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5′-Carbamoyl derivatives of 2′-*C*-methyl-purine nucleosides as selective A_1 adenosine receptor agonists: Affinity, efficacy, and selectivity for A_1 receptor from different species

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Abstract—A series of 5′-carbamoyl and 5′-thionocarbamoyl derivatives of 2′-*C*-methyl analogues of the A_1 adenosine receptor (A_1AR) full agonists N^6-cyclopentyladenosine (CPA), 2-chloro-N^6-cyclopentyladenosine (CCPA), N^6-[3-(*R*)-tetrahydrofuranyl]adenosine (tecadenoson), and 2-chloro analogue (2-Cl-tecadenoson) was synthesized and evaluated for their affinity for adenosine receptor subtypes from bovine, porcine, and human species. In the N^6-cyclopentylamino series, the 5′-substituted derivatives showed a reduced affinity at the bovine A_1AR compared to the parent compounds; however, the selectivity for A_1 versus A_{2A} receptor was retained or increased. The corresponding N^6-3-(*R*)-tetrahydrofuranylamino analogues displayed a very low affinity toward the bovine A_1AR. The 5′-methylthionocarbamoyl derivative of 2′-Me-CCPA showed the best affinity at porcine A_1AR with a K_i value of 13 nM. At human AR subtypes tecadenoson derivatives showed 2.3- to 5-fold lower affinity at A_1AR and very low affinity at the other subtypes (A_{2A}, A_{2B}, and A_3) compared to the corresponding N^6-cyclopentyl analogues. The 5′-carbamoyl and 5′-thionocarbamoyl derivatives of 2′-Me-CCPA **3**, **4**, **7** and tecadenoson derivative **12** were found to be partial A_1 agonists at the porcine receptor. Docking studies explained the lower affinity of N^6-3-(*R*)-tetrahydrofuranyl-substituted compounds at bovine A_1AR compared to that of N^6-cyclopentyl analogues, showing that the oxygen of the tetrahydrofuranyl ring establishes unfavorable electrostatic interactions with the CO oxygen of Asn254. The low binding affinity of the 2′-*C*-methyl-N^6-3-(*R*)-tetrahydrofuranyl adenosine analogues at human A_1AR may be ascribed to the presence of unfavorable interactions between the hydrophilic tetrahydrofuranyl ring and the surrounding hydrophobic residues Leu250 (TM6) and Ile274 (TM7).

1. Introduction

The A_1 adenosine receptor (A_1AR) is the best characterized member of the ARs family that includes the A_{2A}, A_{2B}, and A_3 subtypes.[1] A_1ARs are expressed in high density in the brain (cortex, hippocampus, cerebellum, and thalamus) and in adipose tissue, and in medium to low levels in many tissues such as heart, lung, and kidney.[2] Numerous full agonists with high affinity for the A_1 receptor have been developed, but very few compounds have emerged as truly selective ligands for the human A_1 receptor. Agonists for the A_1AR have many potential therapeutic applications including the control of cardiac rhythm, attenuation of ischemia-reperfusion injury, reduction of neuropathic pain, reduction of plasma lipids, reduction of intestinal peristalsis, and treatment of inflammation.[2] Moreover, adenosine and its N^6-substituted derivatives by A_1 activation produce a neuroprotective effect.[3] Unfortunately, the clinical use of A_1 agonists is hampered by severe cardiovascular side effects caused by the strong hypotensive action and side effects at other organs.[4] Thus, the therapeutic use of an A_1 agonist is highly dependent on the development of

Keywords: 2′-*C*-Methyladenosine derivatives; A_1 adenosine receptor partial agonists; Receptor binding; Species selectivity; Structure–activity relationships.
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strategies to achieve selective action of the drug on the targeted tissue with a minimum of unwanted effects.

One way to circumvent the side effects of full agonists is the use of A_1 selective partial agonists, which may exploit the differences in receptor-effector coupling in various tissues and can achieve selective activity in vivo. The research of partial agonists is an attempt to selectively target those tissues with a high density of receptors and high 'receptor reserve'.[5] Partial agonists may have less side effects and may act more selectively depending on the receptor density of a cell type.[6] In addition, partial agonists may induce less receptor downregulation and desensitization. Thus, for example, partial agonists selective for the A_1AR inhibit lipolysis in vivo while they are devoid of cardiovascular effects.[7]

In the search for potent and selective A_1AR agonists, we have previously investigated a series of 2′-*C*-methyl-ribofuranosyl analogues of selective A_1AR agonists such as (*R*)-PIA, CPA, and CCPA. 2-Chloro-2′-*C*-methyl-N^6-cyclopentyl-adenosine (2′-Me-CCPA) emerged as a potent and highly selective full agonist at rat, bovine, and human A_1 versus A_{2A}, A_{2B}, and A_3ARs.[8,9] Among the N^6-amino substituted adenosine analogues, tecadenoson (*N*-[3-(*R*)-tetrahydrofuranyl]-6-aminopurine riboside, CVT-510, Fig. 1), a derivative structurally related to CPA that proved to be a full agonist selective for the A_1AR, is in phase III trials for the treatment of paroxysmal supraventricular tachycardia (PSVT) and for its conversion to sinus rhythm without lowering blood pressure.[10]

Recently, 5′-carbamates and 5′-thionocarbamates, as well as 5′-aromatic ethers and sulfide derivatives of tecadenoson, demonstrated high affinity for the A_1AR and less than full efficacy resulting in A_1 partial agonists that can be used to provide ventricular rate control during atrial fibrillation.[11,12]

On the basis of these findings, a series of 2′-*C*-methyl-analogues of N^6-cyclopentyladenosine, such as 2′-Me-CPA, 2′-Me-CCPA, and of N^6-[(*R*)-3-tetrahydrofuranyl]-analogues substituted at the 5′-position with a carbamoyl or thionocarbamoyl group were synthesized and evaluated for binding affinity at bovine, porcine, and human ARs and for relative intrinsic efficacy.

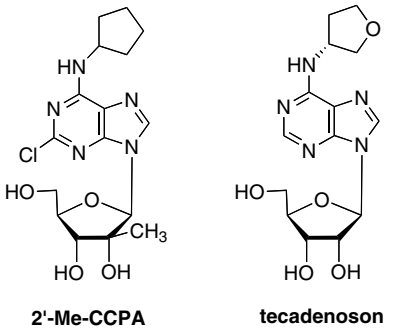


Figure 1. Structures of 2′-Me-CCPA and tecadenoson.

2. Chemistry

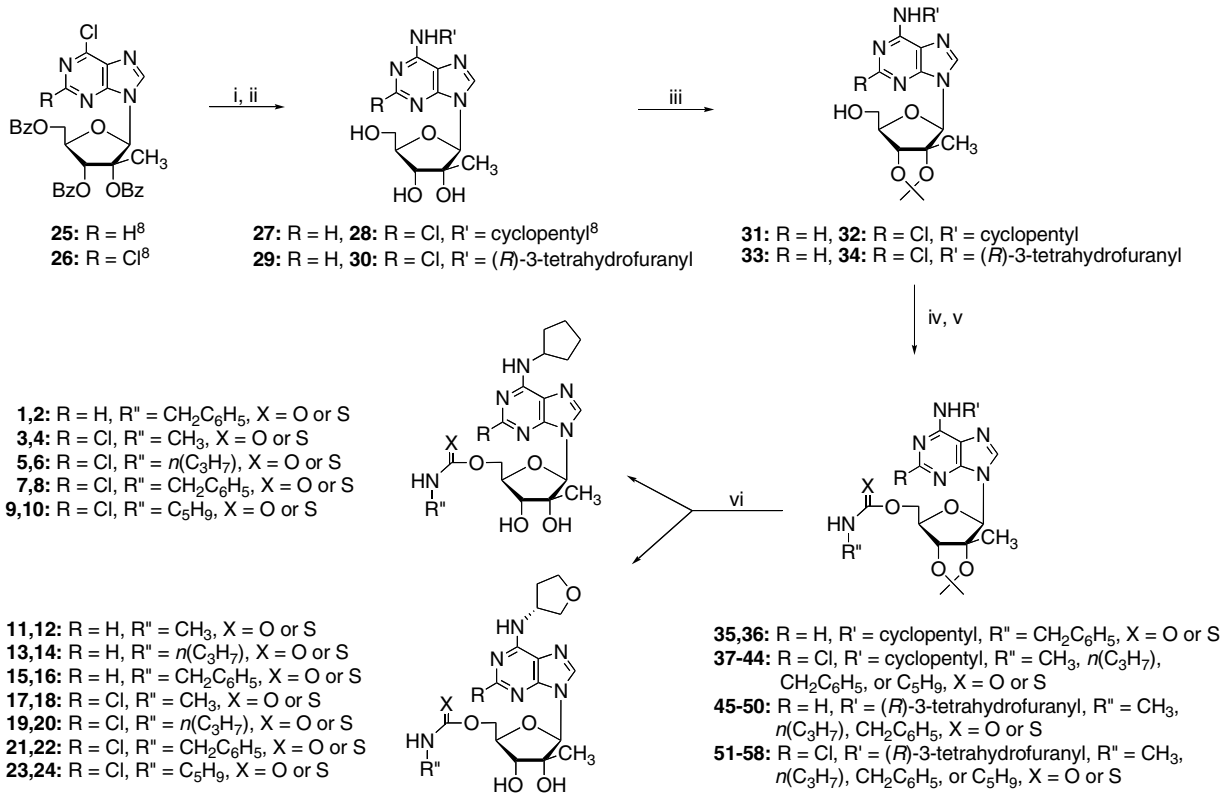
5′-Carbamates and 5′-thionocarbamates under investigation were synthesized as shown in Scheme 1. 2′-*C*-Methyladenosine and the 2-chloro analogues substituted in the N^6 amino group [2′-Me-CPA (**27**),[8] 2′-Me-CCPA (**28**),[8] 2′-Me-tecadenoson (**29**), and 2-Cl-2′-Me-tecadenoson (**30**)] were obtained by nucleophilic displacement of the 6-chlorine atom of 6-chloro-9*H*-[(2-*C*-methyl)-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl]purine (**25**)[8] and of the 2-chloro analogue **26**[8] with an excess of cyclopentylamine or (*R*)-tetrahydro-3-furylamine in ethanol, followed by deprotection of the ribose moiety in basic condition. Compounds **27–30** were converted into the 2′,3′-isopropylidene derivatives **31–34** by treatment with 2,2-dimethoxypropane and camphorsulfonic acid in acetone and, subsequently, reacted with carbonyl- or thiocarbonyldiimidazole and then with the appropriate amine to give the corresponding 5′-carbamoyl derivatives **35–58**. Deisopropylidation of **35–58** with 70% formic acid afforded the 5′-substituted N^6-cyclopentylamino-purine nucleosides **1–10** and the N^6-(*R*)-tetrahydro-3-furanylamino analogues **11–24**. Tecadenoson and 2-chloro-tecadenoson were synthesized as previously reported[13,14] and used for the biological evaluation.

3. Biological evaluation and discussion

The di- and trisubstituted 2′-*C*-methyl-purine nucleosides **1–24**, **29**, and **30** were tested in radioligand binding assays to determine their affinities at A_1 and A_{2A} ARs from bovine and porcine brain cortical and striatum membranes utilizing the agonists [^{3}H]CCPA for A_1 receptor and [^{3}H]CGS21680 for A_{2A} subtype (Table 1). Some representative compounds were also evaluated for affinity at human A_1, A_{2A}, A_{2B}, and A_3ARs. CPA, CCPA, 2′-Me-CPA, 2′-Me-CCPA, tecadenoson, and 2-chloro-tecadenoson were tested as reference compounds.

Binding assay at bovine and porcine A_1AR showed that the replacement of the N^6-cyclopentylamino group in CPA, CCPA, 2′-Me-CPA, and 2′-Me-CCPA with the (*R*)-3-tetrahydrofuranylamino one (tecadenoson, 2-Cl-tecadenoson, and compounds **29** and **30**) resulted in a decrease in affinity. Tecadenoson showed only slightly different affinity toward the A_1AR from bovine and porcine membranes with K_i values of 3.1 and 7.6 nM, respectively. The K_i value at porcine A_1AR appeared to be similar to that reported by Sorbera et al.[10] A similar lack of species selectivity for bovine versus porcine A_1AR was also shown by CPA, CCPA, 2′-Me-CPA, 2′-Me-CCPA, and 2-Cl-tecadenoson, whereas 2′-*C*-methyl-derivatives **29** and **30** exhibited 11- and 5-fold differences in K_i values, respectively, between the two species.

Regarding the di- and trisubstituted 2′-*C*-methyladenosine derivatives, the 5′-carbamoyl derivatives **1–24** showed a decreased affinity for both bovine and porcine A_1AR compared to the parent compounds. The reduction in affinity was more relevant for tetrahydrofuranyl



Scheme 1. Reagents and conditions: (i) R′NH_2, C_2H_5OH, reflux; (ii) CH_3ONa, CH_3OH, rt; (iii) 2,2-dimethoxypropane, camphorsulfonic acid, acetone, Δ; (iv) CDI or thioCDI, THF a, rt; (v) R″NH_2, rt; (vi) 70% HCOOH, Δ.

compounds. However, the A_1 selectivity of these compounds versus the A_{2A} receptor was retained. The 5′-methyl-carbamate **3** and thionocarbamate **4** with K_i values of 72 and 13 nM, respectively, exhibited the best affinity at porcine A_1AR, while the N^6-tetrahydrofuranyl analogues **17** and **18** proved to be 7- and 21-fold less affine, respectively. The 5′-benzyl-carbamate **7** and thionocarbamate **8** had the best affinity at bovine receptor with K_i values of 57 and 42 nM, respectively. In general, comparing the oxo- versus thionocarbamate series with the same alkyl chain length, only in the case of compounds **3** and **4** a different affinity was observed with the thionocarbamate being the more potent derivative in both species.

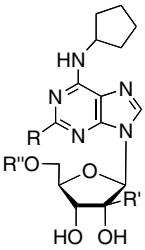
Comparing the effect of varying the substituent in the carbamoyl and thionocarbamoyl moiety within the 2-chloro-N^6-cyclopentyl series, the affinity at bovine A_1AR followed the trend benzyl ⩾ propyl ⩾ cyclopentyl ⩾ methyl, while at porcine receptor the trend was methyl > cyclopentyl = benzyl = propyl. In the N^6-tetrahydrofuranyl series, the 5′-methyl-thionocarbamate **12** and the 2-chloro derivative **18** showed the best affinity at porcine A_1AR (K_i = 173 and 276 nM, respectively), while at bovine A_1 receptor the most affine compound was the 5′-benzyl-thionocarbamate **22** (K_i = 230 nM).

Concerning the effect of the 2-chloro substitution it was observed that in 5′-carbamoyl derivatives of the N^6-cyclopentyl series the replacement of the hydrogen with a chlorine atom at the 2-position of the purine ring induces an increase in affinity at both bovine and porcine A_1AR (compare **1** and **2** with **7** and **8**); this was not always observed in the N^6-tetrahydrofuranyl series.

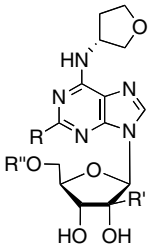
The efficacy of tecadenoson, 2-Cl-tecadenoson, and selected compounds **3**, **4**, **7**, **12**, **29**, and **30** was also determined. The efficacy of these compounds was compared to the level of maximal stimulation of agonist-induced [^{35}S]GTPγS binding in porcine brain tissue by the full agonist CPA (Table 2). The EC_{50} values of tecadenoson and 2-Cl-tecadenoson were in the nanomolar range, while the 2′-*C*-methyl analogues **29** and **30** proved to be much less potent. For these compounds a discrepancy between the EC_{50} and K_i values has been detected. This discrepancy is quite common for A_1AR agonists and has been shown in particular for adenylyl cyclase inhibition versus radioligand binding in numerous papers.[15] As mentioned by Breivogel and Childers,[15e] and Govaerts et al.[15f] the agonist occupancy of G-protein coupled receptors often occurs at lower concentrations than the ones able to stimulate [^{35}S]GTPγS binding to G proteins. Among the 5′-carbamates, compounds **3** and **4** showed the best EC_{50} values (165 and 326 nM, respectively). Tecadenoson, 2-Cl-tecadenoson, and 2′-*C*-methyl analogues **29** and **30** proved to be full agonists, while the 5′-carbamates and thionocarbamates **3**, **4**, **7**, and **12** turned out to be partial agonists with 54–64% of CPA's maximal efficacy.

The affinity of selected compounds at human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes expressed in

Table 1. Binding affinity of compounds **1–24**, **29**, and **30** in radioligand assays at bovine and porcine cortical (A_1) and striatum (A_{2A}) membranes



1-10, CPA, CCPA
2'-Me-CPA, 2'-Me-CCPA



11-24, **29**, **30**, tecadenoson
2-Cl-tecadenoson

Compound	R	R′	R″	K_i (nM) or % displacement at 10^{-5} M			
				bA_1[a]	pA_1[a]	bA_{2A}[b]	pA_{2A}[b]
1	H	CH_3	$CONHCH_2C_6H_5$	538 ± 50	2052 ± 187	0%	14%
2	H	CH_3	$CSNHCH_2C_6H_5$	457 ± 47	1026 ± 96	11000 ± 1000	21%
3	Cl	CH_3	$CONHCH_3$	156 ± 16	72 ± 5.6	16%	24%
4	Cl	CH_3	$CSNHCH_3$	66 ± 6.3	13 ± 1.2	15000 ± 2000	9200 ± 1000
5	Cl	CH_3	$CONH(CH_2)_2CH_3$	65 ± 6.6	513 ± 38	9%	8700 ± 950
6	Cl	CH_3	$CSNH(CH_2)_2CH_3$	75 ± 7.1	337 ± 27	0%	0%
7	Cl	CH_3	$CONHCH_2C_6H_5$	57 ± 6.2	410 ± 36	19300 ± 2000	28%
8	Cl	CH_3	$CSNHCH_2C_6H_5$	42 ± 4.1	380 ± 27	7900 ± 800	15%
9	Cl	CH_3	$CONHC_5H_9$	66 ± 7.2	370 ± 29	12750 ± 950	4700 ± 380
10	Cl	CH_3	$CSNHC_5H_9$	80 ± 9	380 ± 31	8092 ± 820	6845 ± 670
11	H	CH_3	$CONHCH_3$	923 ± 85	760 ± 67	13%	3%
12	H	CH_3	$CSNHCH_3$	615 ± 45	173 ± 18	21%	4%
13	H	CH_3	$CONH(CH_2)_2CH_3$	930 ± 69	3879 ± 267	0%	12%
14	H	CH_3	$CSNH(CH_2)_2CH_3$	770 ± 53	3200 ± 314	0%	5%
15	H	CH_3	$CONHCH_2C_6H_5$	2308 ± 198	16200 ± 1500	15%	12%
16	H	CH_3	$CSNHCH_2C_6H_5$	2703 ± 175	9800 ± 760	5%	7%
17	Cl	CH_3	$CONHCH_3$	769 ± 72	534 ± 37	14%	2%
18	Cl	CH_3	$CSNHCH_3$	615 ± 43	276 ± 19	15%	9%
19	Cl	CH_3	$CONH(CH_2)_2CH_3$	460 ± 35	1848 ± 165	0%	9%
20	Cl	CH_3	$CSNH(CH_2)_2CH_3$	313 ± 29	1450 ± 121	10000 ± 870	13000 ± 1240
21	Cl	CH_3	$CONHCH_2C_6H_5$	384 ± 21	4100 ± 356	0%	1%
22	Cl	CH_3	$CSNHCH_2C_6H_5$	230 ± 18	2870 ± 211	9%	14100 ± 1320
23	Cl	CH_3	$CONHC_5H_9$	307 ± 28	1850 ± 144	12%	29%
24	Cl	CH_3	$CSNHC_5H_9$	460 ± 38	2053 ± 210	7%	16%
CPA	H	H	H	0.4 ± 0.03	0.53 ± 0.06	1220 ± 108	810 ± 78
2′-Me-CPA	H	CH_3	H	4.4 ± 0.33	10.0 ± 1.5	7230 ± 703	11370 ± 1050
CCPA	Cl	H	H	0.34 ± 0.04	0.3 ± 0.02	730 ± 71	650 ± 57
2′-Me-CCPA	Cl	CH_3	H	1.8 ± 0.2	3.6 ± 0.25	4560 ± 385	12000 ± 1000
Tecadenoson	H	H	H	3.1 ± 0.26	7.6 ± 0.45	7300 ± 700	9756 ± 891
29	H	CH_3	H	13.6 ± 0.17	150 ± 14	0%	13%
2-Cl-tecadenoson	Cl	H	H	1.7 ± 0.2	6.6 ± 0.36	4689 ± 511	27%
30	Cl	CH_3	H	13.0 ± 1.23	67 ± 7.0	7%	1%

[a] Displacement of specific [^{3}H]CCPA binding in bovine or porcine cortical membranes expressed as K_i in nM ($n = 3$).
[b] Displacement of specific [^{3}H]CGS21680 binding in bovine or porcine striatum membranes expressed as K_i in nM ($n = 3$).

Table 2. EC_{50} values and maximum level of G-protein activation (±SEM) of selected compounds as determined by [^{35}S]GTPγS binding to porcine brain membranes[a] ($n = 3$)

Compound	EC_{50} (nM)	Efficacy (%)
CPA	7.9 ± 1	100
Tecadenoson	31.6 ± 3	101 ± 1
2-Cl-tecadenoson	18.3 ± 2	97 ± 3
3	165 ± 18	61 ± 3
4	326 ± 26	64 ± 2
7	5000 ± 478	54 ± 2
12	2400 ± 190	56 ± 2
29	3620 ± 358	95 ± 4
30	2760 ± 293	96 ± 3

[a] Measurement of the stimulation of binding of [^{35}S]GTPγS (0.3 nM) to G protein-coupled A_1AR in porcine cortical membranes induced by the test compounds (1 nM–10 μM) as % of CPA.

CHO cells was also determined (Table 3). With the exception of tecadenoson, the N^6-(*R*)-3-tetrahydrofuranylamino derivatives showed only 2.3- to 5-fold lower affinity at human A_1AR in comparison with the corresponding N^6-cyclopentyl analogues. It was confirmed that the introduction of a methyl group in the 2′-position of the ribose moiety induces a moderate reduction of the affinity at A_1 receptor (compare **29** and **30** to tecadenoson and 2-Cl-tecadenoson); however, the decrease in affinity was much more relevant at A_{2A} and A_3 receptors. Thus, 2′-Me-tecadenoson and 2-Cl-2′-Me-tecadenoson turned out to be very selective compounds (>4761- to > 6451-fold A_1 versus human A_{2A}, and > 211- to > 288-fold A_1 versus A_3 receptors, respectively). Among the 5′-carbamates, the thiono-derivatives **4** and **18** showed the best affinity at human A_1 receptor

Table 3. Binding affinity of N^6-cyclopentyl- and N^6-[(*R*)-3-tetrahydrofuranyl]-2′-*C*-methyl-adenosine derivatives at human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes

Compound	K_i (nM)				Selectivity		
A_{2A}/A_3	A_1[a]	A_{2A}[b]	A_{2B}[c]	A_3[b]	A_{2A}/A_1	A_3/A_1	A_{2A}/A_3
CPA	2.25 ± 0.18	794 ± 72	18600 ± 2200	43 ± 6.8	353	19.1	18.5
CCPA	0.8 ± 0.06	2300 ± 215	18800 ± 1950	42 ± 5.4	2875	53	55
2′-Me-CPA	4.52 ± 0.74	10400 ± 2180	26800 ± 2680	879 ± 13	2301	194.4	11.8
2′-Me-CCPA	3.37 ± 0.45	9580 ± 3730	37600 ± 8150	1150 ± 313	2903	348	8.3
Tecadenoson	2.05 ± 0.20	6390 ± 1575	25800 ± 6100	227 ± 49	3117	110.7	28.1
2-Cl-tecadenoson	1.87 ± 0.05	4590 ± 1210	34600 ± 5950	211 ± 67	2455	112.8	21.7
3	1118 ± 25	10400 ± 1360	>100000	8350 ± 1400	9.3	7.5	1.2
4	356 ± 121	8760 ± 1,165	>100000	2810 ± 350	24.6	7.9	3.1
17	4770 ± 660	>100000	>100000	30800 ± 7500	>20.9	6.4	>3.2
18	837 ± 140	15900 ± 3750	>100000	12100 ± 2485	19	14.5	1.3
29	21 ± 2.7	>100000	35500 ± 6500	4440 ± 2174	>4761	211.4	>22.5
30	15.5 ± 4.1	>100000	18900 ± 3300	4470 ± 315	>6451	288.4	>22.4

[a] Displacement of [^{3}H]CCPA binding in CHO cells, stably transfected with human recombinant A_1 adenosine receptor.
[b] Displacement of [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_{2A} or A_3 adenosine receptors.
[c] EC_{50} values (nM) are reported for the agonist-mediated stimulation of adenylyl cyclase activity in a membrane preparation of CHO cells, stably transfected with human recombinant A_{2B} receptor subtype.

with K_i values of 356 and 837 nM, respectively, but their selectivity proved to be reduced in comparison to the non-carbamoylated analogues. Moreover, all tested compounds showed a very poor activity at A_{2B} receptor.

With regard to species selectivity of the 5′-unsubstituted compounds, 2′-*C*-methyl-tecadenoson (**29**) and the 2-chloro analogue **30** appeared to be less affine at porcine than at bovine and human A_1ARs.

Finally, the affinity ranking of 5′-thionocarbamates **4** and **18**, and 5′-carbamates **3** and **17** at the A_1AR of the three species was porcine > bovine > human.

3.1. Molecular modeling

In order to rationalize the lower affinity of N^6-(*R*)-3-tetrahydrofuranyl-substituted derivatives toward the bovine A_1AR compared to that of the corresponding N^6-cyclopentyl analogues, docking simulations of 2-chloro-2′-*C*-methyl-tecadenoson (compound **30**) were carried out using the automatic docking program AutoDock[16] and employing the previously described model of bovine A_1AR.[9] As expected, the top ranking binding solution strongly resembles the one previously described for 2′-Me-CCPA.[9] The N^6-amino group of the ligand forms a H-bond with the CO oxygen of Asn254 (TM6). The ribose moiety is coordinated to several hydrophilic residues in TM3 and TM7. In particular, the 2′-OH, 3′-OH, and 5′-OH groups of the ribose ring are involved in H-bonding with the N$^\delta$ imidazole nitrogen of His278 (TM7) and the OH oxygen of Thr91 (TM3), respectively. Moreover, the ribose 4′-oxygen is engaged in a H-bond with the OH hydrogen of Thr91. In the light of these results, **30** seems still to preserve the ability to bind the bovine A_1AR and activate it.

To evaluate the dynamic stability of the ligand/receptor complex, a 150 ps of molecular dynamics (MD) was undertaken at a constant temperature of 300 K, monitoring the distances between **30** and the key A_1AR residues along the complete MD trajectory (Fig. 1a–f of the supporting information). From the trajectory plots of the distance between the ligand key groups and the receptor amino acids Asn254, His278, and Thr91, it seems clear that **30** is unable to engage a stable H-bond between its 6-NH group and the Asn254 CO oxygen (Fig. 1a), although weak H-bonds are still established between the ligand 3′-OH and His278 N$^\delta$ imidazole nitrogen (Fig. 1c) and the ligand 5′-OH and Thr91 OH oxygen (Fig. 1d). Surprisingly, in contrast to what was previously reported,[9] the H-bonds between the ligand 2′-OH and His278 N$^\delta$ (Fig. 1b) and the ligand 4′-oxygen and Thr91 OH hydrogen (Fig. 1e), initially present in the complex, are gradually lost during the whole length of the monitored simulation period. Moreover, the average root-mean-squared deviation (rmsd) calculated for all the ligand heavy atoms from the initial energy-minimized **30**/bovine A_1AR complex suggests that the predicted binding conformation of the ligand is rather unstable (Fig. 1f).

With the purpose of gaining major insight into the reasons for the instability of **30** in the binding site of bovine A_1AR, electrostatic potentials on the solvent-accessible surface of both ligand and receptor were calculated using 3 MD snapshots taken at 0 ps, 75, and 150 ps, respectively. As depicted in Figure 2, the tetrahydrofuranyl ring oxygen of the ligand, establishing unfavorable electrostatic interactions with the CO oxygen of Asn254 (Fig. 2A), displaces **30** from its original position so as to minimize the cited disadvantageous interaction with the receptor (Fig. 2B and C). Such a displacement results in a loss of the crucial H-bond between the ligand 6-NH group and the CO oxygen of Asn254, thus explaining the reduced affinity of **30** and its derivatives for the bovine A_1AR.

As reported above, the 5′-carbamoyl and 5′-thionocarbamoyl substituents within the 2-chloro-N^6-cyclopentyl (compounds **3**–**10**) and 2-chloro-N^6-tetrahydrofuranyl (compounds **17**–**24**) series are detrimental for the

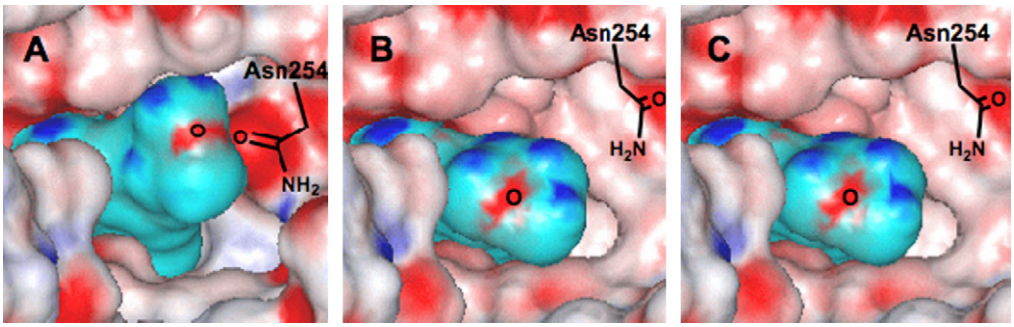


Figure 2. Electrostatic potentials over molecular surfaces of 2-chloro-2′-*C*-methyl-tecadenoson **(30)** in complex with bovine A_1AR computed at 0 ps (A), 75 ps (B), and 150 ps (C) of MD. Red, blue, and white, respectively, indicate negative, positive, and neutral electrostatic potentials of bovine A_1AR. Red, blue, and cyan, respectively, indicate negative, positive, and neutral electrostatic potentials of **30**.

A_1AR activity. Moreover, when comparing series **3**–**10** with series **17**–**24**, the reduction in affinity seems to correlate in a proportional way. This latter evidence suggests that the cited ligands still occupy the bovine A_1AR binding pocket, but the interactions responsible for the receptor activation seem to be weakened or completely lost. In fact, docking of compounds **5** and **19**, chosen as representative members of the two series, revealed that they bind more deeply to the middle of the TM bundle with respect to the 5′-unsubstituted compounds 2′-Me-CCPA[9] and **30**. The carbamoyl carbonyl group H-bonds Asn280 (TM7), while the propylic chain makes hydrophobic interactions with residues Leu90 (TM3) and Phe243 (TM6). These new interactions provoke a slight sliding of the ligands inside the TM binding cavity and determine the weakness or the loss of the crucial interactions with Asn254, His278, and Thr91.

To shed light on the reasons behind the different affinities displayed by CCPA, 2′-Me-CPA, 2′-Me-CCPA, tecadenoson, 2-Cl-tecadenoson, **29**, and **30** toward the human A_1AR, a model of the human A_1AR was built and used for docking experiments. Unexpectedly, for all the inspected compounds, comparable binding solutions were found, in which the key functional groups were within the H-bonding distance from the key receptor residues Asn254, His278, and Thr91. Based on these results, it can be concluded that the above-mentioned compounds bind to the human A_1AR and activate it. However, binding data reported in Table 3 show that replacement of the N^6-cyclopentyl ring with a tetrahydrofuranyl ring reduces binding affinity of derivatives bearing the 2′-*C*-methyl substituent (compare binding affinities of 2′-Me-CPA with **29** and 2′-Me-CCPA with **30**). On the other hand, the same substitution does not influence to a great extent the binding affinity of those derivatives lacking the 2′-*C*-methyl group (compare binding affinities of CPA with tecadenoson and CCPA with 2-Cl-tecadenoson). The discrepancy between docking results and biological data might be ascribed to the fact that the docking experiments do not take into account the receptor plasticity. Therefore, a 150 ps of MD was carried out for CCPA and 2-Cl-tecadenoson complexed with the human A_1AR, in which the cyclopentyl/tetrahydrofuranyl substitution does not greatly affect the binding affinity at human A_1AR. The same simulation was also conducted for 2′-Me-CCPA/A_1AR and **30**/A_1AR complexes, in which the above-cited substitution negatively affects binding affinity. The conformational behavior of the ligands into the binding site together with the distances between their functional groups and the key receptor residues were monitored along the complete MD trajectory (Figs. 2a–e and 3a–e of the supporting information).

It is interesting to outline that the presence of the 2′-*C*-methyl group in 2′-Me-CCPA and 2-Cl-2′-Me-tecadenoson freezes the ribose ring in a North ($^3T_2$)-*anti* conformation. On the other hand, the absence of the methyl group in both CCPA and 2-Cl-tecadenoson allows the complete conformational flexibility of the sugar, which can easily pass from North ($^3T_2$)-*anti* to South ($^2T_3$)-*syn* conformers and vice versa.

The trajectory plots of the distances between the ligand and receptor H-bonding atoms obtained for CCPA and 2-Cl-tecadenoson complexed with the human A_1AR (Fig. 2a–e of the supporting information) strongly indicate that both ligands can establish stable H-bonds with the receptor (see Fig. 3a and b). This is in accordance with the biological data indicating a minor difference in binding affinity for the two ligands (0.8 and 1.87 nM, respectively).

Different results were obtained when analyzing trajectory plots for the interactions of the 2′-Me-CCPA and **30** in complex with the human A_1AR (Fig. 3a–e of the supporting information). From this analysis it seems clear that while the H-bond between 2′-Me-CCPA 6-NH group and the Asn254 CO oxygen is extremely stable during all the simulation period (ranging from 1.55 to 2.62 Å), the same interaction in **30** is gradually lost during the whole length of the monitored simulation (range: 2.01–4.95 Å). In both complexes, all the other remaining interactions remain rather stable during the whole production run (Fig. 3c and d of the supporting information). It is to note that the binding conformation of compound **30** appears rather unstable during the MD simulation, as exemplified by the highly fluctuating average rmsd (from 0 to 2.43 Å^2), calculated for all the ligand heavy atoms from the initial energy-minimized **30**/A_1AR complex. A visual inspection of the ligand/receptor complex suggests that such displacement may be explained by the presence of unfavorable interactions between the hydrophilic tetra-

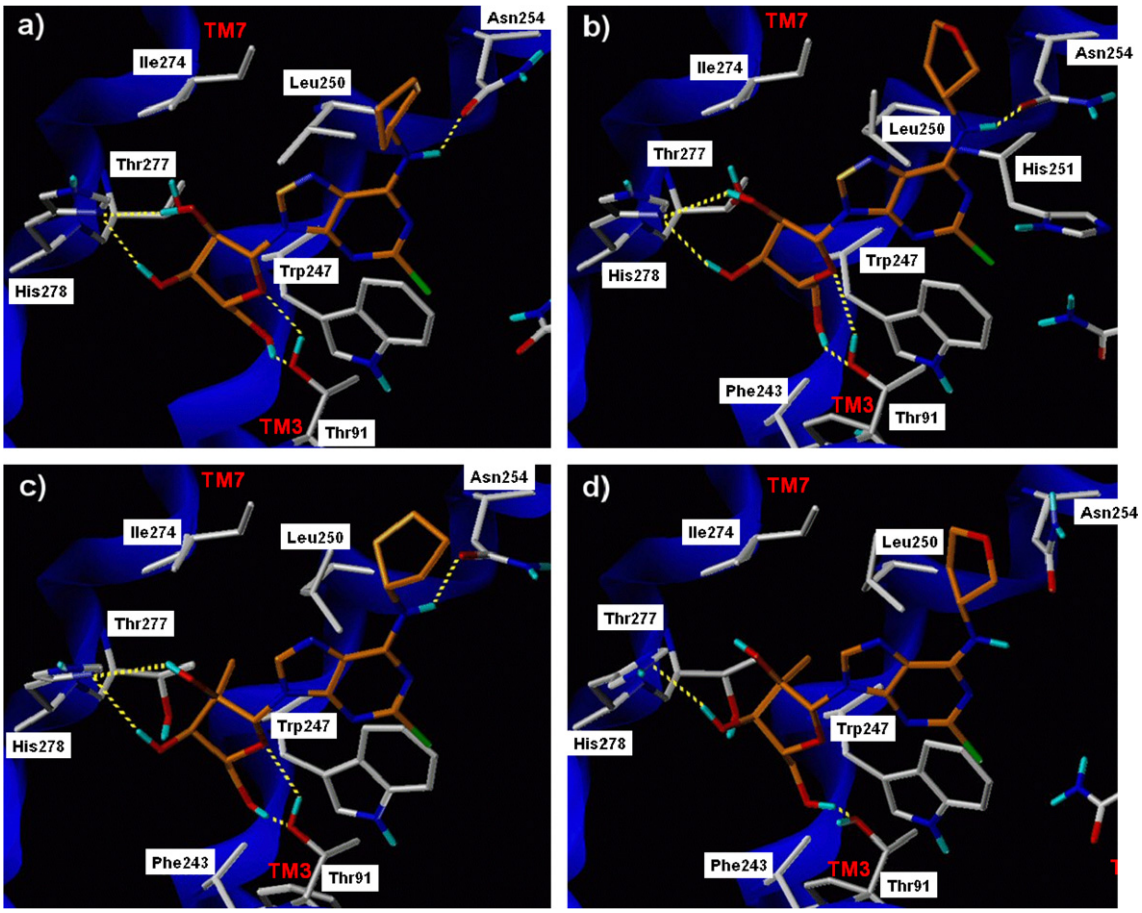


Figure 3. Side view of the CCPA (a), 2-Cl-tecadenoson (b), 2′-Me-CCPA (c), and compound **30** (d) in complex with the human A_1AR. The side chains of the key receptor residues in proximity of the docked molecule (orange) are highlighted and labeled. Nonpolar hydrogens have been removed for clarity. Hydrogen bonds are represented by dashed yellow lines.

hydrofuranyl ring and the surrounding hydrophobic residues Leu250 (TM6) and Ile274 (TM7).

In summary, it can be assumed that the coexistency of the 2′-*C*-methyl group on the ribose and of the tetrahydrofuranyl ring on the N^6 amino group is unfavorable for binding affinity at the human A_1AR for the following reasons: (i) the 2′-*C*-methyl group impedes the formation of the complete H-bonding network responsible for high affinity at the human A_1AR; (ii) the hydrophilic tetrahydrofuranyl ring, if compared with the more hydrophobic cyclopentyl ring, establishes unfavorable interactions with the receptor hydrophobic residues, thus determining a displacement of the entire ligand in the binding site.

4. Conclusions

In summary, a series of di- and trisubstituted analogues of adenosine have been synthesized and evaluated in binding studies at bovine, porcine, and human adenosine receptors. It was found that the combination of modifications at C-2 and N^6-positions of purine moiety and at C-2′ and C-5′ of ribofuranose ring in adenosine resulted in some cases in different affinity profiles at the adenosine receptors from bovine, porcine, and human species. 5′-Carbamoyl substituents in the N^6-cyclopentyl derivatives of 2′-*C*-methyladenosine and 2-chloro analogue caused a decrease in affinity at A_1 receptor from the various species, as compared to the 5′-unsubstituted parent compounds. The same substituents in the N^6-tetrahydrofuranyl analogues brought about a greater reduction in binding affinity. However, the tested nucleosides retained the A_1 versus A_{2A} selectivity at bovine and porcine receptors and versus A_{2A}, A_{2B}, and A_3 human ARs. Molecular modeling analysis explained the lower affinities of N^6-tetrahydrofuranyl derivatives at bovine and human A_1 receptor compared to that of the N^6-cyclopentyl analogues.

[^{35}S]GTPγS binding to porcine brain membranes showed that the 2′-*C*-methyl derivatives of tecadenoson and 2-chloro-tecadenoson (compounds **29** and **30**), which displayed reduced affinity at porcine A_1AR (K_i = 150 and 67 nM, respectively) with respect to the parent compounds (K_i = 7.6 and 6.6 nM, respectively), remain A_1 full agonists. On the contrary, 5′-carbamoyl derivatives of 2′-Me-CCPA (compounds **3**, **4**, and **7**) and tecadenoson analogue **12** proved to be partial agonists at porcine A_1AR.

The selectivity and good affinity of the 5′-methylthionocarbamoyl derivative of 2′-Me-CCPA (compound **4**) for A_1AR from different species combined with partial agonistic activity leads us to believe that this compound deserves further investigation to evaluate its effects on physiological and pathological processes that are selectively regulated by A_1 receptor agonists.

5. Experimental

5.1. General

Elemental analyses (C,H,N) were performed on an EA 1108 CHNS-O (Fisons Instruments) analyzer, and the results were within 0.4% of the theoretical values. Silica gel 60 (70–230 mesh, Merck) for column chromatography was used. Nuclear magnetic resonance ^{1}H NMR spectra were recorded with Varian VXR 300 MHz spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Mass spectra were obtained in a positive-ion mode on a series 1100 MSD detector HP spectrometer using an atmospheric pressure electrospray ionization (API-ESI). Ultraviolet spectra were recorded on an HP 8452 A diode array spectrophotometer.

5.2. Synthesis

5.2.1. General procedure for the amination of 25 and 26 into compounds 27–30. To a stirred solution of **25**[8] or **26**[8] (1.0 mmol) in absolute ethanol (20 mL), (*R*)-3-aminotetrahydrofuran toluene-4-sulfonate (1.6 mmol) and TEA (4.8 mmol) were added. The reaction mixture was refluxed for the time reported below and concentrated in vacuo. The residue was dissolved in anhydrous methanol (10 mL) and treated with sodium methoxide (0.5 M in MeOH) (5.8 mL) for 40 min at room temperature. The obtained solution was neutralized with Dowex 50 W × 8 (H^+ form) ion exchange resin and the resin was filtered off with MeOH washing. The filtrate was evaporated to dryness and the residue was purified by column chromatography.

5.2.2. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-β-D-ribofuranosyl)adenine (29). The title compound was synthesized from **25** (reaction time 17 h) and purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 94:6) as a white solid (95% yield). ^{1}H NMR (DMSO-d_6) δ 0.75 (s, 3H, CH_3), 1.90–2.25 (2m, 2H, tetrahydrofuranyl), 3.55–3.95 (3m, 7H, H-4′, H-5′, tetrahydrofuranyl), 4.05 (m, 1H, H-3′), 4.70 (m, 1H, NHC*H*), 5.20 (m, 3H, OH), 5.95 (s, 1H, H-1′), 7.98 (d, J = 6.6 Hz, 1H, NH), 8.22 (s, 1H, H-2), 8.50 (s, 1H, H-8). MS: *m*/*z* 352.2 $[M+H]^+$. Anal. ($C_{15}H_{21}N_5O_5$) C, H, N.

5.2.3. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-β-D-ribofuranosyl)adenine (30). The title compound was synthesized from **26** (reaction time 3 h) and purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 93:7) as a white solid (83% yield). ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H, CH_3), 2.0–2.20 (2m, 2H, tetrahydrofuranyl), 3.60–4.0 (4m, 8H, H-3′, H-4′, H-5′, tetrahydrofuranyl), 4.65 (m, 1H, NHC*H*), 5.20 (m, 2H, OH), 5.30 (s, 1H, OH), 5.83 (s, 1H, H-1′), 8.50 (s and d, 2H, H-8, NH). MS: *m*/*z* 386.2 $[M+H]^+$. Anal. ($C_{15}H_{20}ClN_5O_5$) C, H, N.

5.2.4. General procedure for the synthesis of 2′,3′-*O*-isopropylidene derivatives 31–34. A mixture of **27**,[8] **28**,[8] **29**, or **30** (1.0 mmol), 2,2-dimethoxypropane (18.1 mmol) and camphorsulfonic acid (1.0 mmol) in anhydrous acetone (10 mL) was stirred at 55 °C for the time reported below. The solvent was removed in vacuo and the residue was purified by column chromatography.

5.2.5. N^6-Cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine (31). The title compound was obtained starting from **27** (reaction time 4 h). Chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1) gave **31** as a white foam (84% yield). ^{1}H NMR (DMSO-d_6) δ 1.08 (s, 3H, CH_3), 1.32, 1.50 (2s, 6H, CH_3), 1.50–1.78 (m, 6H, cyclopentyl), 1.90 (m, 2H, cyclopentyl), 3.65 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.50 (m, 1H, NHC*H*), 4.55 (d, J = 2.2 Hz, 1H, H-3′), 5.38 (t, J = 5.3 Hz, 1H, OH), 6.18 (s, 1H, H-1′), 7.75 (d, J = 7.0 Hz, 1H, NH), 8.15 (s, 1H, H-2), 8.28 (s, 1H, H-8). MS: *m*/*z* 390.3 $[M+H]^+$. Anal. ($C_{19}H_{27}N_5O_4$) C, H, N.

5.2.6. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine (32). The title compound was synthesized starting from **28** (reaction time 4 h). Chromatography on a silica gel column ($CHCl_3$-EtOAc, 94:6) gave **32** as a white foam (77% yield). ^{1}H NMR (DMSO-d_6) δ 1.10 (s, 3H, CH_3), 1.35, 1.55 (2s, 6H, CH_3), 1.50–1.72 (2m, 6H, cyclopentyl), 1.92 (m, 2H, cyclopentyl), 3.70 (q, J = 4.5 Hz, 2H, H-5′), 4.23 (pseudo q, 1H, H-4′), 4.40 (m, 1H, NHC*H*), 4.58 (d, J = 2.2 Hz, 1H, H-3′), 5.26 (t, J = 5.6 Hz, 1H, OH), 6.12 (s, 1H, H-1′), 8.36 (s and d, 2H, H-8, NH). MS: *m*/*z* 424.9 $[M+H]^+$. Anal. ($C_{19}H_{26}ClN_5O_4$) C, H, N.

5.2.7. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine (33). The title compound was synthesized starting from **29** (reaction time 4.5 h). Chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4) gave **33** as a white foam (76% yield). ^{1}H NMR (DMSO-d_6) 1.10 (s, 3H, CH_3), 1.35, 1.55 (2s, 6H, CH_3), 2.0–2.20 (2 m, 2H, tetrahydrofuranyl), 3.60–3.95 (3m, 6H, H-5′, tetrahydrofuranyl), 4.23 (pseudo q, 1H, H-4′), 4.60 (d, J = 2.2 Hz, 1H, H-3′), 4.70 (m, 1H, NHC*H*), 5.40 (t, J = 5.7 Hz, 1H, OH), 6.23 (s, 1H, H-1′), 8.02 (d, J = 6.6 Hz, 1H, NH), 8.22 (s, 1H, H-2), 8.36 (s, 1H, H-8). MS: *m*/*z* 392.2 $[M+H]^+$. Anal. ($C_{18}H_{25}N_5O_5$) C, H, N.

5.2.8. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine (34). The title compound was obtained starting from **30** (reaction time 4.5 h). Chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) gave **34** as a white foam (90% yield). ^{1}H NMR (DMSO-d_6) δ 1.10 (s, 3H, CH_3), 1.35, 1.55 (2s, 6H, CH_3), 1.90–2.30 (2m, 2H, tetrahydrofura-

nyl), 3.60–3.95 (3m, 6H, H-5′, tetrahydrofuranyl), 4.23 (pseudo q, 1H, H-4′), 4.58 (d, $J = 2.2$ Hz, 1H, H-3′), 4.63 (m, 1H, NHC*H*), 5.27 (t, $J = 5.5$ Hz, 1H, OH), 6.14 (s, 1H, H-1′), 8.40 (s, 1H, H-8), 8.58 (d, $J = 6.6$ Hz, 1H, NH). MS: *m*/*z* 426.7 $[M+H]^+$. Anal. ($C_{18}H_{24}ClN_5O_5$) C, H, N.

5.2.9. General procedure for the synthesis of compounds 35–58 and 1–24. To a solution of **31–34** (1.0 mmol) in anhydrous THF (10 mL), CDI or thio CDI (6.0 mmol) was added and the reaction mixture was stirred at room temperature for the time reported below. Then water (50 μL) was added and the solution was stirred for 30 min. After that, the appropriate amine (1.5 mmol) was added stirring at room temperature for the time reported below. The solvent was evaporated to dryness and the residue was purified by chromatography to give the 2′,3′-isopropylidene-5′-substituted derivatives **35–58**. These compounds were deprotected by treatment with 70% HCOOH (10 mL) at 40 or 55 °C for various times, to give the desired compounds **1–24** after chromatographic purification.

5.2.10. N^6-Cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (35). Reaction of **31** with CDI for 8 h, followed by addition of benzylamine and stirring for 2 h, gave **35** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5) as a white foam (88% yield). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3 H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.75 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 4.20 (d, $J = 5.9$ Hz, 2H, CH_2C$_6$H$_5$), 4.35 (m, 3H, H-4′, H-5′), 4.50 (m, 1H, NHC*H*), 4.56 (br s, 1H, H-3′), 6.22 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.76 (d, $J = 7.3$ Hz, 1H, N*H*CH), 7.92 (t, $J = 5.9$ Hz, 1H, N*H*CH$_2$), 8.20 (s, 2H, H-2, H-8). MS: *m*/*z* 523.5 $[M+H]^+$. Anal. ($C_{27}H_{34}N_6O_5$) C, H, N.

5.2.11. N^6-Cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (1). Reaction of **35** with HCOOH at 40 °C for 6 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2), gave **1** as a white solid (50% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 1.50–1.72 (2m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 4.08 (m, 2H, H-5′), 4.18 (d, $J = 5.8$ Hz, 2H, CH_2C$_6$H$_5$), 4.22 (t, $J = 6.4$ Hz, 1H, H-4′), 4.42 (d, $J = 11.9$ Hz, 1H, H-3′), 4.48 (m, 1H, NHC*H*), 5.28 (s, 1H, OH), 5.42 (d, $J = 6.4$ Hz, 1H, OH), 5.95 (s, 1H, H-1′), 7.25 (m, 5H, arom.), 7.72 (d, $J = 7.9$ Hz, 1H, N*H*CH), 7.85 (t, $J = 5.9$ Hz, 1H, N*H*CH$_2$), 8.20 (s, 2H, H-2, H-8). MS: *m*/*z* 483.5 $[M+H]^+$. UV (MeOH): $\lambda_{max}(\varepsilon)$ 210 (23397), 268 (19832). Anal. ($C_{24}H_{30}N_6O_5$) C, H, N.

5.2.12. N^6-Cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (36). Reaction of **31** with thio CDI for 2.5 h, followed by addition of benzylamine and stirring for 4 h, gave **36** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5) as a white foam (58% yield). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.35, 1.55 (2s, 6H, CH_3), 1.50–1.75 (m, 6H, cyclopentyl), 1.92 (m, 2H, cyclopentyl), 4.45 (m, 2H, H-5′), 4.60–4.78 (2m, 5H, H-3′, H-4′, NHC*H*, CH_2C$_6$H$_5$), 6.22 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.75 (d, $J = 8.1$ Hz, 1H, N*H*CH), 8.18 (s, 1H, H-2), 8.22 (s, 1H, H-8), 9.85 (t, $J = 5.9$ Hz, 1H, N*H*CH$_2$). MS: *m*/*z* 539.5 $[M+H]^+$. Anal. ($C_{27}H_{34}N_6O_4S$) C, H, N.

5.2.13. N^6-Cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (2). Reaction of **36** with HCOOH at 40 °C for 6 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2), gave **2** as a white solid (73% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 1.50–1.72 (m, 6H, cyclopentyl), 1.92 (m, 2H, cyclopentyl), 4.10–4.35 (2m, 3H, H-4′, H-5′, NHC*H*), 4.55 (dd, $J = 6.6$, 12.5 Hz, 1H, H-5′), 4.65 (d, $J = 5.9$ Hz, 2H, CH_2C$_6$H$_5$), 4.88 (d, $J = 10.6$ Hz, 1H, H-3′), 5.30 (s, 1H, OH), 5.45 (d, $J = 6.2$ Hz, 1H, OH), 5.98 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.75 (d, $J = 7.7$ Hz, 1H, N*H*CH), 8.20 (s, 1H, H-2), 8.24 (s, 1H, H-8), 9.82 (t, $J = 6.2$ Hz, 1H, N*H*CH$_2$). MS: *m*/*z* 499.5 $[M+H]^+$. $\lambda_{max}(\varepsilon)$ 210 (22850), 269 (19450). Anal. ($C_{24}H_{30}N_6O_4S$) C, H, N.

5.2.14. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)-adenine (37). Reaction of **32** with CDI for 6 h, followed by addition of methylamine and stirring for 2 h, gave **37** as a white foam (62% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3 H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.75 (m, 6H, cyclopentyl), 1.92 (m, 2H, cyclopentyl), 2.58 (d, $J = 4.4$ Hz, 3H, NHCH_3), 4.30 (m, 3H, H-4′, H-5′), 4.40 (m, 1H, NHC*H*), 4.54 (d, $J = 2.2$ Hz, 1H, H-3′), 6.15 (s, 1H, H-1′), 7.2 (q, $J = 4.4$ Hz, 1H, N*H*CH$_3$), 8.20 (s, 1H, H-8), 8.38 (d, $J = 7.7$ Hz, 1H, N*H*CH). MS: *m*/*z* 481.9 $[M+H]^+$. Anal. ($C_{21}H_{29}ClN_6O_5$) C, H, N.

5.2.15. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)adenine (3). Reaction of **37** with HCOOH at 55 °C for 4 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3), gave **3** as a white solid (86% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 1.60, 1.90 (2m, 8H, cyclopentyl), 2.55 (d, $J = 4.8$ Hz, 3H, NHCH_3), 4.02 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (m, 2H, NHC*H*, H-3′), 5.35 (s, 1H, OH), 5.42 (d, $J = 6.2$ Hz, 1H, OH), 5.85 (s, 1H, H-1′), 7.15 (q, $J = 4.5$ Hz, 1H, N*H*CH$_3$), 8.20 (s, 1H, H-8), 8.38 (d, $J = 7.7$ Hz, 1H, N*H*CH). MS: *m*/*z* 441.8 $[M+H]^+$. UV (MeOH): $\lambda_{max}(\varepsilon)$ 214 (25320), 244 (17241), 268 (21300). Anal. ($C_{18}H_{25}ClN_6O_5$) C, H, N.

5.2.16. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (38). Reaction of **32** with thio CDI for 2 h, followed by addition of methylamine and stirring overnight, gave **38** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1) as a white foam (92% yield). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.78 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 2.88 (d, $J = 4.4$ Hz, 3H, NHCH_3), 4.42 (m, 2H, H-5′), 4.62 (d, $J = 3.3$ Hz,

1H, H-3′), 4.70 (m, 2H, H-4′, NHC*H*), 6.15 (s, 1H, H-1′), 8.22 (s, 1H, H-8), 8.38 (d, J = 7.3 Hz, 1H, N*H*CH), 9.25 (q, J = 4.4 Hz, 1H, N*H*CH_3). MS: *m/z* 498.0 $[M+H]^+$. Anal. ($C_{21}H_{29}ClN_6O_4S$) C, H, N.

5.2.17. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (4). Reaction of **38** with HCOOH at 40 °C for 4 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1), gave **4** as a white solid (56% yield). ^1H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 1.60, 1.90 (2m, 8H, cyclopentyl), 2.86 (d, J = 4.4 Hz, 3H, NHCH_3), 4.10 (m, 2H, H-5′), 4.45 (m, 2H, H-4′, NHC*H*), 4.80 (m, 1H, H-3′), 5.35 (s, 1H, OH), 5.48 (d, J = 6.2 Hz, 1H, OH), 5.86 (s, 1H, H-1′), 8.22 (s, 1H, H-8), 8.35 (d, J = 8.1 Hz, 1H, N*H*CH), 7.25 (q, J = 4.4 Hz, 1H, N*H*CH_3). MS: *m/z* 457.9 $[M+H]^+$. UV (MeOH): $\lambda_{max}(\varepsilon)$ 216 (26233), 244 (17718), 272 (22017). Anal. ($C_{18}H_{25}ClN_6O_4S$) C, H, N.

5.2.18. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)-adenine (39). Reaction of **32** with CDI for 3 h, followed by addition of propylamine and stirring for 2 h, gave **39** which was purified by chromatography on a silica gel column ($CHCl_3$) as a white foam (92% yield). ^1H NMR (DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.78 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 2.95 (q, J = 6.8 Hz, 2H, CH_2CH_3), 3.60 (q, J = 6.6 Hz, 2H, NHCH_2), 4.20–4.45 (m, 4H, H-4′, H-5′, NHC*H*), 4.55 (br s, 1H, H-3′), 6.15 (s, 1H, H-1′), 7.35 (t, J = 5.7 Hz, 1H, N*H*CH_2), 8.20 (s, 1H, H-8), 8.38 (d, J = 7.7 Hz, 1H, N*H*CH). MS: *m/z* 510 $[M+H]^+$. Anal. ($C_{23}H_{33}ClN_6O_5$) C, H, N.

5.2.19. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)adenine (5). Reaction of **39** with HCOOH at 40 °C for 4 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1), gave **5** as a white solid (87% yield). ^1H NMR (DMSO-d_6) δ 0.82 (s and t, 6H, CH_3, CH_2CH_3), 1.40 (q, J = 7.0 Hz, 2H, CH_2CH_3), 1.50–1.78 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 2.95 (q, J = 6.6 Hz, 2H, NHCH_2), 4.02 (br s, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (m, 2H, H-3′, NHC*H*), 5.35 (s, 1H, OH), 5.40 (pseudo t, 1H, OH), 5.86 (s, 1H, H-1′), 7.30 (t, J = 5.9 Hz, 1H, N*H*CH_2), 8.20 (s, 1H, H-8), 8.38 (d, J = 7.7 Hz, 1H, N*H*CH). MS: *m/z* 469.9 $[M+H]^+$. Anal. ($C_{20}H_{29}ClN_6O_5$) C, H, N.

5.2.20. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (40). Reaction of **32** with thio CDI for 6 h, followed by addition of propylamine and stirring for 1 h, gave **40** as a white foam (91% yield) after chromatography on a silica gel column ($CHCl_3$). ^1H NMR (DMSO-d_6) δ 0.85 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.78 (m, 6H, cyclopentyl), 1.92 (m, 2H, cyclopentyl), 2.95 (q, J = 6.8 Hz, 2H, CH_2CH_3), 3.70 (q, J = 6.6 Hz, 2H, NHCH_2), 4.20–4.45 (m, 4H, H-4′, H-5′, NHC*H*), 4.70 (m, 1H, H-3′), 6.15 (s, 1H, H-1′), 8.40 (s, d, 2H, H-8, N*H*CH), 9.38 (t, J = 6.0 Hz, 1H, N*H*CH_2). MS: *m/z* 526.1 $[M+H]^+$. Anal. ($C_{23}H_{33}ClN_6O_4S$) C, H, N.

5.2.21. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (6). Reaction of **40** with HCOOH at 55 °C for 2 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5), gave **6** as a white solid (55% yield). ^1H NMR (DMSO-d_6) δ 0.82 (s and t, 6 H, CH_3, CH_2CH_3), 1.44-1.74 (m, 8H, CH_2CH_3, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 3.30 (q, J = 6.6 Hz, 2H, NHCH_2), 4.10 (m, 2H, H-5′), 4.45 (m, 2H, H-4′, NHC*H*), 4.85 (d, J = 11.7 Hz, 1H, H-3′), 5.40 (s, 1H, OH), 5.50 (pseudo t, 1H, OH), 5.90 (s, 1H, H-1′), 8.25 (s, 1H, H-8), 8.40 (d, J = 7.7 Hz, 1H, N*H*CH), 9.32 (t, J = 5.9 Hz, 1H, N*H*CH_2). MS: *m/z* 486.0 $[M+H]^+$. Anal. ($C_{20}H_{29}ClN_6O_4S$) C, H, N.

5.2.22. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)-adenine (41). Reaction of **32** with CDI for 8 h, followed by addition of benzylamine and stirring for 2 h, gave **41** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5) as a white foam (87% yield). ^1H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.76 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 4.20 (d, J = 5.9 Hz, 2H, $CH_2C_6H_5$), 4.35 (m, 4H, H-4′, H-5′, NHC*H*), 4.55 (br s, 1H, H-3′), 6.15 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.95 (t, J = 5.9 Hz, N*H*CH_2), 8.20 (s, 1H, H-8), 8.40 (d, J = 7.7 Hz, 1H, N*H*CH). MS: *m/z* 558.0 $[M+H]^+$. Anal. ($C_{27}H_{33}ClN_6O_5$) C, H, N.

5.2.23. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (7). Reaction of **41** with HCOOH at 55 °C for 6 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3), gave **7** as a white solid (77% yield). ^1H NMR (DMSO-d_6): δ 0.8 (s, 3 H, CH_3), 1.46–1.76 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 4.02 (m, 2H, H-5′), 4.20 (d, J = 6.2 Hz, 2H, $CH_2C_6H_5$), 4.25 (m, 1H, NHC*H*), 4.45 (m, 2H, H-3′, H-4′), 5.35 (s, 1H, OH), 5.45 (m, 1H, OH), 5.86 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.88 (m, N*H*CH_2), 8.23 (s, 1H, H-8), 8.38 (d, J = 8.1 Hz, 1H, N*H*CH). MS: *m/z* 518.0 $[M+H]^+$. UV (MeOH): $\lambda_{max}(\varepsilon)$ 210 (25248), 250 (21365), 265 (19326). Anal. ($C_{24}H_{29}ClN_6O_5$) C, H, N.

5.2.24. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (42). Reaction of **32** with thio CDI for 5 h, followed by addition of benzylamine and stirring for 1 h, gave **42** as a white foam (77% yield) after chromatograpy on a silica gel column ($CHCl_3$). ^1H NMR (DMSO-d_6) δ 1.20 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50–1.75 (m, 6H, cyclopentyl), 1.94 (m, 2H, cyclopentyl), 4.45 (m, 2H, H-5′), 4.60–4.80 (2m, 5H, H-3′, H-4′, $CH_2C_6H_5$, NHC*H*), 6.15 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 8.25 (s, 1H, H-8), 8.40 (d, J = 8.8 Hz, 1H, N*H*CH), 9.88 (t, J = 5.9 Hz, 1H, N*H*CH_2). MS: *m/z* 574.1 $[M+H]^+$. Anal. ($C_{27}H_{33}ClN_6O_4S$) C, H, N.

5.2.25. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (8). Reaction of **42** with HCOOH at 55 °C for 3.5 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3), gave **8** as a white solid (80% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 1.50–1.72 (2m, 6H, cyclopentyl), 1.95 (m, 2H, cyclopentyl), 4.12 (m, 2H, H-5′), 4.20 (m, 1H, NHC*H*), 4.52 (m, 1H, H-4′), 4.65 (m, 2H, C$H_2C_6H_5$), 4.88 (d, J = 11.9 Hz, 1H, H-3′), 5.38 (s, 1H, OH), 5.50 (d, J = 6.4 Hz, 1H, OH), 5.86 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 8.23 (s, 1H, H-8), 8.38 (d, J = 7.6 Hz, 1H, N*H*CH), 9.82 (t, J = 5.9 Hz, 1H, N*H*CH_2). MS: *m*/*z* 534.0 $[M+H]^+$. UV (MeOH): $\lambda_{max}(\varepsilon)$ 210 (25785), 250 (21590), 264 (19920). Anal. ($C_{24}H_{29}ClN_6O_4S$) C, H, N.

5.2.26. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-cyclopentylcarbamoyl-β-D-ribofuranosyl)adenine (43). Reaction of **32** with CDI for 3 h, followed by addition of cyclopentylamine and stirring overnight, gave **43** as a white foam (93% yield) after chromatography on a silica gel column ($CHCl_3$). ^{1}H-NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.30–1.50 (2m, 4H, cyclopentyl), 1.54 (s, 3H, CH_3), 1.50–1.78 (3m, 10H, cyclopentyl), 1.95 (m, 2H, cyclopentyl), 3.78 (m, 1H, NHC*H*), 4.30 (2m, 3H, H-5′, NHC*H*), 4.40 (m, 1H, H-4′), 4.54 (d, J = 2.3 Hz, 1H, H-3′), 6.15 (s, 1H, H-1′), 7.35 (d, J = 7.4 Hz, 1H, N*H*CH), 8.20 (s, 1H, H-8), 8.40 (d, J = 7.4 Hz, 1H, N*H*CH). MS: *m*/*z* 536.0 $[M+H]^+$. Anal. ($C_{25}H_{35}ClN_6O_5$) C, H, N.

5.2.27. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-cyclopentylcarbamoyl-β-D-ribofuranosyl)adenine (9). Reaction of **43** with HCOOH at 40 °C for 2 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2), gave **9** as a white solid (58% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 1.30–2.0 (2m, 16H, cyclopentyl), 3.75 (m, 1H, NHC*H*), 4.0–4.22 (m, 3H, H-5′, NHC*H*), 4.40 (m, 2H, H-3′, H-4′), 5.35 (s, 1H, OH), 5.45 (pseudo t, 1H, OH), 5.86 (s, 1H, H-1′), 7.30 (d, J = 7.3 Hz, 1H, N*H*CH), 8.20 (s, 1H, H-8), 8.40 (d, J = 8.1 Hz, 1H, N*H*CH). MS: *m*/*z* 496.0 $[M+H]^+$. Anal. ($C_{22}H_{31}ClN_6O_5$) C, H, N.

5.2.28. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-cyclopentylthiocarbamoyl-β-D-ribofuranosyl)adenine (44). Reaction of **32** with thio CDI for 4 h, followed by addition of cyclopentylamine and stirring overnight, gave **44** as a white foam (95% yield) after chromatography on a silica gel column ($CHCl_3$).^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.46–1.74 (m, 12H, cyclopentyl), 1.90 (m, 4H, cyclopentyl), 3.70 (m, 1H, NHC*H*), 4.20–4.45 (m, 4H, H-4′, H-5′, NHC*H*), 4.70 (m, 1H, H-3′), 6.15 (s, 1H, H-1′), 7.15 (d, J = 7.1 Hz, 1H, N*H*CH), 8.22 (s, 1H, H-8), 9.35 (d, J = 7.0 Hz, 1H, N*H*CH). MS: *m*/*z* 552.1 $[M+H]^+$. Anal. ($C_{25}H_{35}ClN_6O_4S$) C, H, N.

5.2.29. 2-Chloro-N^6-cyclopentyl-9*H*-(2-*C*-methyl-5-*N*-cyclopentylthiocarbamoyl-β-D-ribofuranosyl)adenine (10). Reaction of **44** with HCOOH at 55 °C for 2 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 99.5:0.5), gave **10** as a white solid (50% yield). ^{1}H NMR (DMSO-d_6) δ 0.82 (s, 3H, CH_3), 1.40–1.78 (m, 12H, cyclopentyl), 1.94 (m, 4H, cyclopentyl), 4.12 (m, 2H, H-5′), 4.30 (m, 1H, NHC*H*), 4.40 (m, 2H, H-4′, NHC*H*), 4.82 (br d, J = 12.1 Hz, 1H, H-3′), 5.38 (s, 1H, OH), 5.50 (pseudo t, 1H, OH), 5.88 (s, 1H, H-1′), 8.22 (s, 1H, H-8), 8.38 (d, J = 7.7 Hz, 1H, N*H*CH), 9.30 (d, J = 7.3 Hz, 1H, N*H*CH). MS: *m*/*z* 512.0 $[M+H]^+$. Anal. ($C_{22}H_{31}ClN_6O_4S$) C, H, N.

5.2.30. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)adenine (45). Reaction of **33** with CDI for 8 h, followed by addition of methylamine and stirring overnight, gave the **45** as a white foam (72% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4). ^{1}H NMR (DMSO-d_6) δ 1.12 (s, 3H, CH_3), 1.32, 1.52 (2s, 6H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 2.58 (d, J = 4.4 Hz, 3H, NHCH_3), 3.60–3.95 (m, 4H, tetrahydrofuranyl), 4.30 (m, 3H, H-4′, H-5′), 4.58 (d, J = 1.8 Hz, 1H, H-3′), 4.70 (m, 1H, NHC*H*), 6.22 (s, 1H, H-1′), 7.20 (q, J = 4.7 Hz, 1H, N*H*CH_3), 8.0 (d, J = 5.7 Hz, 1H, N*H*CH), 8.22 (s, 2H, H-2, H-8). MS: *m*/*z* 448.5 $[M+H]^+$. Anal. ($C_{20}H_{28}N_6O_6$) C, H, N.

5.2.31. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)adenine (11). Reaction of **45** with HCOOH at 55 °C for 3.5 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4), gave **11** as a white solid (71% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 2.55 (d, J = 4.0 Hz, 3H, NHCH_3), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 4.05 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (d, J = 12.5 Hz, 1H, H-3′), 4.65 (m, 1H, NHC*H*), 5.30 (s, 1H, OH), 5.42 (d, J = 5.9 Hz, 1H, OH), 5.96 (s, 1H, H-1′), 7.15 (q, J = 4.7 Hz, 1H, N*H*CH_3), 8.0 (d, J = 5.7 Hz, 1H, N*H*CH), 8.22 (s, 1H, H-2), 8.24 (s, 1H, H-8). MS: *m*/*z* 409.4 $[M+H]^+$. Anal. ($C_{17}H_{24}N_6O_6$) C, H, N.

5.2.32. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (46). Reaction of **33** with thio CDI for 8 h, followed by addition of methylamine and stirring overnight, gave **46** as a white foam (93% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.86 (d, J = 4.8 Hz, 3H, NHCH_3), 3.60–3.95 (m, 4H, tetrahydrofuranyl), 4.45 (m, 1H, H-5′), 4.62 (d, J = 2.6 Hz, 1H, H-3′), 4.70 (m, 4H, H-4′, H-5′, NHC*H*), 6.25 (s, 1H, H-1′), 8.05 (d, J = 5.7 Hz, 1H, N*H*CH), 8.25 (s, 2H, H-2, H-8), 9.28 (q, J = 4.8 Hz, 1H, N*H*CH_3). MS: *m*/*z* 465.4 $[M+H]^+$. Anal. ($C_{20}H_{28}N_6O_5S$) C, H, N.

5.2.33. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (12). Reaction of **46** with HCOOH at 55 °C for 3 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5), gave **12** as a white solid (63% yield). ^{1}H NMR (DMSO-d_6): δ 0.8 (s, 3H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 2.86 (d, J = 4.4 Hz, 3H, NHCH_3), 3.58–3.98 (m, 4H, tetrahydrofuranyl), 4.15 (m, 2H, H-5′), 4.50 (m, 1H, H-4′), 4.70 (m, 1H, NHC*H*),

4.85 (d, J = 12.5 Hz, 1H, H-3′), 5.32 (s, 1H, OH), 5.46 (d, J = 6.2 Hz, 1H, OH), 6.0 (s, 1H, H-1′), 8.0 (d, J = 5.7 Hz, 1H, N*H*CH), 8.25 (s, 2H, H-2, H-8), 9.25 (q, J = 4.4 Hz, 1H, N*H*CH_3). MS: *m/z* 425.5 $[M+H]^+$. Anal. ($C_{17}H_{24}N_6O_5S$) C, H, N.

5.2.34. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)adenine (47). Reaction of **33** with CDI for 6 h, followed by addition of propylamine and stirring for 1 h, gave **47** as a white foam (68% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4). ^{1}H NMR (DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18 (s, 3H, CH_3), 1.38 (s and m, 5H, CH_3, CH_2CH_3), 1.56 (s, 3H, CH_3), 2.05, 2.20 (2m, 2H, tetrahydrofuranyl), 2.95 (q, J = 6.6 Hz, 2H, $CH_2CH_2CH_3$), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.30 (m, 3H, H-4′, H-5′), 4.56 (d, J = 2.2 Hz, 1H, H-3′), 4.70 (m, 1H, NHC*H*), 6.24 (s, 1H, H-1′), 7.38 (t, J = 5.5 Hz, 1H, N*H*CH_2), 8.05 (d, J = 5.9 Hz, 1H, N*H*CH), 8.22 (s, 1H, H-2), 8.26 (s, 1H, H-8). MS: *m/z* 477.5 $[M+H]^+$. Anal. ($C_{22}H_{32}N_6O_6$) C, H, N.

5.2.35. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)adenine (13). Reaction of **47** with HCOOH at 55 °C for 7 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5), gave **13** as a white solid (70% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s and t, 6H, CH_3, CH_2CH_3), 1.38 (m, 2H, CH_2CH_3), 1.98, 2.20 (2m, 2H, tetrahydrofuranyl), 2.90 (q, J = 6.6 Hz, 2H, NHCH_2), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.0–4.20 (m, 3H, H-4′, H-5′), 4.40 (d, J = 11.7 Hz, H-3′), 4.70 (m, 1H, NHC*H*), 5.30 (s, 1H, OH), 5.42 (d, J = 6.2 Hz, 1H, OH), 5.95 (s, 1H, H-1′), 7.28 (t, J = 4.7 Hz, 1H, N*H*CH_2), 8.0 (d, J = 6.2 Hz, 1H, N*H*CH), 8.22 (s, 2H, H-2, H-8). MS: *m/z* 437.5 $[M+H]^+$. Anal. ($C_{19}H_{28}N_6O_6$) C, H, N.

5.2.36. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (48). Reaction of **34** with thio CDI for 8 h, followed by addition of propylamine and stirring overnight, gave **48** as a white foam (64% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.50 (m, 2H, CH_2CH_3), 2.05, 2.20 (2m, 2H, tetrahydrofuranyl), 3.35 (q, J = 6.6 Hz, 2H, NHCH_2), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.45 (m, 2H, H-5′), 4.62 (d, J = 3.0 Hz, 1H, H-3′), 4.72 (m, 2H, H-4′, NHC*H*), 6.26 (s, 1H, H-1′), 8.05 (d, J = 5.9 Hz, 1H, N*H*CH), 8.26 (s, 1H, H-2), 8.28 (s, 1H, H-8), 9.40 (t, J = 5.9 Hz, 1H, N*H*CH_2). MS: *m/z* 492.6 $[M+H]^+$. Anal. ($C_{22}H_{32}N_6O_5S$) C, H, N.

5.2.37. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (14). Reaction of **48** with HCOOH at 55 °C for 4 h gave **14** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4) as a white foam (62% yield). ^{1}H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 0.84 (t, J = 7.3 Hz, CH_2CH_3), 1.50 (m, 2H, CH_2CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.30 (t after exchange with D_2O, J = 6.9 Hz, 2H, NHCH_2), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 4.15 (m, 2H, H-5′), 4.50 (m, 1H, H-4′), 4.70 (m, 1H, NHC*H*), 4.84 (d, J = 10.6 Hz, 1H, H-3′), 5.40 (br s, 2H, OH), 6.0 (s, 1H, H-1′), 8.03 (d, J = 5.5 Hz, 1H, N*H*CH), 8.25 (s, 1H, H-2), 8.27 (s, 1H, H-8), 9.30 (t, J = 5.9 Hz, 1H, N*H*CH_2). MS: *m/z* 453.6 $[M+H]^+$. Anal. ($C_{19}H_{28}N_6O_5S$) C, H, N.

5.2.38. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (49). Reaction of **33** with CDI for 9 h, followed by addition of benzylamine and stirring overnight, gave **49** as a white foam (92% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2). ^{1}H NMR (DMSO-d_6) δ 1.16 (s, 1H, CH_3), 1.35, 1.56 (s, 6H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.60–3.90 (m, 4H, tetrahydrofuranyl), 4.20 (d, J = 6.2 Hz, 2H, $CH_2C_6H_5$), 4.35 (m, 3H, H-4′, H-5′), 4.58 (d, J = 2.1 Hz, 1H, H-3′), 4.72 (m, 1H, NHC*H*), 6.25 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 7.94 (t, J = 6.0 Hz, 1H, N*H*CH_2), 8.04 (d, J = 4.4 Hz, 1H, N*H*CH). 8.24 (s, 2H, H-2, H-8). MS: *m/z* 525.5 $[M+H]^+$. Anal. ($C_{26}H_{32}N_6O_6$) C, H, N.

5.2.39. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (15). Reaction of **49** with HCOOH at 55 °C for 2 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4), gave **15** as a white solid (60% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 2.0, 2.18 (2m, 2H, tetrahydrofuranyl), 3.60–3.95 (m, 4H, tetrahydrofuranyl), 4.18 (m, 5H, $CH_2C_6H_5$, H-4′, H-5′), 4.45 (d, J = 10.6 Hz, 1H, H-3′), 4.70 (m, 1H, NHC*H*), 5.30 (s, 1H, OH), 5.42 (d, J = 5.9 Hz, 1H, OH), 5.97 (s, 1H, H-1′), 7.25 (m, 5H, arom.), 7.85 (t, J = 6.2 Hz, 1H, N*H*CH_2), 8.0 (d, J = 6.6 Hz, 1H, N*H*CH), 8.22 (s, 2H, H-2, H-8). MS: *m/z* 485.4 $[M+H]^+$. Anal. ($C_{23}H_{28}N_6O_6$) C, H, N.

5.2.40. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (50). Reaction of **33** with thio CDI for 9 h, followed by addition of benzylamine and stirring overnight, gave **50** as a white foam (92% yield) after chromatography on a silica gel column ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 1H, CH_3), 1.38, 1.58 (s, 6H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.60–3.95 (m, 4H, tetrahydrofuranyl), 4.44 (m, 2H, H-5′), 4.60–4.80 (2m, 5H, H-3′, H-4′, $CH_2C_6H_5$, NHC*H*), 6.25 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 8.0 (d, J = 5.9 Hz, 1H, N*H*CH), 8.22 (s, 2H, H-2, H-8), 9.82 (t, J = 5.7 Hz, 1H, N*H*CH_2). MS: *m/z* 541.5 $[M+H]^+$. Anal. ($C_{26}H_{32}N_6O_5S$) C, H, N.

5.2.41. N^6-(*R*)-3-Tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (16). Reaction of **50** with HCOOH at 55 °C for 4.5 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3), gave **16** as a white solid (60% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.60–3.95 (m, 4H, tetrahydrofuranyl), 4.20 (m, 2H, H-5′), 4.50 (m, 1H, H-4′), 4.60 (br s, 2H, $CH_2C_6H_5$), 4.70 (m, 1H, NHC*H*), 4.88 (d,

J = 11.7 Hz, 1H, H-3′), 5.32 (s, 1H, OH), 5.50 (d, J = 6.0 Hz, 1H, OH), 5.98 (s, 1H, H-1′), 7.28 (m, 5H, arom.), 8.0 (d, J = 5.7 Hz, 1H, NHCH), 8.22 (s, 1H, H-2), 8.26 (s, 1H, H-8), 9.82 (t, J = 5.7 Hz, 1H, $NHCH_2$). MS: m/z 501.5 $[M+H]^+$. Anal. ($C_{23}H_{28}N_6O_5S$) C, H, N.

5.2.42. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)adenine (51). Reaction of **34** with CDI for 6 h, followed by addition of methylamine and stirring for 2 h, gave **51** as a white foam (93% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2). ^1H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.58 (d, J = 4.4 Hz, 3H, $NHCH_3$), 3.58–3.94 (m, 4H, tetrahydrofuranyl), 4.30 (m, 3H, H-4′, H-5′), 4.55 (d, J = 2.2 Hz, 1H, H-3′), 4.62 (m, 1H, NHC*H*), 6.15 (s, 1H, H-1′), 7.20 (q, J = 4.8 Hz, 1H, $NHCH_3$), 8.24 (s, 1H, H-8), 8.60 (d, J = 5.7 Hz, 1H, NHCH). MS: m/z 483.9 $[M+H]^+$. Anal. ($C_{20}H_{27}ClN_6O_6$) C, H, N.

5.2.43. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-methylcarbamoyl-β-D-ribofuranosyl)adenine (17). Reaction of **51** with HCOOH at 55 °C for 6 h gave **17** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4) as a white solid (69% yield). ^1H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.58 (d, J = 4.0 Hz, 3H, $NHCH_3$), 3.60–3.96 (m, 4H, tetrahydrofuranyl), 4.05 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (d, J = 11.2 Hz, 1H, H-3′), 4.62 (m, 1H, NHC*H*), 5.38 (s, 1H, OH), 5.45 (br s, 1H, OH), 5.9 (s, 1H, H-1′), 7.18 (m, 1H, $NHCH_3$), 8.24 (s, 1H, H-8), 8.60 (d, J = 5.7 Hz, 1H, NHCH). MS: m/z 443.9 $[M+H]^+$. Anal. ($C_{17}H_{23}ClN_6O_6$) C, H, N.

5.2.44. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (52). Reaction of **34** with thio CDI for 6.5 h, followed by addition of methylamine and stirring for 2 h, gave **52** as a white foam (69% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1). ^1H NMR (DMSO-d_6) δ 1.18 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.86 (d, J = 4.0 Hz, 3H, $NHCH_3$), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.45 (m, 1H, H-4′), 4.62 (d, J = 2.9 Hz, 1H, H-3′), 4.70 (m, 3H, H-5′, NHC*H*), 6.15 (s, 1H, H-1′), 8.24 (s, 1H, H-8), 8.60 (d, J = 6.2 Hz, 1H, NHCH), 9.28 (q, J = 4.4 Hz, 1H, $NHCH_3$). MS: m/z 499 $[M+H]^+$. Anal. ($C_{20}H_{27}ClN_6O_5S$) C, H, N.

5.2.45. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-methylthiocarbamoyl-β-D-ribofuranosyl)adenine (18). Reaction of **52** with HCOOH at 55 °C for 3 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 94:6), gave **18** as a white solid (88% yield). ^1H NMR (DMSO-d_6) δ 0.8 (s, 3H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.86 (d, J = 4.0 Hz, 3H, $NHCH_3$), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.12 (m, 2H, H-5′), 4.60 (m, 2H, H-4′, NHC*H*), 4.85 (m, 1H, H-3′), 5.40 (s, 1H, OH), 5.50 (pseudo t, 1H, OH), 5.90 (s, 1H, H-1′), 8.30 (s, 1H, H-8), 8.62 (d, J = 5.7 Hz, 1H, NHCH), 9.25 (q, J = 4.4 Hz, 1H, $NHCH_3$). MS: m/z 459.9 $[M+H]^+$. Anal. ($C_{17}H_{23}ClN_6O_5S$) C, H, N.

5.2.46. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)adenine (53). Reaction of **34** with CDI for 7 h, followed by addition of propylamine and stirring for 1 h, gave **53** as a white foam (77% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2). ^1H NMR (DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18 (s, 3H, CH_3), 1.40 (s and q, 5H, CH_3, CH_2CH_3), 1.56 (s, 3H, CH_3), 2.0, 2.20 (m, 2H, tetrahydrofuranyl), 2.95 (q, J = 6.6 Hz, 2H, $NHCH_2$), 3.58–3.96 (m, 4H, tetrahydrofuranyl), 4.30 (m, 3H, H-4′, H-5′), 4.56 (br s, 1H, H-3′), 4.62 (m, 1H, NHC*H*), 6.16 (s, 1H, H-1′), 7.36 (t, J = 5.9 Hz, 1H, $NHCH_2$), 8.24 (s, 1H, H-8), 8.60 (d, J = 6.2 Hz, 1H, NHCH). MS: m/z 511.9 $[M+H]^+$. Anal. ($C_{22}H_{31}ClN_6O_6$) C, H, N.

5.2.47. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-propylcarbamoyl-β-D-ribofuranosyl)adenine (19). Reaction of **53** with HCOOH at 55 °C for 3 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 96:4), gave **19** as a white solid (75% yield). ^1H NMR (DMSO-d_6) δ 0.80 (s and t, 6H, CH_3, CH_2CH_3), 1.40 (m, 2H, CH_2CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 2.92 (q, J = 6.6 Hz, 2H, $NHCH_2$), 3.58–3.94 (m, 4H, tetrahydrofuranyl), 4.0 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (d, J = 11.0 Hz, H-3′), 4.60 (m, 1H, NHC*H*), 5.38 (s, 1H, OH), 5.45 (pseudo t, 1H, OH), 5.90 (s, 1H, H-1′), 7.30 (t, J = 5.9 Hz, 1H, $NHCH_2$), 8.25 (s, 1H, H-8), 8.60 (d, J = 5.7 Hz, 1H, NHCH). MS: m/z 471.9 $[M+H]^+$. Anal. ($C_{19}H_{27}ClN_6O_6$) C, H, N.

5.2.48. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (54). Reaction of **34** with thio CDI for 6 h, followed by addition of propylamine and stirring overnight, gave **54** as a white foam (75% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2). ^1H NMR (DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.18, 1.38 (s, 6H, CH_3), 1.54, (s and q, 3H, $CH_3CH_2CH_3$), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.30 (t after exchange with D_2O, J = 6.9 Hz, 2H, $NHCH_2$), 3.58–3.94 (m, 4H, tetrahydrofuranyl), 4.44 (m, 2H, H-5′), 4.60 (d, J = 3.0 Hz, 1H, H-3′), 4.70 (m, 2H, H-4′, NHC*H*), 6.16 (s, 1H, H-1′), 8.26 (s, 1H, H-8), 8.58 (d, J = 5.9 Hz, 1H, NHCH), 9.35 (t, J = 5.5 Hz, 1H, $NHCH_2$). MS: m/z 528.1 $[M+H]^+$. Anal. ($C_{22}H_{31}ClN_6O_5S$) C, H, N.

5.2.49. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-propylthiocarbamoyl-β-D-ribofuranosyl)adenine (20). Reaction of **54** with HCOOH at 55 °C for 3 h gave **20** which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (59% yield). ^1H NMR (DMSO-d_6) δ 0.80 (s and t, 6H, CH_3, CH_2CH_3), 1.50 (m, 2H, CH_2CH_3), 1.95, 2.20 (2m, 2H, tetrahydrofuranyl), 3.30 (t after exchange with D_2O, J = 6.9 Hz, 2H, $NHCH_2$), 3.58–3.94 (m, 4H, tetrahydrofuranyl), 4.12 (m, 2H, H-5′), 4.48 (m, 1H, H-4′), 4.62 (m, 1H, NHC*H*), 4.84 (d, J = 12.2 Hz,

1H, H-3′), 5.36 (s, 1H, OH), 5.48 (pseudo t, 1H, OH), 5.88 (s, 1H, H-1′), 8.25 (s, 1H, H-8), 8.58 (d, J = 5.7 Hz, 1H, N*H*CH), 9.30 (t, J = 5.5 Hz, 1H, N*H*CH_2). MS: *m*/*z* 487.9 $[M+H]^+$. Anal. ($C_{19}H_{27}ClN_6O_5S$) C, H, N.

5.2.50. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (55). Reaction of **34** with CDI for 5 h, followed by addition of benzylamine and stirring for 1.5 h, gave **55** as a white foam (55% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3). ^{1}H NMR (DMSO-d_6) δ 1.15 (s, 1H, CH_3), 1.35, 1.54 (2s, 6H, CH_3), 1.95, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.92 (m, 4H, tetrahydrofuranyl), 4.20 (m, 3H, H-4′, H-5′), 4.32 (m, 2H, C*H*$_2$$C_6H_5$), 4.55 (d, J = 2.1 Hz, 1H, H-3′), 4.60 (m, 1H, NHC*H*), 6.16 (s, 1H, H-1′), 7.25 (m, 5H, arom.), 7.90 (t, J = 5.7 Hz, 1H, N*H*CH_2), 8.25 (s, 1H, H-8), 8.58 (d, J = 5.9 Hz, 1H, N*H*CH). MS: *m*/*z* 560.1 $[M+H]^+$. Anal. ($C_{26}H_{31}ClN_6O_6$) C, H, N.

5.2.51. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-benzylcarbamoyl-β-D-ribofuranosyl)adenine (21). Reaction of **55** with HCOOH at 55 °C for 8 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5), gave **21** as a white solid (58% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 4.08 (m, 2H, H-5′), 4.20 (m, 2H, C*H*$_2$$C_6H_5$), 4.28 (m, 1H, H-4′), 4.45 (d, J = 10.6 Hz, 1H, H-3′), 4.62 (m, 1H, NHC*H*), 5.38 (s, 1H, OH), 5.48 (pseudo t, 1H, OH), 5.90 (s, 1H, H-1′), 7.30 (m, 5H, arom.), 7.88 (t, J = 5.6 Hz, 1H, N*H*CH_2), 8.28 (s, 1H, H-8), 8.58 (d, J = 5.7 Hz, 1H, N*H*CH). MS: *m*/*z* 519.9 $[M+H]^+$. Anal. ($C_{23}H_{27}ClN_6O_6$) C, H, N.

5.2.52. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (56). Reaction of **34** with thio CDI for 6 h, followed by addition of benzylamine and stirring for 1.5 h, gave **56** as a white foam (53% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3). ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 1H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 4.46 (m, 1H, H-4′), 4.55–4.80 (m, 5H, H-3′, H-5′, C*H*$_2$$C_6H_5$, NHC*H*), 6.18 (s, 1H, H-1′), 7.26 (m, 5H, arom.), 8.30 (s, 1H, H-8), 8.62 (d, J = 5.9 Hz, 1H, N*H*CH), 9.88 (t, J = 5.7 Hz, 1H, N*H*CH_2), MS: *m*/*z* 576.1 $[M+H]^+$. Anal. ($C_{26}H_{31}ClN_6O_5S$) C, H, N.

5.2.53. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-benzylthiocarbamoyl-β-D-ribofuranosyl)adenine (22). Reaction of **56** with HCOOH at 55 °C for 2 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2), gave **22** as a white solid (60% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 4.08 (m, 2H, H-5′), 4.30 (m, 1H, H-4′), 4.60 (m, 3H, NHC*H*, C*H*$_2$$C_6H_5$), 4.85 (d, J = 11.7 Hz, 1H, H-3′), 5.38 (s, 1H, OH), 5.50 (m, 1H, OH), 5.90 (s, 1H, H-1′), 7.30 (m, 5H, arom.), 8.28 (s, 1H, H-8), 8.60 (d, J = 5.7 Hz, 1H, N*H*CH), 9.85 (t, J = 5.7 Hz, 1H, N*H*CH_2). MS: *m*/*z* 536.1 $[M+H]^+$. Anal. ($C_{23}H_{27}ClN_6O_5S$) C, H, N.

5.2.54. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-cyclopentylcarbamoyl-β-D-ribofuranosyl)adenine (57). Reaction of **34** with CDI for 7 h, followed by addition of cyclopentylamine and stirring overnight, gave **57** as a white foam (97% yield) after chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2). ^{1}H NMR (DMSO-d_6) δ 1.15 (s, 3H, CH_3), 1.38, 1.56 (2s, 6H, CH_3), 1.42–1.60 (m, 6H, cyclopentyl), 1.76 (m, 2H, cyclopentyl), 1.95, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.92 (m, 5H, NHC*H*, tetrahydrofuranyl), 4.20–4.35 (2m, 3H, H-4′, H-5′), 4.55 (d, J = 2.7 Hz, 1H, H-3′), 4.62 (m, 1H, NHC*H*), 6.15 (s, 1H, H-1′), 7.32 (d, J = 7.0 Hz, 1H, N*H*CH), 8.22 (s, 1H, H-8), 8.58 (d, J = 6.3 Hz, 1H, N*H*CH). MS: *m*/*z* 538.0 $[M+H]^+$. Anal. ($C_{24}H_{33}ClN_6O_6$) C, H, N.

5.2.55. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-cyclopentylcarbamoyl-β-D-ribofuranosyl)adenine (23). Reaction of **57** with HCOOH at 55 °C for 5 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2), gave **23** as a white solid (54% yield). ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 1.30–1.80 (3m, 8H, cyclopentyl), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.95 (m, 5H, NHC*H*, tetrahydrofuranyl), 4.05 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.40 (br d, 1H, H-3′), 4.60 (m, 1H, NHC*H*), 5.32 (s, 1H, OH), 5.42 (pseudo t, 1H, OH), 5.88 (s, 1H, H-1′), 7.26 (d, J = 7.7 Hz, 1H, N*H*CH), 8.25 (s, 1H, H-8), 8.58 (d, J = 6.2 Hz, 1H, N*H*CH). MS: *m*/*z* 486.0 $[M+H]^+$. Anal. ($C_{21}H_{29}ClN_6O_6$) C, H, N.

5.2.56. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-2,3-*O*-isopropylidene-5-*N*-cyclopentylthiocarbamoyl-β-D-ribofuranosyl)adenine (58). Reaction of **34** with CDI for 12 h, followed by addition of cyclopentylamine and stirring overnight, gave **58** as a white foam (79% yield) after chromatography on a silica gel column ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 3H, CH_3), 1.38, 1.58 (2s, 6H, CH_3), 1.42–1.70 (m, 9H, cyclopentyl), 1.90 (m, 2H, cyclopentyl), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.92 (m, 4H, tetrahydrofuranyl), 4.30 (m, 1H, NHC*H*), 4.45 (m, 1H, H-4′), 4.60–4.80 (m, 4H, H-3′, H-5′, NHC*H*), 6.18 (s, 1H, H-1′), 8.30 (s, 1H, H-8), 8.60 (d, J = 6.9 Hz, 1H, N*H*CH), 9.40 (d, J = 7.0 Hz, 1H, N*H*CH). MS: *m*/*z* 554 $[M+H]^+$. Anal. ($C_{24}H_{33}ClN_6O_5S$) C, H, N.

5.2.57. 2-Chloro-N^6-(*R*)-3-tetrahydrofuranyl-9*H*-(2-*C*-methyl-5-*N*-cyclopentylthiocarbamoyl-β-D-ribofuranosyl)adenine (24). Reaction of **56** (1.0 mmol) with HCOOH at 55 °C for 3 h, followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 99:1), gave **24** as a white solid (55% yield). ^{1}H NMR (DMSO-d_6) δ 0.82 (s, 3H, CH_3), 1.40–1.70 (m, 6H, cyclopentyl), 1.90 (m, 2H, cyclopentyl), 2.0, 2.20 (2m, 2H, tetrahydrofuranyl), 3.58–3.95 (m, 4H, tetrahydrofuranyl), 3.88 (m, 2H, tetrahydrofuranyl), 4.12 (m, 2H, H-5′), 4.30 (m, 1H, NHC*H*), 4.45 (m, 1H, H-4′), 4.62 (m, 1H, NHC*H*), 4.85 (br d, J = 11.4 Hz, 1H, H-3′), 5.45 (br s, 2H, OH), 5.88 (s, 1H, H-1′), 8.28 (s, 1H, H-8), 8.60 (d,

J = 7.0 Hz, 1H, NHCH), 9.30 (d, J= 7.7 Hz, 1H, NHCH). MS: m/z 513 $[M+H]^+$. Anal. ($C_{21}H_{29}ClN_6O_5S$) C, H, N.

5.3. Computational chemistry

Molecular modeling and graphics manipulations were performed using the SYBYL software package (Sybyl Molecular Modeling System, version 7.0, Tripos Inc., St. Louis, MO), running it on a Silicon Graphics Tezro R16000 workstation. Model building of compounds CCPA, 2-Cl-tecadenoson, 2′-Me-CCPA, and **30** was accomplished with the TRIPOS force field[17] available within SYBYL. Energy minimizations and MD simulations were realized by employing the AMBER software packages AMBER 8.0 program,[18] using the parm99 force field.[19]

5.3.1. Homology model of the human A_1 adenosine receptor. The structural model of the human A_1AR was built using the recently reported 2.8 Å crystal structure of bovine rhodopsin (PDB entry code: 1F88)[20] as a structural template. Briefly, sequences of the human A_1AR transmembrane domains were amended by comparison to the corresponding domains of rhodopsin, according to a published sequence alignment.[21] Individual TM helical segments were built as ideal helices (using $\phi - \psi$ angles of $-63.0\,^\circ$ and $-41.6\,^\circ$) with side chains placed in prevalent rotamers and representative proline kink geometries. Each model helix was capped with an acetyl group at the N-terminus and an *N*-methyl group at the C-terminus. These structures were then grouped by adding one at a time until a helical bundle (TM region), matching the overall characteristics of the crystallographic structure of rhodopsin, had been obtained. The relative orientations and interactions between the helices were adjusted based on incorporated structural inferences from available experimental data, such as mutation and ligand binding studies,[22] cysteine scanning data,[23] and site-directed mutation experiments.[24] Because earlier work showed that polarity conserved positions cluster together in the cores of proteins to create conserved hydrogen-bonding interactions,[25] we refined the model by applying the additional hydrogen-bonding constraints between the conserved polar residues Asn27, Asp55, and Asn284 in accordance with data from site-directed mutagenesis.[24,25] The helical bundle was subjected to energy-minimization using the SANDER module of the AMBER suite of programs until the rms value of the coniugate gradient was 0.001 kcal/mol per Å. An energy penalty force constant of 5 kcal/Å^2/mol on the protein backbone atoms was applied throughout these calculations.

For the conformational refinement of the human A_1AR, the minimized structure was then used as the starting point for subsequent 200 ps of MD, during which the positional constraints on the protein backbone atoms were gradually released from 5 to 0.05 kcal/Å^2/mol. The options of MD at 300 K with 0.2 ps coupling constant were a time step of 1 fs and a nonbonded update every 25 fs. The lengths of bonds with hydrogen atoms were constrained according to the SHAKE algorithm.[26] The average structure from the last 50 ps trajectory of MD was re-minimized with backbone constraints in the secondary structure. The conformational validity of main chain and side chain torsions in each residue within the protein models was analyzed using the PROCHECK program.[27] Also, all ω angles for the peptide planarity were measured. The chirality of all Cα atoms, which in naturally occurring amino acids is of the L-configuration, was checked. Rms deviations between backbone atoms in all helices were compared to the X-ray structure of rhodopsin as a template.

5.3.2. Docking simulations. Docking was performed with version 3.05 of the program AutoDock.[16] It combines a rapid energy evaluation through pre-calculated grids of affinity potentials with a variety of search algorithms to find suitable binding positions for a ligand on a given protein. While the protein is required to be rigid, the program allows torsional flexibility in the ligand. Docking to both bovine[9] and human A_1AR models was carried out using the empirical free energy function and the Lamarckian genetic algorithm, applying a standard protocol, with an initial population of 50 randomly placed individuals, a maximum number of 1.5×106 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. Proportional selection was used, where the average of the worst energy was calculated over a window of the previous 10 generations. For the local search, the so-called pseudo-Solis and Wets algorithm was applied using a maximum of 300 iterations per local search. The probability of performing local search on an individual in the population was 0.06, and the maximum number of consecutive successes or failures before doubling or halving the local search step size was 4. Fifty independent docking runs were carried out for each ligand. Results differing by less than 1.5 Å in positional rmsd were clustered together and represented by the result with the most favorable free energy of binding.

5.3.3. Ligand set up. The core structures of CCPA, 2-Cl-tecadenoson, 2′-Me-CCPA, and **30** were retrieved from the Cambridge Structural Database (CSD)[28] and modified using standard bond lengths and bond angles of the SYBYL fragment library. Geometry optimizations were realized with the SYBYL/Maximin2 minimizer by applying the BFGS (Broyden, Fletcher, Goldfarb and Shannon) algorithm[29] and setting a rms gradient of the forces acting on each atom of 0.05 kcal/mol Å as the convergence criterion. Atomic charges were assigned using the Gasteiger–Marsili formalism,[30] which is the type of atomic charges used in calibrating the AutoDock empirical free energy function. Finally, the two compounds were set up for docking with the help of AutoTors, the main purpose of which is to define the torsional degrees of freedom to be considered during the docking process. The number of flexible torsions defined for each ligand is 5.

5.3.4. Protein set up. The energy-minimized structure of either bovine[9] and human A_1AR models were set up for docking as follows: polar hydrogens were added using

the biopolymers module of the SYBYL program (Arg, Lys, Glu, and Asp residues were considered ionized, while all His were considered neutral by default), and Kollman united-atom partial charges were assigned. Solvation parameters were added to the final protein file using the Addsol utility of AutoDock. The grid maps representing the proteins in the actual docking process were calculated with AutoGrid. The grids (one for each atom type in the ligand, plus one for electrostatic interactions) were chosen to be sufficiently large to include not only the active site but also significant portions of the surrounding surface. The dimensions of the grids were thus 50 Å × 40 Å × 40 Å, with a spacing of 0.375 Å between the grid points.

5.3.5. Molecular dynamics simulations. Refinement of the ligand/receptor complexes was achieved by in vacuo energy minimization with the SANDER module of AMBER (50 000 steps; distance dependent dielectric function of $\varepsilon = 4r$), applying an energy penalty force constant of 5 kcal/mol on the protein backbone atoms. The geometry-optimized complexes were then used as the starting point for subsequent 150 ps MD simulation, during which the protein backbone atoms were constrained as done in the previous step. The additional parameters and the AM1-BCC charges were assigned to ligands by using the ANTECHAMBER module[31] in AMBER. A time step of 1 fs and a nonbonded pairlist updated every 25 fs were used for the MD simulations. The temperature was mantained at 300 K using the Berendsen algorithm[32] with a 0.2 ps coupling constant. An average structure was calculated from the last 100 ps trajectory and energy-minimized using the steepest descent and conjugate gradient methods as specified above. Rms deviations from the initial structures and interatomic distances were monitored using the CARNAL module in AMBER. Finite difference solutions to the linearized Poisson-Boltzmann equation, as implemented in the DelPhi module of INSIGHT II (Accelrys, 2001, San Diego, CA), were used to calculate MEPs. For MEP calculations on compound **30** and the putative bovine A_1AR binding site, cubic grids with a resolution of 1.0 Å were centered on the molecular systems considered, and the charges were distributed onto the grid points. AMBER charges and radii were used. Solvent-accessible surfaces, calculated with a spherical probe with a radius of 1.4 Å, defined the solute boundaries, and a minimum separation of 10 Å was left between any solute atom and the borders of the box. The potentials at the grid points delimiting the box were calculated analytically by treating each charge atom as a Debye–Hückel sphere.

5.4. Biological methods

5.4.1. Materials. [^{3}H]2-Chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) (specific activity 43 Ci/mmol), [^{3}H]2-[4-(2-carboxyethyl)phenyl]ethyl-amino-5′-*N*-ethylcarboxyamido-adenosine ([^{3}H]CGS21680) (specific activity 47 Ci/mmol) were obtained from Perkin-Elmer Life Sciences. [^{35}S]GTPγS was obtained from Amersham Biosciences. Adenosine deaminase (ADA) was from Sigma Chemical Co. (St. Louis, MO). All other reagents were from standard commercial sources and of the highest commercially available grade.

5.4.2. Radioligand binding assays. Affinities of new synthesized compounds at A_1AR were determined with [^{3}H]CCPA in cortical bovine and porcine membranes. Cerebral cortex membranes were prepared as described by Lohse et al.[33] The [^{3}H]CCPA binding assay was performed according to Klotz et al.[15b] with minor modifications.

Briefly, the [^{3}H]CCPA binding assay was performed by incubating aliquots of cortical membrane homogenate (0.04 and 0.12 mg/protein for bovine and porcine membranes, respectively) at 25 °C for 120 min in 0.5 mL of 50 mM Tris/HCl buffer, pH 7.7, containing 1 mM EDTA, 2 mM $MgCl_2$, with 1 nM [^{3}H]CCPA and a range of concentrations of the tested compounds (1 nM–10 μM). The tested compounds were dissolved in DMSO and the percentage of organic solvent did not exceed 2% of final assay volume. Nonspecific binding was determined in the presence of 20 μM of (*R*)-N^6-phenylisopropyladenosine ((*R*)-PIA). The incubation was stopped by sample filtration under vacuum through GF/C glass fiber filters. After two washes with 4 mL of ice-cold buffer, the filters were placed in vials containing 4 mL of scintillation cocktail and the radioactivity was counted using a scintillation counter (Tri-Carb 2800 TR, Perkin-Elmer). Affinities of the new synthesized compounds at A_{2A}AR were determined with [^{3}H]CGS21680 in striatal bovine and porcine membranes as described by Jarvis et al.[34] Protein concentration was estimated according to the method of Bradford (Bradford).[35] The experiments ($n = 4$), carried out in triplicate, were analyzed by an iterative curve fitting procedure (GraphPad, Prism program, San Diego, CA), which provided IC_{50} and SEM values for tested compounds. IC_{50} values were converted to K_i values according to the equation of Cheng and Prusoff.[36]

5.4.3. [^{35}S]GTPγS membrane binding assay. [^{35}S]GTPγS binding was performed as previously described[37] with minor modifications. Briefly, the porcine cerebral cortex was homogenized with a Teflon pestle in a Potter Elvehjem glass homogenizer in 0.32 M sucrose (9 mL of sucrose/g of tissue) and centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was centrifuged at 46,000*g* for 20 min at 4 °C and the resulting pellet was suspended in 50 mM Tris/HCl, pH 7.4, homogenized, and recentrifuged at 46,000*g* for 20 min at 4 °C. The final pellet was used immediately or stored at −80 °C until binding assays.

For the radioligand binding assay, the incubation mixture contained, in a total volume of 100 μL, 50 mM Tris/HCl, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 10 μM GDP, 1 mM dithiothreitol, 100 mM NaCl, 0.2 U/mL ADA, 0.3 nM [^{35}S]GTPγS, 0.5% bovine serum albumin, and a range of concentrations of tested compounds (1 nM–10 μM). The incubation (45 min at 25 °C) was started by addition of 7.5 μg/protein of membranes, which were pre-incubated for 15 min with 3 mU/mL ADA at 37 °C. Incubations were terminated by rapid fil-

tration under vacuum through glass fiber filters (Whatman GF/C), followed by three washes with 50 mM Tris/HCl, 5 mM $MgCl_2$, pH 7.4, cold buffer. The filters were placed in vials containing 4 mL of scintillation cocktail and the radioactivity was counted using a scintillation counter (Tri-Carb 2800 TR, Perkin-Elmer). Non-specific binding was measured with 10 μM unlabeled GTPγS and was less than 10% of total binding. Protein concentration was estimated according to the method of Bradford.[35] The EC_{50} values were derived from three independent experiments and are presented as means ± SEM. Intrinsic activities are expressed as relative to CPA.

5.4.4. Binding assay and adenylyl cyclase assay at cloned human adenosine receptors. K_i values were determined in competition experiments with membranes from CHO cells stably transfected with the individual human adenosine receptor subtypes.[38] For A_1 receptors 1 nM [^{3}H]CCPA was used as a radioligand, and [^{3}H]NECA was used for the A_{2A} (30 nM) and A_3 subtypes (10 nM). The relative potency (EC_{50} values) at A_{2B} adenosine receptors was determined measuring the activation of adenylyl cyclase in a membrane preparation of CHO cells stably transfected with the human A_{2B} subtype following the procedure described earlier.[38]

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Supplementary data

Information available: Plots of the monitored distance between the key groups of 2-chloro-2′-*C*-methyl-tecadenoson (compound **30**), CCPA, 2-Cl-tecadenoson, 2′-Me-CCPA and the A_1AR amino acids Asn254, His278, and Thr91 along the complete MD trajectory (Figs. 1a–f, 2a–e, 3a–e), and elemental analytical data for all new compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.09.035.

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