)-7*H*-adenine (17a). Mp: 233 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.13 (d, 3H, J = 6.3 Hz CH_3), 4.05 (m, 1H, *H*–CHN), 4.12 (m, 1H, CHOH), 4.33 (m, 1H, *H*–CHN), 5.15 (d, 1H, J = 5.1 Hz, OH), 7.74 (s, 1H, H-2), 7.85 (br s, 2H, NH_2), 8.20 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.88; H, 5.92; N, 36.05.

4.1.1.25. 9-(2,3-Dihydroxypropyl)-9*H*-adenine (18). Mp: 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 3.31 (m, 2H, CH_2OH), 3.80 (m, 1H CHOH), 3.97 (m, 1H, *H*–CHN), 4.27 (m, 1H, *H*–CHN), 4.83 (t, 1H, J = 6.0 Hz, CH_2–OH), 5.08 (d, 1H, J = 5.1 Hz, CHOH), 7.17 (br s, 2H, NH_2), 8.01 (s, 1H, H-8), 8.10 (s, 1H, H-2). Anal. Calcd for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 46.15; H, 5.72; N, 33.27.

4.1.1.26. 7-(2,3-Dihydroxypropyl)-7*H*-adenine (18a). Vitreous solid; ^{1}H NMR (DMSO-d_6) δ 3.35 (m, 2H, CH_2OH), 3.95 (m, 1H CHOH), 4.12 (m, 1H, *H*–CHN), 4.50 (m, 1H, *H*–CHN), 5.14 (t, 1H, J = 6.0 Hz, CH_2OH), 5.24 (d, 1H, J = 5.4 Hz, CHOH), 7.75 (s, 1H, H-2), 7.87 (br s, 2H, NH_2), 8.19 (s, 1H, H-8). Anal. Calcd for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 46.12; H, 5.62; N, 33.19.

4.1.1.27. 9-Benzyl-9*H*-adenine (19). Mp: 205–207 °C (lit.[50] mp: 233–236 °C); ^{1}H NMR (DMSO-d_6) δ 5.37 (s, 2H, CH_2), 7.25 (br s, 2H, NH_2); 7.33 (m, 5H, H–Ph), 8.15 (s, 1H, H-2), 8.26 (s, 1H, H-8). Anal. Calcd for $C_{12}H_{11}N_5$: C, 63.99; H, 4.92; N, 31.09. Found: C, 64.13; H, 5.23; N, 31.00.

4.1.1.28. 7-Benzyl-7*H*-adenine (19a). Mp: > 250 °C; ^{1}H NMR (DMSO-d_6) δ 5.51 (s, 2H, CH_2), 7.33 (m, 3H, H–Ph), 7.46 (d, 2H, J = 7.8 Hz, H–Ph), 7.75 (s, 1H, H-2), 7.92 (br s, 2H, NH_2), 8.55 (s, 1H, H-8 Anal. Calcd for $C_{12}H_{11}N_5$: C, 63.99; H, 4.92; N, 31.09. Found: C, 64.36; H, 5.22; N, 30.82.

4.1.1.29. 9-Phenylethyl-9*H*-adenine (20). Mp: 183–185 °C (lit.[51] mp: 179–180 °C); ^{1}H NMR (DMSO-d_6) δ 3.11 (t, 2H, J = 7.0 Hz, CH_2Ph), 4.36 (t, 2H, J = 7.2 Hz, CH_2N), 7.18 (m, 7H, H–Ph and NH_2), 7.90 (s, 1H, H-8), 8.12 (s, 1H, H-2). Anal. Calcd for $C_{13}H_{13}N_5$: C, 65.25; H, 5.48; N, 29.27. Found: C, 65.55; H, 5.71; N, 29.16.

4.1.1.30. 7-Phenylethyl-7*H*-adenine (20a). Mp: 225–229 °C; ^{1}H NMR (DMSO-d_6) δ 3.24 (t, 2H, J = 7.1 Hz, CH_2Ph), 4.54 (t, 2H, J = 7.2 Hz, CH_2 N), 7.21 (m, 5H, H–Ph), 7.81 (s, 1H, H-2), 7.87 (br s, 2H, NH_2); 8.12 (s, 1H, H-8). Anal. Calcd for $C_{13}H_{13}N_5$: C, 65.25; H, 5.48; N, 29.27. Found: C, 65.48; H, 5.68; N, 29.02.

4.1.1.31. 9-Allyl-8-bromo-9*H*-adenine (44). Vitreous solid; ^{1}H NMR (DMSO-d_6) δ 4.75 (d, 2H, J = 4.4 Hz, CH_2 N), 4.97–5.26 (m, 2H, CH=CH_2), 6.19 (m, 1H, CH=CH_2), 7.63 (br s, 2H, NH_2), 8.18 (s, 1H, H-2). Anal. Calcd for $C_8H_8BrN_5$: C, 37.82; H, 3.17; N, 27.56. Found: C, 37.95; H, 3.44; N, 27.35.

4.1.1.32. 8-Bromo-9-(but-3-enyl)-9*H*-adenine (45). Mp: 140 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.56 (m, 2H, CH_2CH_2N), 4.21 (t, 2H, J = 7.0 Hz, CH_2N), 4.97 (m, 2H, CH=CH_2), 5.80 (m, 1H, CH=CH_2), 7.37 (br s, 2H, NH_2), 8.14 (s, 1H, H-2). Anal. Calcd for $C_9H_{10}Br\ N_5$: C, 40.32; H, 3.76; N, 26.12. Found: C, 40.62; H, 3.87; N, 25.98.

4.1.2. General procedure for the synthesis of 9-alkyl-8-bromoadenines (21–37)

To 1.0 mmol of the appropriate 9-alkyladenine (**2–20**) dissolved in 4 mL of dry DMF, *N*-bromosuccinimide (2.0 mmol) was added. The mixture was stirred under a nitrogen atmosphere at room temperature for the time listed in Table 2. In the case of compound **2**, the bromination was carried out in the same quantity of CH_3CN. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents listed in Table 2 to give compounds **21–37**.

4.1.2.1. 8-Bromo-9-methyl-9*H*-adenine (21). Mp: > 250 °C[43]; ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H, CH_3), 7.38 (br s, 2H, NH_2), 8.15 (s, 1H, H-2). Anal. Calcd for $C_6H_6BrN_5$: C, 31.60; H, 2.65; N, 30.71. Found: C, 31.66; H, 2.75; N, 30.50.

4.1.2.2. 8-Bromo-9-ethyl-9*H*-adenine (22). Mp: 218–220 °C[10]; ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 7.3 Hz, CH_3), 4.17 (q, 2H, J = 7.1 Hz, CH_2), 7.39 (br s, 2H, NH_2), 8.15 (s, 1H, H-2). Anal. Calcd for $C_7H_8BrN_5$: C, 34.73, H, 3.33, N, 28.93. Found: C, 35.02, H, 3.41, N, 28.67.

4.1.2.3. 8-Bromo-9-propyl-9*H*-adenine (23). Mp: 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 7.4 Hz, CH_3), 1.79 (m, 2H, CH_2CH_3), 4.10 (t, 2H, J = 7.2 Hz, CH_2 N), 7.41 (br s, 2H, NH_2), 8.15 (s, 1H, H-2). Anal. Calcd for $C_8H_{10}BrN_5$: C, 37.52; H, 3.94; N, 27.35. Found: C, 37.62; H, 4.01; N, 27.19.

4.1.2.4. 8-Bromo-9-isopropyl-9*H*-adenine (24). Mp: 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 1.62 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 4.80 (m, 1H, CH), 7.36 (br s, 2H, NH_2), 8.12 (s, 1H, H-2). Anal. Calcd for $C_8H_{10}BrN_5$: C, 37.52; H, 3.94; N, 27.35. Found: C, 37.69; H, 3.99; N, 27.14.

4.1.2.5 8-Bromo-9-(*sec*-butyl)-9*H*-adenine (25). Mp: 152–154 °C; ^{1}H NMR (DMSO-d_6) δ 0.71 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.61 (d, 3H, J = 6.8 Hz, CHCH_3), 1.90 (m, 1H, *H*–CH), 2.32 (m, 1H, *H*–CH), 4.54 (m, 1H, CH), 7.38 (br s, 2H, NH_2), 8.11 (s, 1H, H-2). Anal. Calcd for $C_9H_{12}BrN_5$: C, 40.02; H, 4.48; N, 25.93. Found: C, 40.25; H, 4.56; N, 25.80.

4.1.2.6. 8-Bromo-9-isobutyl-9*H*-adenine (26). Mp: 226–228 °C; ^{1}H NMR (DMSO-d_6) δ 0.89 (d, 6H, J = 6.7 Hz, CH(CH_3)$_2$), 2.25 (m, 1H, CH), 3.95 (d, 2H, J = 7.5 Hz, CH_2), 7.40 (br s, 2H, NH_2), 8.14 (s, 1H, H-2). Anal. Calcd for $C_9H_{12}BrN_5$: C, 40.02; H, 4.48; N, 25.93. Found: C, 40.36; H, 4.59; N, 25.87.

4.1.2.7. 8-Bromo-9-(2-butyl)-9*H*-adenine (27). Mp: 173–175 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, J = 7.2 Hz, CH_3), 1.29 (m, 2H, CH_2CH_3), 1.75 (m, 2H, $CH_2CH_2CH_3$), 4.13 (t, 2H, J = 7.1 Hz, CH_2 N), 7.41 (br s, 2H, NH_2), 8.15 (s, 1H, H-2). Anal. Calcd for $C_9H_{12}BrN_5$: C, 40.02; H, 4.48; N, 25.93. Found: C, 40.14; H, 4.53; N, 25.88.

4.1.2.8. 8-Bromo-9-cyclobutyl-9*H*-adenine (28). Mp: 207–210 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 2H, H cyclobutyl), 2.38 (m, 2H, H cyclobutyl), 3.16 (m, 2H, H cyclobutyl), 5.01 (m, 1H, H-1 cyclobutyl), 7.37 (br s, 2H, NH_2), 8.16 (s, 1H, H-2). Anal. Calcd for $C_9H_{10}BrN_5$: C, 40.32; H, 3.76; N, 26.12. Found: C, 40.85; H, 3.84; N, 26.01.

4.1.2.9. 8-Bromo-9-cyclopentyl-9*H*-adenine (29). Mp: 195–197 °C; ^{1}H NMR (DMSO-d_6) δ 1.68 (m, 2H, H cyclopentyl), 2.00 (m, 4H, H cyclopentyl), 2.32 (m, 2H, H cyclopentyl), 4.89 (m, 1H, H-1 cyclopentyl), 7.38 (br s, 2H, NH_2), 8.12 (s, 1H, H-2). Anal. Calcd for $C_{10}H_{12}BrN_5$: C, 42.57; H, 4.29; N, 24.82. Found: C, 42.68; H, 4.38; N, 24.61.

4.1.2.10. 8-Bromo-9-cyclohexyl-9*H*-adenine (30). Mp: 199–201 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (m, 3H, H cyclohexyl), 1.71 (m, 1H, H cyclohexyl), 1.85 (m, 4H, H cyclohexyl), 2.48 (m, 2H, H cyclohexyl), 4.36 (m, 1H, H-1 cyclohexyl), 7.35 (br s, 2H, NH_2), 8.11 (s, 1H, H-2). Anal. Calcd for $C_{11}H_{14}BrN_5$: C, 44.61; H, 4.76; N, 23.65. Found: C, 44.72; H, 4.85; N, 23.35.

4.1.2.11. 8-Bromo-9-cycloheptyl-9*H*-adenine (31). Vitreous solid; ^{1}H NMR (DMSO-d_6) δ 1.62 (m, 5H, H cycloheptyl), 1.85 (m, 5H, H cycloheptyl), 2.4 (m, 2H, H cycloheptyl), 4.59 (m, 1H, H-1 cycloheptyl), 8.36 (s, 1H, H-2), 8.56 (br s, 2H, NH_2). Anal. Calcd for $C_{12}H_{16}BrN_5$: C, 46.46; H, 5.20; N, 22.58. Found: C, 46.65; H, 5.35; N, 22.38.

4.1.2.12. 8-Bromo-9-(2-hydroxyethyl)-9*H*-adenine (32). Mp: 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (m, 2H, CH_2OH), 4.19 (t, 2H, J = 5.8 Hz, CH_2 N), 5.00 (t, 1H, J = 5.8 Hz, OH), 7.38 (br s, 2H, NH_2), 8.14 (s, 1H, H-2). Anal. Calcd for $C_7H_8BrN_5O$: C, 32.58; H, 3.12; N, 27.14. Found: C, 32.76; H, 3.41; N, 27.02.

4.1.2.13. 8-Bromo-9-(3-hydroxypropyl)-9*H*-adenine (33). Mp: 205–208 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 2H, CH_2CH_2OH), 3.44 (m, 2H, CH_2OH), 4.19 (t, 2H, J = 4.8 Hz, CH_2 N), 4.66 (t, 1H, J = 3.4 Hz, OH), 7.39 (br s, 2H, NH_2), 8.13 (s, 1H, H-2). Anal. Calcd for $C_8H_{10}BrN_5O$: C, 35.31; H, 3.70; N, 25.74. Found: C, 35.61; H, 3.92; N, 25.57.

4.1.2.14. 8-Bromo-9-(2-hydroxypropyl)-9*H*-adenine (34). Mp: 177–179 °C; ^{1}H NMR (DMSO-d_6) δ 1.11 (d, 3H, J = 6.0 Hz, CH_3), 4.10 (m, 3H, *CH*OH and CH_2 N), 5.03 (d, 1H, J = 4.8 Hz, OH), 7.38 (br s, 2H, NH_2), 8.13 (s, 1H, H-2). Anal. Calcd for $C_8H_{10}BrN_5O$: C, 35.31; H, 3.70; N, 25.74. Found: C, 35.56; H, 3.85; N, 25.67.

4.1.2.15. 8-Bromo-9-(2,3-dihydroxypropyl)-9*H*-adenine (35). Mp: 248–250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.40 (m, 2H, CH_2OH), 4.07 (m, 3H *CH*OH and CH_2 N), 4.86 (t, 1H, J = 5.8 Hz, CH_2OH), 5.04 (d, 1H, J = 4.9 Hz, CH–*OH*), 7.39 (br s, 2H, NH_2), 8.13 (s, 1H, H-2). Anal. Calcd for $C_8H_{10}BrN_5O_2$: C, 33.35; H, 3.50; N, 24.31. Found: C, 33.61; H, 3.61; N, 24.09.

4.1.2.16. 9-Benzyl-8-bromo-9*H*-adenine (36). Mp: 227–230 °C; ^{1}H NMR (DMSO-d_6) δ 5.37 (s, 2H, CH_2), 7.32 (m, 5H, H–Ph), 7.49 (br s, 2H, NH_2), 8.18 (s, 1H, H-2). Anal. Calcd for $C_{12}H_{10}BrN_5$: C, 47.39; H, 3.31; N, 23.03. Found: C, 47.48; H, 3.43; N, 22.98.

4.1.2.17. 8-Bromo-9-phenylethyl-9*H*-adenine (37). Mp: 182–185 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (t, 2H, J = 7.1 Hz, CH_2Ph), 4.36 (t, 2H, J = 7.1 Hz, CH_2 N), 7.11 (d, 2H, J = 7.4 Hz, H–Ph), 7.26 (m, 3H, H–Ph), 7.39 (br s, 2H, NH_2), 8.15 (s, 1H, H-2). Anal. Calcd for $C_{13}H_{12}BrN_5$: C, 49.07; H, 3.80; N, 22.01. Found: C, 49.33; H, 3.97; N, 21.86.

4.1.3. General procedure for the synthesis of 6-chloro-N^4-alkylpirimidines-4,5-diammines (39, 40)

To 10.0 mmol of 5-ammino-4,6-dichloropyrimidine (**38**) dissolved in 60 mL of EtOH, 5 mL of Et_3N and 1.1 equiv of the suitable amine were added. The mixtures were left at 150 °C for 27 h or at 120 °C for 40 h, respectively. Solvents were removed in vacuo and the residues were chromatographed on a silica gel column eluting with the suitable mixture of solvents, to give compounds **39** and **40**, respectively.

4.1.3.1. 6-Chloro-N^4-ethylpyrimidine-4,5-diamine (39). Obtained as white solid after chromatography eluting with $CHCl_3$–CH_3OH 99.5:0.5 in 88% yield. Mp: 142–144 °C (lit.[24] mp: 196–198 °C); ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, J = 7.2 Hz, CH_3), 3.39 (m, 2H, CH_2), 5.01 (br s, 2H, NH_2), 6.79 (t, 1H, J = 5.0, NH), 7.72 (s, 1H, H-2). Anal. Calcd for $C_6H_9ClN_4$; C, 41.75; H, 5.26; N, 32.46. Found: C, 41.89; H, 5.33; N, 32.19.

4.1.3.2. 6-Chloro-N^4-cyclohexylpyrimidine-4,5-diamine (40). Obtained as white solid after chromatography eluting with cC_6H_{12}–AcOEt 95:15 to 85:15 in 89% yield. Mp: 132–134 °C (lit.[25] mp: 114 °C); ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 5H, H cyclohexyl), 1.62 (m, 1H, H cyclohexyl), 1.75 (m, 2H, H cyclohexyl), 1.92 (m, 2H, H cyclohexyl), 3.90 (m, 1H, H1 cyclohexyl), 5.08 (br s, 2H, NH_2), 6.55 (d, 1H, J = 7.3, NH), 7.72 (s, 1H, H-2). Anal. Calcd for $C_{10}H_{15}ClN_4$; C, 52.98; H, 6.67; N, 24.71. Found: C, 53.34; H, 6.73; N, 24.62.

4.1.4. General procedure for the synthesis of 6-chloro-9-alkylpurines (41, 42)

Compounds **39** and **40** (3.0 mmol) have been added of diethoxymethyl acetate (8 mL) and left under stirring at room temperature 48 h. The reagent was removed under vacuo and the residues were chromatographed on a silica gel column eluting with $CHCl_3$–CH_3OH 98:2 and 99.5:0.5, respectively.

4.1.4.1. 6-Chloro-9-ethyl-9*H*-purine (41). Compound **41** has been obtained in 87% yield. Mp: 77–79 °C (lit.[24] mp: 81–84 °C); ^{1}H NMR (DMSO-d_6) δ 1.46 (t, 3H, J = 7.2 Hz, CH_3), 4.32 (m, 2H, CH_2N), 8.74 (s, 1H, H-8), 8.79 (s, 1H, H-2). Anal. Calcd for $C_7H_7ClN_4$: C, 46.04; H, 3.86; N, 30.68. Found: C, 46.30; H, 3.94; N, 30.47.

4.1.4.2. 6-Chloro-9-cyclohexyl-9*H*-purine (42). Compound **42** has been obtained in 70% yield. Crystallized by petroleum ether. Mp: 86–89 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (m, 1H, H cyclohexyl), 1.40 (m, 2H, H cyclohexyl), 1.72 (m, 1H, H cyclohexyl), 1.95 (m, 6H, H cyclohexyl), 4.36 (m, 1H, H-1 cyclohexyl), 4.56 (m, 2H, H-1 cyclohexyl), 8.78 (s, 1H, H-2), 8.82 (s, 1H, H-8). Anal. Calcd for $C_{11}H_{13}ClN_4$: C, 55.82; H, 5.54; N, 23.67. Found: C, 55.97; H, 5.75; N, 23.32.

4.1.5. General procedure for the synthesis of 9-alkyladenines (3, 13)

A solution of compound **41** or **42** (1.5 mmol) in liquid ammonia (2 mL) was sealed in a stainless steel tube and set aside at room temperature for 24 h. The solvent was removed in vacuo and the residue was purified by flash chromatography on a silica gel column, eluting with the suitable solvent, to give compound **3** or **13**, respectively.

4.1.5.1. 9-Ethyl-9*H*-adenine (3). Obtained as white solid after chromatography on a silica gel column eluting with $CHCl_3$–CH_3OH 97:3, in 77% yield.

4.1.5.2. 9-Cyclohexyl-9*H*-adenine (13). Obtained as white solid after chromatography on a silica gel column eluting with $CHCl_3$–CH_3OH 98:2, in 92% yield.

4.2. Biological assays

All pharmacological methods followed the procedures as described earlier.[42] 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 (1000 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 adenosine AA_1R receptors 1 nM [^{3}H]CCPA was used, whereas 30 and 10 nM [^{3}H]NECA were used for $AA_{2A}R$ and AA_3R, respectively. Nonspecific 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.[52] At $AA_{2B}R$, 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.[53]

4.3. Molecular modeling

4.3.1. Computational methodologies

All molecular modeling studies were performed on a 2 CPU (PIV 2.0–3.0 GHZ) Linux PC. Homology modeling and docking studies have been carried out using Molecular Operating Environment (MOE, version 2008.10) suite.[54] All ligands structures were optimized using RHF/AM1 semiempirical calculations, and the software package MOPAC implemented in MOE was utilized these calculations.[55]

4.3.2. Human $AA_{2A}R$ crystal structure refinement

The recently solved X-ray crystal structure of the human $AA_{2A}R$ in complex with ZM241385 was retrieved from the RCSB Protein Data Bank (pdb code: 3EML;[48] 2.6-Å resolution). All hydrogen atoms were added, and the protein coordinates were then minimized with MOE using the AMBER99 force field.[56] The minimizations were performed by 1000 steps of steepest descent followed by conjugate gradient minimization until the RMS gradient of the potential energy was less than 0.05 kJ mol^{-1} Å^{-1}.

4.3.3. Molecular docking of the human $AA_{2A}R$ antagonists

The human $AA_{2A}R$ crystal structure was solved in complex with ZM241385 antagonist. The ligand was removed and the cavity occupied by this molecule was employed as binding site region for the docking analysis. All antagonists structures were hence docked by using the MOE Dock tool. This method is divided into a number of stages: Conformational Analysis of ligands. The algorithm generated conformations from a single 3D conformation by conducting a systematic search. In this way, all combinations of angles were created for each ligand. Placement. A collection of poses was generated from the pool of ligand conformations using Alpha

Triangle placement method. Poses were generated by superposition of ligand atom triplets and triplets points in the receptor binding site. The receptor site points are alpha sphere centres which represent locations of tight packing. At each iteration a random conformation was selected, a random triplet of ligand atoms and a random triplet of alpha sphere centres were used to determine the pose. Scoring. Poses generated by the placement methodology were scored using two available methods implemented in MOE, the *London dG* scoring function which estimates the free energy of binding of the ligand from a given pose, and *Affinity dG Scoring* which estimates the enthalpic contribution to the free energy of binding. Top 30 poses for each ligand were output in a MOE database. Each resulting ligand pose was then subjected to MMFF94[57–63] energy minimization until the RMS gradient of the potential energy was less than 0.05 kJ mol^{-1} $Å^{-1}$. In this phase, water molecules originally present in the human $AA_{2A}R$ were recovered and subjected to energy minimization together with the ligands. AMBER99 partial charges of receptor and water molecules and MOPAC output partial charges of ligands were utilized. Top pose for each ligand was then selected and subjected to rescoring within MOE, by using the already cited *London dG* and *Affinity dG* scoring functions and the *dock-pK_i* predictor. The last tool allows to estimate the pK_i for each ligand using the 'scoring.svl' script retrievable at the SVL exchange service (Chemical Computing Group, Inc. SVL exchange: http://svl.chemcomp.com) The estimated pK_i for these structures were calculated by choosing the dock_pK_i descriptor with default settings for the molecular database.

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