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2,8-Disubstituted Adenosine Derivatives as Partial Agonists for the Adenosine A_{2A} Receptor

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Abstract—Novel 2,8-disubstituted adenosine derivatives were synthesized in good overall yields starting from 2-iodoadenosine. Binding affinities were determined for rat adenosine A_1 and A_{2A} receptors and human A_3 receptors. Some compounds displayed good adenosine A_{2A} receptor affinities, with most of the 2-(1-hexynyl)- and 2-[(*E*)-1-hexenyl]-substituted derivatives having K_i values in the nanomolar range. Although the introduction of an 8-alkylamino substituents decreased the affinity for the adenosine A_{2A} receptor somewhat, the selectivity for this receptor compared to A_3 was improved significantly. The 8-methylamino (**12**) and 8-propylamino (**14**) derivatives of 2-(1-hexynyl)adenosine (**3**), showed reasonable A_{2A} receptor affinities with K_i values of 115 and 82 nM, respectively, and were 49- and 26-fold selective for the adenosine A_{2A} receptor compared to the A_3 receptor. The compounds were also evaluated for their ability to stimulate the cAMP production in CHO cells expressing the human adenosine A_{2A} receptor. 2-(1-Hexynyl)adenosine (**3**) and 2-[(*E*)-1-hexenyl]adenosine (**4**) both showed submaximal levels of produced cAMP, compared to the reference full agonist CGS 21680, and thus behaved as partial agonists. Most 8-alkylamino-substituted derivatives of **3**, displayed similar cAMP production as **3**, and behaved as partial agonists as well. Introduction of alkylamino groups at the 8-position of **4**, showed a slight reduction of the efficacy compared to **4**, and these compounds were partial agonists also.

Introduction

Adenosine mediates a wide variety of effects as a result of its activation of specific membrane-bound receptors called P_1-purinoceptors. The three subclasses of P_1-purinoceptors are A_1, A_2 and A_3, with A_2 further subdivided into A_{2A} and A_{2B}. All adenosine receptors are coupled to the enzyme adenylate cyclase; activation of the A_1 and A_3 adenosine receptors inhibit the adenylate cyclase, whereas activated A_{2A} and A_{2B} receptors stimulate it. The target receptor in this study, the adenosine A_{2A} receptor, can be found throughout the whole body, and A_{2A} receptor agonists might be used to inhibit platelet aggregation in thrombosis, in the diagnosis of diseases in coronary arteries, in ischemia and reperfusion.[1,2] Furthermore, activation of adenosine A_{2A} receptors has been shown to alter the binding characteristics of other receptors. Stimulation of adenosine A_{2A} receptors in rat striatal membranes reduces the affinity of agonist binding to dopamine D_2 receptors.[3,4] This raises the possibility of using adenosine A_{2A} receptor agonists as a novel therapeutic approach in the treatment of psychosis. Additionally, selective activation of adenosine A_{2A} receptors has also been shown to improve inappropriate and/or extensive inflammatory responses in cells such as mast cells and neutrophils.[5] However, these desired actions of agonists for the adenosine A_{2A} receptor may be accompanied with undesired effects such as cardiovascular actions, since the receptor is ubiquitously distributed.[6] The design of partial agonists for the adenosine A_1 receptor has already been shown to be a useful tool for achievement of selectivity of action in vivo by exploitation of the differences in receptor–effector coupling in various tissues.[7–10] Hence, partial agonists for the adenosine A_{2A} receptor might have potential as antipsychotic agents. However, whether they will be devoid of undesired cardiovascular actions, remains to be investigated in vivo, particularly since vascular A_{2A} receptors are highly expressed.[11]

Selectivity for the adenosine A_{2A} receptor can be obtained by the introduction of a 2-substituent, such as

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a 1-hexynyl or a (*E*)-1-hexenyl group that have been shown to induce high affinity for the adenosine A_{2A} receptor compared to A_1.[12–14] Partial agonism for adenosine receptor in general has been achieved by the introduction of alkylthio-substituents at the 5′-position[10,15] or by removing the 2′-hydroxy group.[16] However, partial agonism for the adenosine A_1 receptor has also successfully been accomplished by introducing alkylamino substituents at the 8-position of adenosine by our laboratory.[17] 8-Alkylamino substituted CPA (N^6-cyclopentyladenosine) derivatives have proven to be adenosine A_1 receptor partial agonists in vivo when assessed for cardiovascular activity, although with modest affinities.[8,18,19]

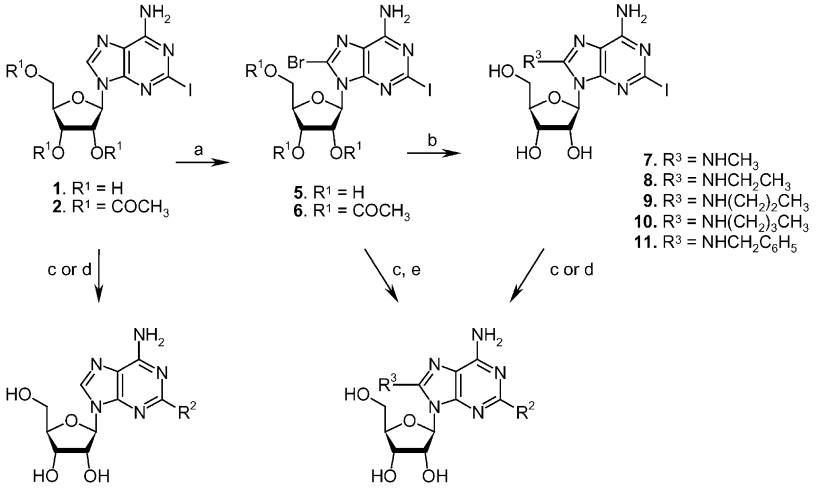
In this study, the synthesis and biological evaluation of a series of adenosine analogues substituted at the 2-position with a 1-hexynyl or (*E*)-1-hexenyl group for A_{2A} selectivity and at the 8-position with alkylamino groups for potential partial agonism are described. The compounds were tested in radioligand binding assays for affinity. Their intrinsic activities were determined in cAMP assays.

Chemistry

The synthesis of the 2,8-disubstituted adenosine derivatives **3–5**, **7–23** is illustrated in Scheme 1. 2-(1-Hexynyl)adenosine (**3**) was prepared in good yield (85%) via a slightly modified traditional palladium-catalyzed cross-coupling reaction[12] by reacting 2-iodoadenosine[20] (**1**) with 1-hexyne.[12,13] In literature, classical cross-coupling of terminal alkenes with compound **1** has been shown to be disappointing, with 2-(*E*)-alkenyl derivatives obtained in very low yields only.[14] Therefore an alternative route was used here,[14] that includes the preparation of a (*E*)-1-(borocatechol)-1-alkene complex, by letting catecholborane react with the appropriate terminal alkyne. These (*E*)-1-(borocatechol)-1-alkene complexes were coupled successfully with 2-iodoadenosine derivatives. Treatment of **1** with (*E*)-1-(borocatechol)-1-hexene, gave 2-[(*E*)-1-hexenyl]adenosine (**4**) in reasonable yield.

To obtain the 2,8-disubstituted derivatives (**12–23**), either unprotected 2-iodoadenosine (**1**) or 2′,3′,5′-tri-*O*-acetyl-2-iodoadenosine (**2**) was brominated at the 8-position. Using a standard bromination procedure, for example stirring 2-iodoadenosine (**1**) or 2′,3′,5′-tri-*O*-acetyl-2-iodoadenosine (**2**) in dioxane and adding bromine water (in phosphate buffer, 10% w/v, pH = 7),[21] yielded the corresponding 8-bromo derivatives, although workup often was quite laborious. With the use of a different buffer (NaOAc buffer, 1.0 M, pH = 4), 2-iodoadenosine (**1**) readily dissolved (50 °C) and after addition of Br_2, stirring overnight and subsequent adjustment of the pH to 7, the 8-brominated compound (**5**) was obtained as a white solid that could easily be collected by filtration.[22]

Subsequently, different amines could be introduced at this position by stirring either **5** or **6** with the appro-



Scheme 1. (a) (i) NaOAc buffer (1.0 M, pH 4), Br_2 (ii) $NaHSO_3$, aq NaOH; (b) the appropriate amine; (c) CH_3CN, Et_3N, CuI, $PdCl_2$, Ph_3P and 1-hexyne; (d) CH_3CN/DMF (1:1), tetrakis-(triphenylphosphine)-palladium(0), K_2CO_3 and (*E*)-1-(borocatechol)-1-hexene; (e) benzyl-amine, reflux.

priate amine overnight (less nucleophilic amines required some heating). Under these reaction conditions the acetyl protecting groups, when present, were readily removed and compounds **7**–**11** were obtained in good yields (70–91%).[23] Finally, the 1-hexynyl and the (*E*)-1-hexenyl substituents were introduced at the 2-position as described above for compounds **3** and **4**, and yielded the desired compounds **12**–**21**. The alternative synthesis of compounds **12**–**21** by the introduction of the 2-substituent prior to the 8-substituent failed. For example, compound **3** could indeed be brominated at the 8-position, however, addition of bromine at the triple bond of its 2-substituent occurred as well. Compounds **22** and **23** were obtained by treating 8-bromo-2-iodoadenosine (**5**) with an excess of either 1-hexyne or benzylamine.

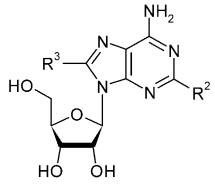
Biological Evaluation

All compounds were tested in radioligand binding assays to determine their affinities for the adenosine A_1 receptor in rat brain cortex, the A_{2A} receptor in rat striatum and the human A_3 receptor as expressed in HEK 293 cells (Table 1). For the adenosine A_1 receptor, the tritiated antagonist, [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX), and for the adenosine A_{2A} receptor, the tritiated antagonist [^{3}H]ZM 241385 (7-amino-2-(2-furyl)-5-[2-(4-hydroxyphenyl)ethyl]amino[1,2,4]-triazolo[1,5-a][1,3,5]triazine) were used. At the time the compounds were tested, no suitable radiolabeled antagonists were commercially available for the adenosine A_3 receptor. Therefore [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)-5′-methylcarboxamidoadenosine), an A_3 receptor agonist, was utilized. Displacement experiments were performed in the absence of GTP (guanosine 5′-triphosphate).

All compounds were also tested in a functional assay. The ability of the compounds (**3**–**5**, **7**–**23**) to produce cAMP by activation of human adenosine A_{2A} receptors expressed in CHO cells was assessed and compared to the reference full agonist CGS 21680 (2-[4-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine, 100%).

Table 1 displays radioligand-binding data for all synthesized final products **1**, **3**–**5**, **7**–**23**. From this table, it is clear that most synthesized compounds had low affinities for the adenosine A_1 and A_3 receptor. The affinities of the compounds for the adenosine A_{2A} receptor were higher, depending on the 2-substituent present. The compounds with an 8-alkylamino group had lower adenosine A_{2A} receptor affinities than the 8-unsubstituted derivatives **3** and **4**. The introduction of either an (*E*)-1-hexenyl or a 1-hexynyl substituent at the 2-position of 8-alkylamino adenosine derivatives led to

Table 1. Affinities of 2,8-disubstituted adenosine analogues at adenosine A_1, A_{2A} and A_3 receptors expressed as K_i values (±SEM in nM, $n=3$) or percentage displacement ($n=2$, mean value) at 10 μM



Compd	R^2	R^3	K_i (nM) or% displacement at 10^{-5} M[a]			
			A_1[b]	A_{2A}[c]	A_3[d]	A_3/A_{2A}
1	I	H	36%	4200±80	297±17	0.07
3	C≡C(CH$_2$)$_3$CH$_3$	H	64%	6.0±1.0	17±4.1	2.8
4	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	H	53%	26±1.8	74±7.7	2.8
5	I	Br	22%	43%	6%	—
7	I	NHCH$_3$	35%	43%	7310±440	—
8	I	NHCH$_2$CH$_3$	35%	41%	5110±890	—
9	I	NH(CH$_2$)$_2$CH$_3$	40%	51%	8670±2000	—
10	I	NH(CH$_2$)$_3$CH$_3$	28%	3110±1650	43%	—
11	I	NHCH$_2$C$_6$H$_5$	36%	45%	40%	—
12	C≡C(CH$_2$)$_3$CH$_3$	NHCH$_3$	32%	115±8.0	5640±780	49
13	C≡C(CH$_2$)$_3$CH$_3$	NHCH$_2$CH$_3$	31%	253±29	8830±1230	35
14	C≡C(CH$_2$)$_3$CH$_3$	NH(CH$_2$)$_2$CH$_3$	48%	82±10	2160±120	26
15	C≡C(CH$_2$)$_3$CH$_3$	NH(CH$_2$)$_3$CH$_3$	539±380	149±29	64%	—
16	C≡C(CH$_2$)$_3$CH$_3$	NHCH$_2$C$_6$H$_5$	756±483	35%	7970±610	—
17	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	NHCH$_3$	18%	663±106	13440±2130	20
18	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	NHCH$_2$CH$_3$	30%	1840±300	17530±2100	9.5
19	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	NH(CH$_2$)$_2$CH$_3$	50%	678±25	14330±2160	21
20	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	NH(CH$_2$)$_3$CH$_3$	906±264	580±250	47%	—
21	(*E*)CH=CH(CH$_2$)$_3$CH$_3$	NHCH$_2$C$_6$H$_5$	2110±560	26%	24%	—
22	C≡C(CH$_2$)$_3$CH$_3$	C≡C(CH$_2$)$_3$CH$_3$	50%	29%	254±34	—
23	NHCH$_2$C$_6$H$_5$	NHCH$_2$C$_6$H$_5$	50%	12%	54%	—

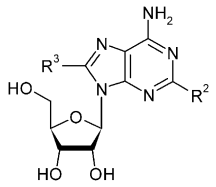
[a]Percentages were given as the means of two independent determinations (SE < 15%).
[b]Displacement of [^{3}H]DPCPX from rat cortical membranes.[39]
[c]Displacement of [^{3}H]ZM241385 from rat striatal membranes.[40]
[d]Displacement of [^{125}I]AB MECA from the human A_3 receptor expressed in HEK 293 cells.[37,42]

an increase in affinity for the adenosine A_{2A} receptor, up to approximately 7- or 60-fold, respectively (Table 1).[17] Remarkably, these compounds had adenosine A_{2A} receptor affinities in the nanomolar range, with compound **14** having the highest A_{2A} receptor affinity (K_i value of 82 nM). This is consistent with previously reported data on 8-substituted adenosine analogues. The introduction of 8-substituents at adenosine decreases the affinity of the resulting compounds for the adenosine receptors compared to adenosine itself.[17] 8-Alkylamino adenosine derivatives had adenosine A_1 and A_{2A} receptor affinities in the μM range, without substantial preference for either receptor, whereas introduction of a cyclopentyl group at the N^6-position led to an increase in adenosine A_1 receptor affinity up to 23-fold.[18] The 2-(1-hexynyl) adenosine derivatives **12**–**16** generally had higher adenosine A_{2A} receptor affinities than the 2-[(*E*)-1-hexenyl]-substituted compounds **17**–**21**, in line with the receptor affinities of compounds **3** and **4** as well as data from literature.[14] Within both 2-substituted series (**12**–**16** and **17**–**21**, Table 1), the 8-propylamino substituent seemed to be tolerated best on the adenosine A_{2A} receptor. This is in contrast with the 2-unsubstituted derivatives, for which the 8-methylamino group appeared optimal for adenosine A_{2A} receptor affinity.[17] In general, all compounds were more adenosine A_{2A} receptor selective compared to the A_1 receptor than when they are compared to the A_3 receptor. Exceptions were compounds **16** and **21**, containing the large benzylamino group at the 8-position. These compounds displayed higher adenosine A_1 receptor affinities compared to A_{2A} and A_3, indicating that the adenosine A_1 receptor seemed best capable of accommodating this group. A 1-hexynyl group at the 8-position strongly decreased the A_{2A} receptor affinity as well (compounds **3** and **22**). The adenosine A_3 receptor seemed better able to accommodate the 1-hexynyl group (**22**) than the benzylamino group at the 8-position (**16**, **21**).[24] Finally, the adenosine A_{2A} receptor affinity of compound **16**, with a 1-hexynyl group at the 2-position, was higher than that of the 2-benzylamino-substituted derivative (**23**), also in line with receptor (functional) data on either 2-(1-hexynyl)-[12] or 2-benzylamino-substituted[25] derivatives. Other adenosine derivatives substituted at the 8-position have not displayed very high receptor affinities.[18] In the present study, however, 8-alkylamino-substituted adenosine derivatives were obtained with affinities in the low micromolar (**17**–**20**) or even nanomolar range (**12**–**15**) for the adenosine A_{2A} receptor. Although the adenosine A_{2A} receptor affinity was somewhat decreased with the introduction of the 8-alkylamino groups, the A_{2A} receptor selectivity compared to (A_1 and) A_3 was increased. Many 2-substituted adenosine derivatives have been described as potent ligands for the A_{2A} receptor, although binding data for the adenosine A_3 receptor is often lacking.[12,14,26–28] Some 2-alkynyl adenosine derivatives have been tested on the adenosine A_3 receptor.[29–32] These compounds, such as HENECA [2-(1-hexynyl)-5′-deoxy-5′-*N*-ethylcarboxamidoadenosine], had high affinity for the A_3 receptor, and were often more selective for the adenosine A_3 compared to the A_{2A} receptor. Here, the 8-methylamino (**12**) and 8-propylamino (**14**) derivatives of 2-(1-hexynyl)adenosine (**3**), showed reasonable A_{2A} receptor affinities with K_i values of 115 and 82 nM, respectively. Furthermore, the selectivity for the adenosine A_{2A} receptor compared to A_3 was approx. 49- and 26-fold, respectively. Although the affinities of the 2-[(*E*)-1-hexenyl]adenosines were somewhat lower, the selectivity for the A_{2A} receptor was also increased. It must be mentioned that the affinity of some (2-) substituted adenosine derivatives for the human A_{2A} receptor has been shown to be somewhat lower than their rat A_{2A} receptor affinity. Examples are compounds such as CPA, CGS 21680, NECA, DMPA (N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine) and HENECA, which all display lower affinity for the human adenosine A_{2A} receptor than for the rat A_{2A} receptor.[30,33,34] These data suggest that our synthesized compounds might also have somewhat lower affinities for the human adenosine A_{2A} receptor and therefore their selectivity compared the (human) A_3 receptor might be somewhat less.

The nanomolar adenosine A_{2A} receptor affinities of compounds **12**–**15**, and the acceptable A_{2A} receptor affinities of compounds **17**–**20**, indicate that the (fairly large) size of the substituents is not the main determining factor for the affinities of these compounds. The 8-substituents may have an influence on the conformation of the ligand itself (by forcing the ribose ring into the *syn* conformation). The conformation of 8-bromoadenosine in the crystal structure is *syn*, suggesting to cause the low affinity of this compound for the adenosine receptors.[35–37] The bulkiness of 8-alkylamino groups was initially thought to force the ribose ring into the *syn* conformation as well. However, from the X-ray structure of 8-(cyclopentylamino)-N^6-ethyladenosine it became apparent that the *anti* conformation is compatible with 8-substitution.[18] The difference in energy between the *syn* and the *anti* conformation is often not very large, and a direct steric hindrance at the receptor-binding site seems to be a more probable explanation. Furthermore, the electronic (hydrogen bond formation) and lipophilic characteristics of the 8-substituents influence receptor affinity too.[17]

Table 2 shows the effects of the synthesized compounds in cAMP assays. For determination of the amount of cAMP produced via adenosine A_{2A} receptor agonism, all compounds were first tested at a single concentration (approx. 100× the K_i value). Compounds **3** and **4**, without an alkylamino group at the 8-position, displayed partial agonism compared to the reference full agonist CGS 21680. The intrinsic activity of compound **3** was less than that of compound **4**, consistent with data previously obtained in our laboratory.[38] Compound **1**, with iodine at the 2-position, behaved as a full agonist. Introduction of the 8-alkylamino groups to compound **1**, in most cases led (except compound **11**) to a decrease in intrinsic activity. Compound **9** showed a cAMP production of only 4%, compared to CGS 21680. Within the 2-(hexynyl) series (**12**–**16**), compounds **14**–**16** had similar intrinsic activities as the 8-unsubstituted compound **3**, while **12** had a somewhat higher intrinsic activity. They all behaved as partial agonists compared to CGS 21680, whereas compound

Table 2. Percentage of cAMP production ($n=2$, mean value, both values in parentheses) via the human adenosine A_{2A} receptor expressed in CHO cells (at approx. 100 × the K_i value), compared to the reference full agonist CGS 21680 (10 μM)



Compd	R^2	R^3	% cAMP production hA_{2A}[a]
CGS 21680			100
1	I	H	126
3	$C\equiv C(CH_2)_3CH_3$	H	61
4	(*E*) $CH=CH(CH_2)_3CH_3$	H	82
5	I	Br	n.d.
7	I	$NHCH_3$	23
8	I	$NHCH_2CH_3$	77
9	I	$NH(CH_2)_2CH_3$	4
10	I	$NH(CH_2)_3CH_3$	28
11	I	$NHCH_2C_6H_5$	125
12	$C\equiv C(CH_2)_3CH_3$	$NHCH_3$	85
13	$C\equiv C(CH_2)_3CH_3$	$NHCH_2CH_3$	134
14	$C\equiv C(CH_2)_3CH_3$	$NH(CH_2)_2CH_3$	58
15	$C\equiv C(CH_2)_3CH_3$	$NH(CH_2)_3CH_3$	72
16	$C\equiv C(CH_2)_3CH_3$	$NHCH_2C_6H_5$	83
17	(*E*) $CH=CH(CH_2)_3CH_3$	$NHCH_3$	54
18	(*E*) $CH=CH(CH_2)_3CH_3$	$NHCH_2CH_3$	65
19	(*E*) $CH=CH(CH_2)_3CH_3$	$NH(CH_2)_2CH_3$	73
20	(*E*) $CH=CH(CH_2)_3CH_3$	$NH(CH_2)_3CH_3$	36
21	(*E*) $CH=CH(CH_2)_3CH_3$	$NHCH_2C_6H_5$	73
22	$C\equiv C(CH_2)_3CH_3$	$C\equiv C(CH_2)_3CH_3$	99
23	$NHCH_2C_6H_5$	$NHCH_2C_6H_5$	95

[a]Percentages were given as the means of two independent determinations (SE < 20%).

13 behaved as a full agonist. Within the 2-(*E*)-alkenyl substituted series (**17**–**21**), the introduction of methyl-, ethyl-, and butylamino groups all decreased the intrinsic activity compared to compound **4**. The intrinsic activities of compounds **19** and **21** were similar to that of **4** and they all behaved as partial agonists as well. Most compounds with very bulky 8-substituents (**11**, **16**, **22** and **23**) behaved as full agonists at the adenosine A_{2A} receptor in this assay. Actually, CGS 21680 appeared to be a partial agonist here, since its intrinsic activity was lower than that of compounds **1**, **11** and **13**.

Conclusions

The 2,8-disubstituted adenosine derivatives described in the present study were synthesized in good overall yields starting from 2-iodoadenosine. Most compounds appeared to have adenosine A_{2A} receptor affinities in the low micromolar or nanomolar range. Although the affinity for the adenosine A_{2A} receptor was decreased somewhat with the introduction of the 8-alkylamino substituents, the selectivity for this receptor compared to the A_3 receptor was improved significantly. The 8-methylamino (**12**) and 8-propylamino (**14**) derivatives of 2-(1-hexynyl)adenosine (**3**), showed high A_{2A} receptor affinities with K_i values of 115 and 82 nM, respectively, and were 49- and 26-fold selective for the adenosine A_{2A} receptor compared to the A_3 receptor. The adenosine A_{2A} receptor seemed to accommodate the 8-propyl- or 8-butylamino substituents best, whereas the even larger 8-substituents [8-benzylamino or 8-(1-hexynyl)] were not well tolerated. As for the intrinsic activity, the 8-unsubstituted compounds **3** and **4** displayed a reduced intrinsic activity compared to the reference agonist CGS 21680. In general, the introduction of 8-alkylamino substituents did either not affect the intrinsic activity much, or as in most cases, further reduced it, and most of the compounds behaved as partial agonists. Most of the 8-substituted derivatives of 2-iodoadenosine (**1**) behaved as partial agonists as well, except for the very bulky ones. Whether these novel adenosine derivatives will be devoid of undesired cardiovascular actions, remains to be investigated in vivo. However, these compounds may be useful pharmacological tools, and may have reduced side effects on the cardiovascular system, while possibly retaining their useful antipsychotic properties upon adenosine A_{2A} receptor activation.

Experimental

Chemicals and solvents

All reagents were from standard commercial sources and of analytic grade. [^{3}H]DPCPX, [^{3}H]CGS 21680 and [^{125}I]AB-MECA were purchased from NEN (Hoofddorp, The Netherlands).

Chromatography

Thin-layer chromatography (TLC) was carried out using aluminum sheets (20×20 cm) with silica gel F_{254} from Merck. Spots were visualized under UV (254 nm). Preparative column chromatography was performed on silica gel (230–400 mesh ASTM).

Instruments and analyses

Elemental analyses were performed for C, H, N (Department of analytical Chemistry, Leiden University, The Netherlands). ^{13}C NMR spectra were measured at 50.1 MHz with a Jeol JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier-transform mode. ^{1}H NMR spectra were measured at 200 MHz, using the above mentioned spectrometer, or at 300 MHz, using a Brüker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier-transform mode. Chemical shifts for ^{1}H and ^{13}C NMR are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard.

All high resolution mass spectra were measured on a Finnigan MAT900 mass spectrometer equipped with a direct insertion probe for EI experiments (70 eV with resolution 1000) or on a Finnigan MAT TSQ-70 spectrometer equipped with an electrospray interface for ESI experiments. Spectra were collected by constant infusion of the analyte dissolved in 80/20 methanol/H_2O. ESI is a soft ionization technique resulting in

protonated, sodiated species in positive ionization mode and deprotonated species in the negative ionization mode.

Resolution of the compounds was achieved by reverse-phase HPLC (Gilson HPLC system, 712 system controller software. Gilson Netherlands, Meyvis en Co BV, Bergen op Zoom, the Netherlands) using as a mobile phase either: A: 20% CH_3CN in H_2O–100% CH_3CN in 35 min or B: 30% MeOH in H_2O–100% MeOH in 40 min; an Alltima C18 5 μm (250 × 4.6 mm) column (Alltech Nederland BV, Breda, The Netherlands) at a flow rate of 1 mL/min. The peaks were defined by measurement of UV absorbance (254 nm). Retention times are given.

Melting points (uncorrected) were determined in a Büchi capillary melting point apparatus.

Syntheses

2-Iodoadenosine (1). This was prepared according to literature.[20] Yield 80%; mp 185–187 °C; R_f 0.21 (10% MeOH in CH_2Cl_2). Anal. calcd for $C_{10}H_{12}IN_5O_4$: C, 32.18; H, 3.49; N, 16.60. Found (•0.33 $CH_3CO_2C_2H_5$) C, 32.48; H, 3.10; N, 16.72.

2′,3′,5′-tri-*O*-Acetyl-2-iodoadenosine (2). Acid anhydride (0.34 mL, 3.60 mmol) was added to a suspension of 2-iodoadenosine (**1**, 400 mg, 1.02 mmol) and dimethylaminopyridine (DMAP, 9.32 mg, 0.08 mmol) in a mixture of acetonitrile (13 mL) and Et_3N (0.56 mL, 4.04 mmol) at room temperature. After stirring for 1 h, the solution became clear. Methanol (5 mL) was added and stirring was continued for 5 min. The mixture was concentrated in vacuo. The residue was extracted with H_2O (15 mL) and EtOAc (15 mL). The organic layer was dried ($MgSO_4$), concentrated and purified further by column chromatography (eluent EtOAc). Yield 434 mg (0.84 mmol, 82%), R_f 0.70 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H, H-8), 7.79 (bs, 2H, NH_2), 6.12 (d, 1H, J = 5.15 Hz, H-1′), 5.84 (t, 1H, J = 5.84 Hz, H-2′), 5.59 (t, 1H, J = 5.14 Hz, H-3′), 4.40–4.25 (m, 3H, H-4′,5′), 2.11 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$) ppm.

8-Bromo-2-iodoadenosine (5). 2-Iodoadenosine (**1**, 2.93 g, 7.45 mmol) was dissolved in NaOAc buffer (1.0 M, pH 4, 50 mL) at 50 °C. The solution was cooled to room temperature and bromine (0.46 mL, 8.94 mmol) was added. After stirring overnight at room temperature, adding $NaHSO_3$ destroyed the excess of bromine and the pH of the solution was adjusted to 7 with NaOH solution (5 M). The reaction mixture was kept at 4 °C for 5 h and the precipitate was filtered. The white solid was washed with water and dried. Yield 2.43 mg (5.14 mmol, 69%), R_f 0.69 (10% MeOH in EtOAc); ^{1}H NMR (DMSO-d_6) δ 7.91 (s, 2H, NH_2), 5.77 (d, 1H, J = 6.52 Hz, H-1′), 5.47 (d, 1H, J = 5.84 Hz, OH-2′), 5.24 (d, 1H, J = 4.80 Hz, OH-5′), 5.03–4.84 (m, 2H, OH-3′, H-2′), 4.20–4.09 (m, 1H, H-3′), 3.97–3.88 (m, 1H, H-4′), 3.71–3.42 (m, 2H, H-5′) ppm.

2′,3′,5′-tri-*O*-Acetyl-8-bromo-2-iodoadenosine (6). To an aqueous Na_2HPO_4 solution (10% w/v, 10 mL) was added bromine (83.5 μL). The mixture was stirred vigorously for 15 min until most of the bromine had dissolved. Then 2.6 mL of the decanted bromine solution was added to 2′,3′,5′-tri-*O*-acetyl-2-iodoadenosine (**2**, 132 mg, 0.25 mmol) in dioxane (2.6 mL) cooled in an ice/water bath. After 20 min the icebath was removed and the reaction mixture was stirred overnight at room temperature. The mixture was cooled again (icebath) and $NaHSO_3$ (1.8 M) was added dropwise until it became colorless. The waterlayer was extracted with CH_2Cl_2 (1 × 25 mL) and EtOAc (2 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (eluent EtOAc). Yield 94.2 mg (0.16 mmol, 63%), R_f 0.61 (5% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 7.95 (bs, 2H, NH_2), 6.01–5.99 (m, 2H, H-1′,2′), 5.75–5.73 (m, 1H, H-3′), 4.37–4.17 (m, 3H, H-4′,5′), 2.10, 2.08, 1.97 (3×s, 9H, 3×$COCH_3$) ppm.

General procedure for amination of compound 5 or 6 to obtain the 8-alkylamino-2-iodoadenosines 7–10. To 8-bromo-2-iodoadenosine (5) or 2′,3′,5′-tri-*O*-acetyl-8-bromo-2-iodoadenosine (6) (0.17 mmol) was added the appropriate alkylamine (excess) and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the product was crystallized from water.

2-Iodo-8-methylaminoadenosine (7). The reaction was performed with 2′,3′,5′-tri-*O*-acetyl-8-bromo-2-iodoadenosine (6, 100 mg, 0.17 mmol) and methylamine (16 mL, 40% w/v in water). Yield 54.6 mg (0.13 mmol, 77%), mp 162–164 °C; ^{1}H NMR (DMSO-d_6) δ 7.02 (d, 1H, J = 5.15 Hz, NH), 6.93 (s, 2H, NH_2), 5.77 (d, 1H, J = 7.56 Hz, H-1′), 5.66 (t, 1H, J = 4.81 Hz, OH-2′), 5.31 (d, 1H, J = 6.52 Hz, OH-5′), 5.18 (d, 1H, J = 4.46 Hz, OH-3′), 4.56 (q, 1H, J = 5.15 Hz, H-2′), 4.09–4.04 (m, 1H, H-3′), 3.98–3.91 (m, 1H, H-4′), 3.68–3.57 (m, 2H, H-5′), 2.86 (d, 3H, J = 4.46 Hz, CH_3); MS m/z 423 $(M+H)^+$. Anal. calcd for $C_{11}H_{15}IN_6O_4$: C, 29.00; H, 4.14; N, 18.44. Found (•1.9H_2O) C, 29.19; H, 4.21; N, 18.18.

8-Ethylamino-2-iodoadenosine (8). The reaction was performed with 8-bromo-2-iodoadenosine (5, 90 mg, 0.19 mmol) and ethylamine (2.5 mL, 70% w/v in water). Yield 58.3 mg (0.13 mmol, 70%), mp 205–207 °C, R_f 0.15 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 6.97 (t, 1H, J = 5.15 Hz, NH), 6.89 (s, 2H, NH_2), 5.79 (d, 1H, J = 7.56 Hz, H-1′), 5.65–5.61 (m, 1H, OH-2′), 5.30 (d, 1H, J = 6.18 Hz, OH-5′), 5.18 (d, 1H, J = 3.77 Hz, OH-3′), 4.55 (q, 1H, J = 6.17 Hz, H-2′), 4.08–4.04 (m, 1H, H-3′), 3.94 (bs, 1H, H-4′), 3.65–3.61 (m, 2H, H-5′), 2.49 (m, 2H, CH_2), 1.16 (t, 3H, J = 7.21 Hz, CH_3); MS m/z 437 $(M+H)^+$. Anal. calcd for $C_{12}H_{17}IN_6O_4$: C, 33.41; H, 4.64; N, 17.68. Found (•1.2CH_3OH) C, 33.11; H, 4.91; N, 17.72.

2-Iodo-8-propylaminoadenosine (9). The reaction was performed with 8-bromo-2-iodoadenosine (5, 90 mg, 0.19 mmol) and propylamine (2.5 mL, 70% w/v in water). Yield 68.8 mg (0.15 mmol, 80%), mp 160–162 °C, R_f 0.20 (10% MeOH in CH_2Cl_2); ^{1}H NMR

(DMSO-d_6) δ 6.99 (t, 1H, J = 4.80 Hz, NH), 6.87 (s, 2H, NH_2), 5.80 (d, 1H, J = 8.24 Hz, H-1′), 5.67–5.63 (m, 1H, OH-2′), 5.30 (d, 1H, J = 6.18 Hz, OH-5′), 5.18 (d, 1H, J = 3.77 Hz, OH-3′), 4.56–4.51 (m, 1H, H-2′), 4.09–4.03 (m, 1H, H-3′), 3.96–3.94 (m, 1H, H-4′), 3.62–3.59 (m, 4H, H-5′, NHCH_2), 1.57 (q, 2H, J = 7.20 Hz, CH_2CH$_3$), 0.88 (t, 3H, J = 7.50 Hz, CH_3); MS m/z 451 $(M+H)^+$. Anal. calcd for $C_{13}H_{19}IN_6O_4$: C, 38.04; H, 5.49; N, 18.78. Found [•1.8HCON$(CH_3)_2$] C, 37.95; H, 5.71; N, 18.80.

8-Butylamino-2-iodoadenosine (10). The reaction was performed with 8-bromo-2-iodoadenosine (5, 1.15 g, 2.44 mmol) and *n*-butylamine (25 mL). Some drops of water were added to dissolve everything. Yield 0.92 g (1.98 mmol, 81%), mp 142–144 °C, R_f 0.23 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 6.94–6.86 (m, 3H, NH, NH_2), 5.79 (d, 1H, J = 7.56 Hz, H-1′), 5.67–5.62 (m, 1H, OH-2′), 5.30–5.16 (m, 2H, OH-3′,5′), 4.54–4.49 (m, 1H, H-2′), 4.07–4.04 (m, 1H, H-3′), 3.95–3.92 (m, 1H, H-4′), 3.64–3.57 (m, 2H, H-5′), 2.49–2.42 (m, 2H, NHCH_2), 1.55–1.26 (m, 4H, $CH_2CH_2CH_3$), 0.88 (t, 3H, J = 7.21 Hz, CH_3); MS m/z 464 $(M+H)^+$. Anal. calcd for $C_{14}H_{21}IN_6O_4$: C, 36.22; H, 4.56; N, 18.10. Found C, 36.55; H, 4.24; N, 18.22.

8-Benzylamino-2-iodoadenosine (11). 8-Bromo-2-iodoadenosine (5, 1.28 g, 2.14 mmol) was dissolved in benzylamine (21.4 mmol, 2.34 mL) and some drops of water were added to dissolve everything. The mixture was stirred overnight at 60 °C and concentrated in vacuo. The white solid was stirred in CH_2Cl_2, filtered and dried. Yield 0.97 g (1.95 mmol, 91%), mp 128–130 °C, R_f 0.19 (5% MeOH in EtOAc); ^{1}H NMR (DMSO-d_6) δ 7.65 (t, 1H, NH), 7.37–7.21 (m, 5H, phenyl), 6.91 (bs, 2H, NH_2), 5.86 (d, 1H, J = 7.90 Hz, H-1′), 5.63 (t, 1H, J = 3.43 Hz, OH-2′), 5.37 (d, 1H, J = 6.52 Hz, OH-5′), 5.20 (d, 1H, J = 3.77 Hz, OH-3′), 4.65–4.55 (m, 3H, H-2′, NHCH_2), 4.12–4.07 (m, 1H, H-3′), 4.07–3.97 (m, 1H, H-4′), 3.64–3.61 (m, 2H, H-5′); MS m/z 498 $(M+H)^+$. Anal. calcd for $C_{17}H_{19}IN_6O_4$: C, 40.98; H, 3.84; N, 16.87. Found C, 40.59; H, 4.05; N, 16.50.

General procedure for the introduction of an 1-hexynyl group at derivatives 1 and 7–11 to obtain compounds 3 and 12–16. To a solution of 2-iodoadenosine (**1**) or the appropriate 8-(ar)alkylamino-2-iodoadenosine (**7**–**11**) (0.65 mmol) in dry acetonitrile (5 mL) and Et_3N (5 mL) under an atmosphere of nitrogen were added CuI (9.3 mg, 48.8 μmol), $PdCl_2$ (6.0 mg, 33.8 μmol), Ph_3P (19.5 mg, 74.3 μmol) and 1-hexyne (3.15 mmol, 362 μL). The mixture was stirred overnight at room temperature under an atmosphere of nitrogen. The mixture was filtered, concentrated and purified by column chromatography.

2-(1-Hexynyl)adenosine (3).[12] The reaction was performed with 2-iodoadenosine (1, 255 mg, 0.65 mmol) and 1-hexyne (3.15 mmol). The mixture was purified by column chromatography (5% MeOH in CH_2Cl_2). Yield 192 mg (0.55 mmol, 85%), mp 106–109 °C; R_f 0.10 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H, H-8), 7.41 (bs, 2H, NH_2), 5.84 (d, J = 6.18 Hz, 1H, H-1′), 5.45 (d, J = 6.18 Hz, 1H, OH-2′), 5.22–5.16 (m, 1H, OH-5′), 5.22–5.16 (m, 1H, OH-3′), 4.52 (q, J = 5.15 Hz, 1H, H-2′), 4.11 (q, J = 3.43 Hz, 1H, H-3′), 3.93 (d, J = 3.43 Hz, 1H, H-4′), 3.65–3.48 (m, 2H, H-5′), 2.39 (t, J = 6.86 Hz, 2H, ≡CCH_2), 1.51–1.39 (m, 4H, CH_2CH_2), 0.90 (t, J = 6.87 Hz, 3H, CH_3); MS m/z 348 $(M+H)^+$. Anal. calcd for $C_{16}H_{21}N_5O_4$: C, 53.25; H, 5.91; N, 19.15. Found (•0.22CH_2Cl_2) C, 53.31; H, 5.82; N, 19.05.

2-(1-Hexynyl)-8-methylaminoadenosine (12). The reaction was performed with 2-iodo-8-methylaminoadenosine (7, 50 mg, 0.12 mmol) and 1-hexyne (0.58 mmol). The mixture was purified by column chromatography (5% MeOH in CH_2Cl_2). Yield 36 mg (0.09 mmol, 79%), mp 161–163 °C; R_f 0.23 (5% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 7.01 (d, 1H, J = 4.81 Hz, NH), 6.61 (s, 2H, NH_2), 5.84 (d, 1H, J = 7.55 Hz, H-1′), 5.78 (t, 1H, J = 4.47 Hz, OH-2′), 5.26 (d, 1H, J = 6.87 Hz, OH-5′), 5.15 (d, 1H, J = 4.12 Hz, OH-3′), 4.58 (q, 1H, J = 5.83 Hz, H-2′), 4.10–4.03 (m, 1H, H-3′), 3.95 (bs, 1H, H-4′), 3.62 (bs, 2H, H-5′), 2.87 (s, 3H, J = 4.46 Hz, NHCH_3), 2.36 (t, 2H, J = 6.87 Hz, ≡CCH_2), 1.53–1.39 (m, 4H, CH_2CH_2), 0.89 (t, 3H, J = 6.87 Hz, CH_3); MS m/z 376 $(M+H)^+$. Anal. calcd for $C_{17}H_{24}N_6O_4$: C, 47.69; H, 7.00; N, 19.63. Found (•2.9H_2O) C, 47.66; H, 7.21; N, 19.73.

8-Ethylamino-2-(1-hexynyl)adenosine (13). The reaction was performed with 8-ethylamino-2-iodoadenosine (8, 67 mg, 0.15 mmol) and 1-hexyne (0.73 mmol). The mixture was purified by column chromatography (EtOAc–10% MeOH in EtOAc). Yield 49 mg (0.12 mmol, 83%), mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 6.97 (t, 1H, J = 4.80 Hz, NH), 6.57 (s, 2H, NH_2), 5.86 (d, 1H, J = 7.55 Hz, H-1′), 5.74 (t, 1H, J = 4.80 Hz, OH-2′), 5.25 (d, 1H, J = 6.52 Hz, OH-5′), 5.15 (d, 1H, J = 4.12 Hz, OH-3′), 4.57 (q, 1H, J = 6.18 Hz, H-2′), 4.08 (m, 1H, H-3′), 3.95 (m, 1H, H-4′), 3.64–3.60 (m, 2H, H-5′), 3.05 (m, 2H, NHCH_2), 2.36 (t, 2H, J = 7.21 Hz, ≡CHCH_2), 1.51–1.40 (m, 4H, CH_2CH_2CH$_3$), 1.16 (t, 3H, J = 7.20 Hz, NHCH$_2$CH_3), 0.89 (t, 3H, J = 5.15 Hz, CH_3); MS m/z 391 $(M+H)^+$. Anal. calcd for $C_{18}H_{26}N_6O_4$: C, 54.21; H, 6.81; N, 21.07. Found (•0.5H_2O) C, 54.10; H, 7.01; N, 21.4.

2-(1-Hexynyl)-8-propylaminoadenosine (14). The reaction was performed with 2-iodo-8-propylaminoadenosine (9, 68 mg, 0.15 mmol) and 1-hexyne (0.73 mmol). The mixture was purified by column chromatography (EtOAc–10% MeOH in EtOAc). Yield 49 mg (0.12 mmol, 80%), mp 184–186 °C; R_f 0.60 (10% MeOH in EtOAc); ^{1}H NMR (DMSO-d_6) δ 6.98 (t, 1H, J = 5.15 Hz, NH), 6.54 (bs, 2H, NH_2), 5.87 (d, 1H, J = 7.55 Hz, H-1′), 5.75 (t, 1H, J = 4.46 Hz, OH-2′), 5.26 (d, 1H, J = 6.86 Hz, OH-5′), 5.15 (d, 1H, J = 4.12 Hz, OH-3′), 4.55 (q, 1H, J = 6.86 Hz, H-2′), 4.06 (m, 1H, H-3′), 3.96 (bs, 1H, H-4′), 3.61 (bs, 2H, H-5′), 2.49 (m, 2H, NHCH_2), 2.36 (m, 2H, ≡CCH_2), 1.60–1.42 (m, 6H, NHCH$_2$CH_2CH$_3$, CH_2CH_2), 0.89 (t, 6H, J = 7.55 Hz, 2 × CH_3); MS m/z 405 $(M+H)^+$. Anal. calcd for $C_{19}H_{28}N_6O_4$: C, 52.63; H, 7.26; N, 19.38. Found (•1.6H_2O) C, 52.70; H, 7.23; N, 19.19.

8-Butylamino-2-(1-hexynyl)adenosine (15). The reaction was performed with 8-butylamino-2-iodoadenosine (10, 200 mg, 0.43 mmol) and 1-hexyne (2.08 mmol). The mixture was purified by column chromatography (5% MeOH in CH_2Cl_2). Yield 111 mg (0.26 mmol, 62%), mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 6.95 (t, 1H, J = 5.49 Hz, NH), 6.55 (bs, 2H, NH_2), 5.87 (d, 1H, J = 7.90 Hz, H-1′), 5.77–5.72 (m, 1H, OH-2′), 5.26 (d, 1H, J = 6.52 Hz, OH-5′), 5.15 (d, 1H, J = 4.12 Hz, OH-3′), 4.54 (q, 1H, J = 5.84 Hz, H-2′), 4.09–4.04 (m, 1H, H-3′), 3.95–3.93 (m, 1H, H-4′), 3.64–3.59 (m, 2H, H-5′), 3.09 (m, 2H, $NHCH_2$), 2.36 (m, 2H, $\equiv CCH_2$), 1.51–1.30 (m, 8H, 2 × CH_2CH_2), 0.89 (t, 6H, J = 7.55 Hz, 2 × CH_3); MS m/z 419 $(M+H)^+$. Anal. calcd for $C_{20}H_{30}N_6O_4$: C, 54.16; H, 7.45; N, 18.95. Found (•1.4H_2O) C, 54.11; H, 7.72; N, 19.14.

8-Benzylamino-2-(1-hexynyl)adenosine (16). The reaction was performed with 8-benzylamino-2-iodoadenosine (11, 230 mg, 0.46 mmol) and 1-hexyne (2.23 mmol). The mixture was purified by column chromatography (EtOAc). Yield 146 mg (0.32 mmol, 70%), mp 141–143 °C; R_f 0.26 (5% MeOH in EtOAc); ^{1}H NMR (DMSO-d_6) δ 7.64 (t, 1H, J = 6.10 Hz, NH), 7.36–7.18 (m, 5H, phenyl), 6.59 (bs, 2H, NH_2), 5.92 (d, 1H, J = 7.71 Hz, H-1′), 5.77 (t, 1H, J = 4.85 Hz, OH-2′), 5.32 (d, 1H, J = 6.74 Hz, OH-5′), 5.18 (d, 1H, J = 4.18 Hz, OH-3′), 4.64 (q, 1H, J = 4.64 Hz, H-2′), 4.58 (t, 2H, J = 4.65 Hz, $NHCH_2$), 4.09 (t, 1H, J = 4.09 Hz, H-3′), 3.98 (d, 1H, J = 1.60 Hz, H-4′), 3.62 (q, 2H, J = 2.58 Hz, H-5′), 2.36 (t, 2H, J = 6.92 Hz, $\equiv CCH_2$), 1.55–134 (m, 4H, CH_2CH_2), 0.89 (t, 3H, J = 7.11 Hz, CH_3); MS m/z 453 $(M+H)^+$. Anal. calcd for $C_{23}H_{28}N_6O_4$: C, 59.43; H, 6.71; N, 18.65. Found (•1.0H_2O) C, 59.39; H, 6.69; N, 18.28.

(*E*)-1-(borocatechol)-1-hexene.[14] Yield 75%; R_f 0.28 (EtOAc/PE40/60 40/60 = 1:1).

General procedure for the introduction of an (*E*)-1-hexene group at derivatives 1 and 7–11 to obtain compounds 3 and 17–21. To a solution of 2-iodoadenosine (1) or the appropriate 8-(ar)alkylamino-2-iodoadenosine (7–11) (0.82 mmol) in 20 mL of CH_3CN–DMF (1:1) was added 50 mg of tetrakis(triphenylphosphine)palladium(0) and the mixture was stirred at room temperature for 15 min. Then 500 mg each of K_2CO_3 and (*E*)-1-(borocatechol)-1-hexene (2.46 mmol) were added, and the suspension was refluxed for 5 h. The mixture was filtered, concentrated in vacuo and purified by column chromatography.

2-[(*E*)-1-Hexenyl]adenosine (4).[14] **2-[(*E*)-1-Hexenyl]-8-methylaminoadenosine (17).** The reaction was performed with 2-iodo-8-methylaminoadenosine (7, 520 mg, 1.23 mmol) and (*E*)-1-(borocatechol)-1-hexene (3.70 mmol). The mixture was purified by column chromatography (20% MeOH in CH_2Cl_2). Yield 42 mg (1.11 mmol, 9%), mp 161–166 °C; R_f 0.60 (20% MeOH in CH_2Cl_2); ^{1}H NMR (MeOD-d_4) δ 6.91–6.83 (m, 1H, $=CHCH_2$), 6.29 (d, 1H, J = 15.44 Hz, =CH), 5.98 (d, 1H, J = 7.55 Hz, H-1′), 4.81–4.74 (m, 1H, H-2′), 4.29 (t, 1H, J = 5.49 Hz, H-3′), 4.13 (m, 1H, H-4′), 3.82–3.79 (m, 2H, H-5′), 2.97 (m, 3H, $NHCH_3$), 2.29–2.22 (m, 2H, $=CHCH_2$), 1.52–1.36 (m, 4H, CH_2CH_2), 0.94 (t, 3H, J = 6.52 Hz, CH_3); MS m/z 379 $(M+H)^+$; HPLC, System A: 20–100% CH_3CN in H_2O in 35 min, retention time = 6.15 min; System B: 30–100% CH_3OH in H_2O in 40 min, retention time = 18.67 min.

8-Ethylamino-2-[(*E*)-1-hexenyl]adenosine (18). The reaction was performed with 8-ethylamino-2-iodoadenosine (8, 740 mg, 1.70 mmol) and (*E*)-1-(borocatechol)-1-hexene (5.09 mmol). The mixture was purified by column chromatography (10–20% MeOH in CH_2Cl_2).Yield 23 mg (0.06 mmol, 5%), mp 128–130 °C; R_f 0.61 (20% MeOH in CH_2Cl_2); ^{1}H NMR (MeOD-d_4) δ 6.92–6.82 (m, 1H, $=CHCH_2$), 6.29 (d, 1H, J = 14.04 Hz, =CH), 6.02 (d, 1H, J = 7.64 Hz, <H-1′), 4.80–4.76 (m, 1H, H-2′), 4.29–4.27 (m, 1H, H-3′), 4.13–4.11 (m, 1H, H-4′), 3.81 (q, 2H, J = 10.89 Hz, H-5′), 3.42 (q, 2H, J = 6.02 Hz, $NHCH_2$), 2.24 (q, 2H, J = 6.00 Hz, $=CHCH_2$), 1.53–1.33 (m, 4H, CH_2CH_2), 1.28 (t, 3H, J = 7.72 Hz, $NHCH_2CH_3$), 0.94 (t, 3H, J = 7.11 Hz, CH_3); MS m/z 393 $(M+H)^+$; HPLC, System A: 20–100% CH_3CN in H_2O in 35 min, retention time = 7.42 min; System B: 30–100% CH_3OH in H_2O in 40 min, retention time = 20.16 min.

2-[(*E*)-1-Hexenyl]-8-propylaminoadenosine (19). The reaction was performed with 2-iodo-8-propylaminoadenosine (9, 400 mg, 0.89 mmol) and (*E*)-1-(borocatechol)-1-hexene (2.67 mmol). The mixture was purified by column chromatography (10% MeOH in CH_2Cl_2). Yield 43 mg (0.11 mmol, 12%), mp 170–172 °C; R_f 0.41 (10% MeOH in CH_2Cl_2); ^{1}H NMR (MeOD-d_4) δ 6.93–6.82 (m, 1H, $=CHCH_2$), 6.29 (d, 1H, J = 15.48 Hz, =CH), 6.04 (d, 1H, J = 7.65 Hz, H-1′), 4.79–4.74 (m, 1H, H-2′), 4.29–4.26 (m, 1H, H-3′), 4.13–4.12 (m, 1H, H-4′), 3.81 (q, 2H, J = 8.24 Hz, H-5′), 3.37–3.32 (m, 2H, $NHCH_2$), 2.24 (q, 2H, J = 7.16 Hz, $=CHCH_2$), 1.89–1.66 (m, 2H, $NHCH_2CH_2$), 1.51–1.35 (m, 4H, CH_2CH_2), 1.02–0.91 (m, 6H, 2 × CH_3); MS m/z 407 $(M+H)^+$; HPLC, System A: 20–100% CH_3CN in H_2O in 35 min, retention time = 8.10 min; System B: 30–100% CH_3OH in H_2O in 40 min, retention time = 20.25 min.

8-Butylamino-2-[(*E*)-1-hexenyl]adenosine (20). The reaction was performed with 8-butylamino-2-iodoadenosine (10, 380 mg, 0.82 mmol) and (*E*)-1-(borocatechol)-1-hexene (2.46 mmol). The mixture was purified by column chromatography (10% MeOH in CH_2Cl_2). Yield 42 mg (0.10 mmol, 12%); R_f 0.44 (10% MeOH in CH_2Cl_2); ^{1}H NMR (MeOD-d_4) δ 6.94–6.80 (m, 1H, $=CHCH_2$), 6.28 (d, 1H, J = 15.42 Hz, =CH), 6.02 (d, 1H, J = 7.62 Hz, H-1′), 4.81–4.69 (m, 1H, H-2′), 4.31–4.20 (m, 1H, H-3′), 4.20–4.05 (m, 1H, H-4′), 3.87–3.70 (m, 2H, H-5′), 3.35–3.23 (m, 2H, $NHCH_2$), 2.29–2.05 (m, 2H, $=CHCH_2$), 1.72–1.20 (m, 8H, 2 × CH_2CH_2), 1.00–0.83 (m, 6H, 2 × CH_3); MS m/z 422 $(M+H)^+$; HPLC, System A: 20–100% CH_3CN in H_2O in 35 min, retention time = 8.53 min; System B: 30–100% CH_3OH in H_2O in 40 min, retention time = 20.47 min.

8-Benzylamino-2-[(*E*)-1-hexenyl]adenosine (21). The reaction was performed with 8-benzylamino-2-iodoadenosine (**11**, 410 mg, 0.82 mmol) and (*E*)-1-(borocatechol)-1-hexene (2.47 mmol). The mixture was

purified by column chromatography (10% MeOH in CH_2Cl_2). Yield 41 mg (0.10 mmol, 11%), mp 153–155 °C; ^{1}H NMR (MeOD-d_4) δ 7.64 (t, 1H, J = 6.10 Hz, NH), 7.36–7.18 (m, 5H, phenyl), 6.59 (bs, 2H, NH_2), 6.94–6.80 (m, 1H, =C*H*CH$_2$), 6.28 (d, 1H, J = 15.42 Hz, =CH), 5.92 (d, 1H, J = 7.71 Hz, H-1′), 5.77 (t, 1H, J = 4.85 Hz, OH-2′), 5.32 (d, 1H, J = 6.74 Hz, OH-5′), 5.18 (d, 1H, J = 4.18 Hz, OH-3′), 4.64 (q, 1H, J = 4.64 Hz, H-2′), 4.58 (t, 2H, J = 4.65 Hz, NHC*H*$_2$), 4.09 (t, 1H, J = 4.09 Hz, H-3′), 3.98 (d, 1H, J = 1.60 Hz, H-4′), 3.62 (q, 2H, J = 2.58 Hz, H-5′), 2.29–2.05 (m, 2H, =CHC*H*$_2$), 1.72–1.20 (m, 4H, C*H*$_2$C*H*$_2$), 1.00–0.83 (m, 3H, CH_3) ppm; MS m/z 456 $(M+H)^+$; HPLC, System A: 20–100% CH_3CN in H_2O in 35 min, retention time = 9.61 min; System B: 30–100% CH_3OH in H_2O in 40 min, retention time = 26.10 min.

2,8-di-(1-Hexynyl)adenosine (22). 8-Bromo-2-iodoadenosine (5, 150 mg, 0.32 mmol) was dissolved in 3 mL CH_3CN and 3 mL Et_3N (dry). Then 4.5 mg CuI (23.6 μmol), 2.9 mg $PdCl_2$ (16.4 μmol) and 9.6 mg Ph_3P (36.4 μmol) were added. Subsequently, 1.55 mmol (178 μL) 1-hexyne was added and the mixture was stirred under nitrogen atmosphere at room temperature overnight. The mixture was concentrated and purified by column chromatography (10% MeOH in CH_2Cl_2). Yield 96 mg (0.22 mmol, 70%), mp 146–148 °C; R_f 0.48 (10% MeOH in CH_2Cl_2); ^{1}H NMR (MeOD-d_4) δ 7.61 (bs, 2H, NH_2), 5.89 (d, 1H, J = 6.86 Hz, H-1′), 5.39 (d, 1H, J = 6.18 Hz, OH-2′), 5.29–5.22 (m, 1H, OH-5′), 5.16 (d, 1H, J = 4.12 Hz, OH-3′), 4.94 (q, 1H, J = 5.83 Hz, H-2′), 4.15–4.13 (m, 1H, H-3′), 3.94–3.92 (m, 1H, H-4′), 3.72–3.44 (m, 2H, H-5′), 2.56 (t, 2H, J = 6.52 Hz, ≡C*H*$_2$), 2.44–2.34 (m, 2H, ≡C*H*$_2$), 1.57–1.37 (m, 8H, CH_2CH_2), 0.93–0.86 (m, 6H, CH_3) ppm; MS m/z 429 $(M+H)^+$. Anal. calcd for $C_{22}H_{29}N_5O_4$: C, 60.27; H, 7.18; N, 16.72. Found [•0.8HCON$(CH_3)_2$], C, 60.10; H, 7.00; N, 16.46.

2,8-di-Benzylaminoadenosine (23). 8-Bromo-2-iodoadenosine (5, 1.4 g, 2.34 mmol) was dissolved in benzylamine (23.4 mmol, 2.56 mL). The mixture was heated at 140 °C for 2 h and was poured into $CHCl_3$. A white precipitate was formed which was removed by filtration. The filtrate was concentrated and purified by column chromatography (0–10% MeOH in EtOAc). Yield 838 mg (1.76 mmol, 75%), mp 133–135 °C; R_f 0.24 (5% MeOH in EtOAc); ^{1}H NMR (MeOD-d_4) δ 7.32–6.91 (m, 11H, 2 × phenyl, NH), 6.35–6.29 (m, 1H, NH), 6.05 (bs, 2H, NH_2), 5.81 (d, 1H, J = 6.86 Hz, H-1′), 5.66–5.54 (m, 1H, OH-2′), 5.20 (d, 1H, J = 6.18 Hz, OH-5′), 5.04 (d, 1H, J = 4.80 Hz, OH3′), 4.75–4.62 (m, 1H, H-2′), 4.56–4.38 (m, 4H, 2×CH_2), 4.13–4.04 (m, 1H, H-3′), 3.92–3.84 (m, 1H, H-4′), 3.65–3.52 (m, 2H, H-5′) ppm; MS m/z 479 $(M+H)^+$. Anal. calcd for $C_{24}H_{27}N_7O_4$: C, 60.37; H, 5.70; N, 20.53. Found C, 60.65; H, 5.37; N, 20.44.

Radioligand binding studies

Measurements with [^{3}H]DPCPX in the absence of GTP were performed according to a protocol published previously.[39] Adenosine A_{2A} receptor affinities were determined according to Gao et al.[40] Adenosine A_3 receptor affinities were determined essentially as described.[37,41] Briefly, assays were performed in 50/10/1 buffer [50 mM Tris/10 mM $MgCl_2$/1 mM ethylenediaminetetra-acetic acid (EDTA) and 0.01% 3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate (CHAPS)] in glass tubes and contained 50 μL of a HEK 293 cell membrane suspension (10–30 μg), 25 μL [^{125}I]AB MECA (final concentration 0.15 nM), and 25 μL of ligand. Incubations were carried out for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD, USA). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 10^{-5} M *R*-PIA.

cAMP assay. A_{2A}

CHO cells expressing the human adenosine A_{2A} receptors were grown overnight as a monolayer in 24 wells tissue culture plates (400 μL/well; 2×10^5 cells/well). cAMP generation was performed in Dulbecco's Modified Eagles Medium (DMEM)/N-2-hydroxyethylpiperazin-*N*′-2-ethansulfonic acid (HEPES) buffer (0.60 g HEPES/ 50 mL DMEM pH 7.4). To each well, washed three times with DMEM/HEPES buffer (250 μL), 100 μL DMEM/HEPES buffer, 100 μL adenosine deaminase (final concentration 5 IU/mL) and 100 μL of a mixture of rolipram and cilostamide (final concentration 50 μM each) were added. After incubation for 40 min at 37 °C, 100 μL of agonist-solution was added. After 15 min at 37 °C, the reaction was terminated by removing the medium and adding 200 μL 0.1 M HCl. Wells were stored at −20 °C until assay.

The amounts of cAMP were determined after a protocol with cAMP binding protein[17] with the following minor modifications. As a buffer was used 150 mM K_2HPO_4/ 10 mM EDTA/0.2% bovine serum albumin (BSA) at pH 7.5. Samples (20 + 30 μL 0.1 M HCl) were incubated for at least 2.5 h at 0 °C before filtration over Whatman GF/B filters. Filters were additionally rinsed with 2 × 2 mL Tris–HCl buffer (pH 7.4, 4 °C). Filters were counted in Packard Emulsifier Safe scintillation fluid (3.5 mL) after 24 h of extraction.

Data analysis

Apparent K_i were computed from the displacement curves by nonlinear regression of the competition curves with the software package Prism (Graph Pad, San Diego, CA, USA).

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