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Synthesis and biological evaluation of disubstituted N^6-cyclopentyladenine analogues: the search for a neutral antagonist with high affinity for the adenosine A_1 receptor

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Abstract—Novel 3,8- and 8,9-disubstituted N^6-cyclopentyladenine derivatives were synthesised in moderate overall yield from 6-chloropurine. The derivatives were made in an attempt to find a new neutral antagonist with high affinity for adenosine A_1 receptors. N^6-Cyclopentyl-9-methyladenine (N-0840) was used as a lead compound. Binding affinities of the new analogues were determined for human adenosine A_1 and A_3 receptors. Their intrinsic activity was assessed in [^{35}S]GTPγS binding experiments. Elongation of the 9-methyl of N-0840 to a 9-propyl substituent was very well tolerated. A 9-benzyl group, on the other hand, caused a decrease in adenosine A_1 receptor affinity. Next, the 8-position was examined in detail, and affinity was increased with appropriate substitution. Most derivatives were A_1-selective and 20 of the new compounds (**6**–**9**, **15**–**21**, **23**–**26**, **28**, **31**, **33**, **35**, and **36**) had higher adenosine A_1 receptor affinity than the reference substance, N-0840. Compound **31** (N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine, LUF 5608) had the highest adenosine A_1 receptor affinity, 7.7 nM. In the [^{35}S]GTPγS binding experiments, derivatives **5**, **14**, **22**, **23**, **25**, **26**, **33** and **34** did not significantly change basal [^{35}S]GTPγS binding, thus behaving as neutral antagonists. Moreover, four of these compounds (**23**, **25**, **26**, and **33**) displayed a 4- to 10-fold increased adenosine A_1 receptor affinity (75–206 nM) compared to N-0840 (852 nM). In summary, we synthesised a range of N-0840 analogues with higher affinity for adenosine A_1 receptors. In addition, four new derivatives, LUF 5666 (**23**), LUF 5668 (**25**), LUF 5669 (**26**) and LUF 5674 (**33**), behaved as neutral antagonists when tested in [^{35}S]GTPγS binding studies. Thus, these compounds have improved characteristics as neutral adenosine A_1 receptor antagonists.

1. Introduction

Most of the 'classical' adenosine A_1 receptor antagonists, for example DPCPX (1,3-dipropyl-8-cyclopentylxanthine) and CGS 15943 (9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine), have been shown to act as inverse agonists.[1] In the same study, [^{35}S]GTPγS binding experiments revealed also three neutral antagonists for human adenosine A_1 receptors, N^6-cyclopentyl-9-methyladenine (N-0840, compound **3**), (±)-N^6-(endonorbornan-2-yl)-9-methyladenine (N-0861), and N^6-(5′-endohydroxy-endonorbornan-2-yl)-9-methyl-adenine (WRC-0342). However, these compounds had relatively poor affinity for the human adenosine A_1 receptor, 1549, 1023, and 1047 nM, respectively.[1] The purpose of our research was, therefore, to design novel neutral antagonists with higher affinity for human adenosine A_1 receptors.

N-0840 was used as a lead compound, which was predominantly substituted at two different positions (Fig. 1).

The N9-position was primarily explored to introduce substituents with the capacity for radiolabelling. Moreover, to increase adenosine A_1 receptor affinity, we focussed

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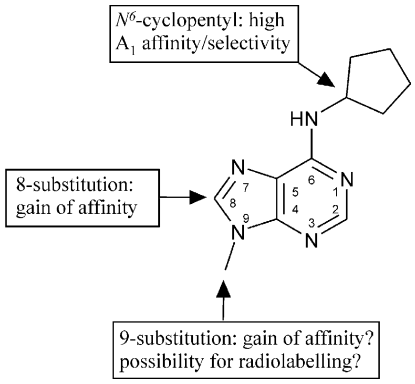


Figure 1. Chemical structure of N^6-cyclopentyl-9-methyladenine (N-0840) and effects on adenosine A_1 receptor affinity induced by modifications at different positions of the adenine molecule.

on substitution at the 8-position, since other positions had already been examined closely. For example, the N^6-cyclopentyl is known to induce high adenosine A_1 receptor affinity and selectivity.[2,3] Furthermore, small substituents, for example a chlorine atom, at the 2-position of adenines[3] or adenosines[4] only have limited effects on adenosine A_1 receptor affinity. Additionally, larger and bulkier substituents at this position of adenosines seem to favour A_{2A} and/or A_3 receptor selectivity,[5–8] and this finding may also be true for corresponding substitution at adenines.[9]

Finally, Linden and co-workers[10] investigated the C8-position of adenines to some extent. They synthesised 8-substituted N^6-norbornyl-9-methyladenines and found that nitrogen-containing groups at this position enhance adenosine A_1 receptor affinity. Moreover, introduction of alkynyl chains on the C8-position of adenosine led to selective adenosine A_3 receptor antagonists.[11]

In this paper, we describe the synthesis of 32 novel N-0840 derivatives, which were all tested in radioligand binding experiments for adenosine A_1 and A_3 receptor affinity. Furthermore, their intrinsic activity was determined with [^{35}S]GTPγS binding studies to establish whether these compounds acted as adenosine A_1 receptor neutral antagonists.

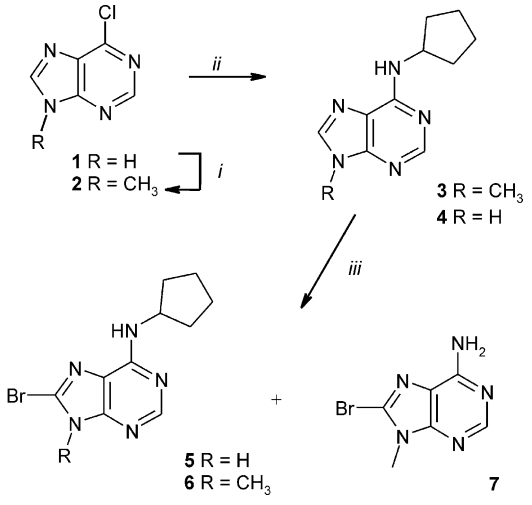
2. Results and discussion

2.1. Chemistry

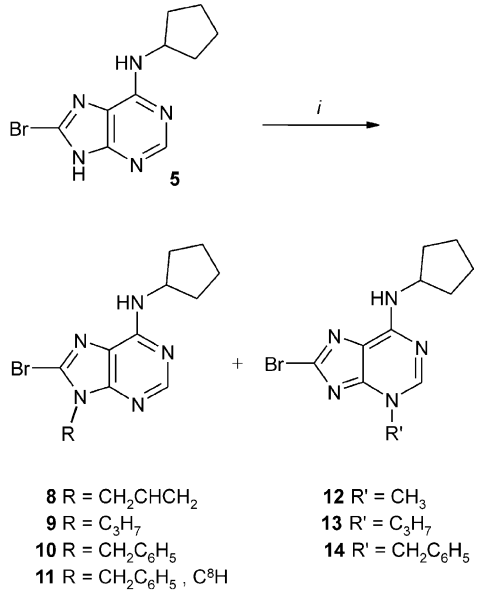
The synthesis of the 3,8- and 8,9-disubstituted N^6-cyclopentyladenines (compounds **5–37**) was accomplished via the routes illustrated in Schemes 1–4.

First, 8-bromo-N^6-cyclopentyladenine (**5**) and 8-bromo-N^6-cyclopentyl-9-methyl-adenine (**6**) were prepared from 6-chloropurine (compound **1**, Scheme 1).[3,12,13] During the bromination of N^6-cyclopentyl-9-methyladenine (**3**), partial dealkylation of the N^6-position occurred, which resulted in product **7**.[14]

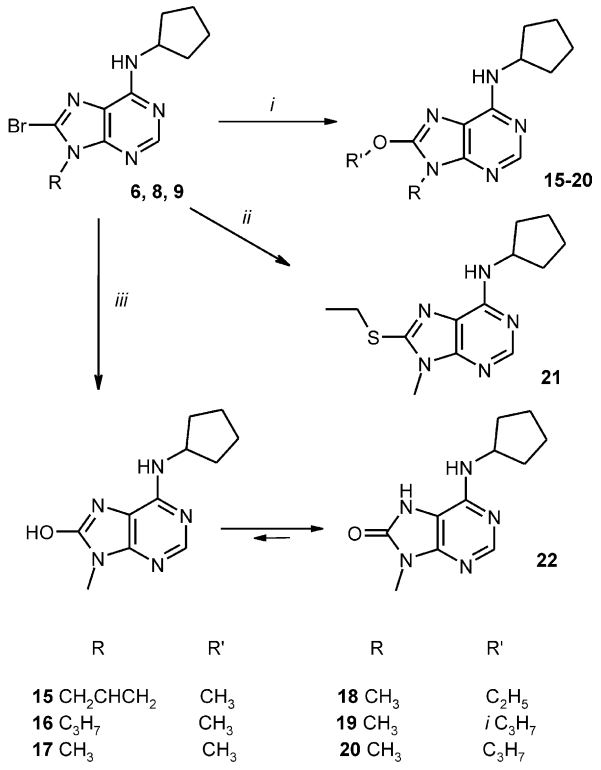
To investigate the effect of N9-substitution (Fig. 1), the appropriate alkyl halide was reacted with compound 5 in the presence of K_2CO_3 and DMF.[15] Although 9-alkylation is usually dominant, minor amounts of alkylation at N3 and/or N7 have been reported.[16,17] Probably due to the bromine atom on C8 a mixture of two compounds with the same mass was formed. These mixtures were separated and purified with column chromatography to provide the products **8–14** in moderate to low yields (Scheme 2). Assignment of the two compounds was made by NMR analysis and by comparison of the methylated products with compound 6, which was prepared via another route (Scheme 1). Chemical shifts of the H-2 proton for N9-substituted compounds (**6**, **8**, **9**, **10**) were between 8.35 and 8.41 ppm and for the N3-substituted compounds (**12**, **13**, **14**) between 7.95 and 7.97 ppm. Similarly, chemical shifts of



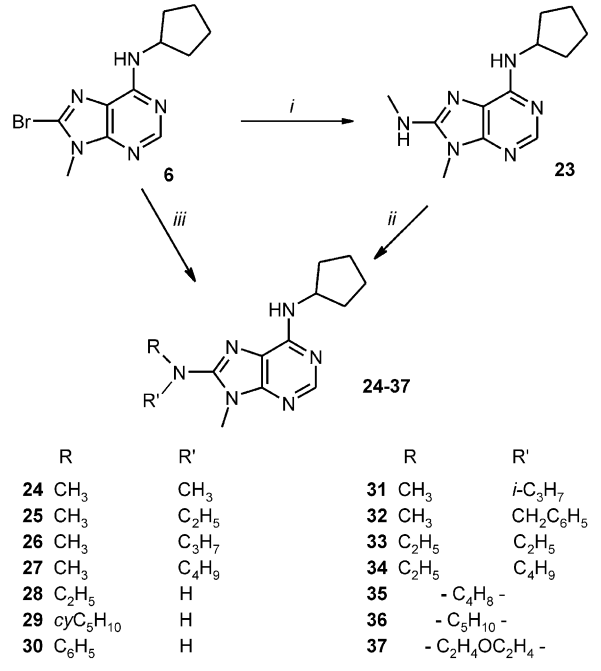
Scheme 1. Synthesis of 8-bromo-N^6-cyclopentyladenine (5), 8-bromo-N^6-cyclopentyl-9-methyladenine (**6**) and 8-bromo-9-methyladenine (**7**): (i) NaH/DMF, MeI; (ii) cyclo-pentylamine, *n*-BuOH/Et_3N, 120 °C; (iii) Br_2 in Na_2HPO_4 buffer.



Scheme 2. Synthesis of N3- and N9-substituted N^6-cyclopentyladenines: (i) alkylhalide, K_2CO_3, DMF.



Scheme 3. Synthesis of 9-alkyl-N^6-cyclopentyl-8-oxoadenines and N^6-cyclopentyl-8-(ethylthio)-9-methyladenine: (i) R′-OH, K*t*BuO; (ii) EtSH, K*t*BuO, EtOH, 50 °C; (iii) 1 M aq NaOH, reflux, 3 h.



Scheme 4. Synthesis of 8-amino substituted N^6-cyclopentyl-9-methyladenines: (i) H_3CNH_2/H_2O, rt; (ii) NaH/DMF, alkylhalide, rt; (iii) amine/dioxane, Δ.

the N^6–H proton were between 5.58 and 5.74 ppm for the N9 substituted compounds and between 6.23 and 6.50 ppm for the N3 substituted compounds. NOESY experiments on **12**, **13** and **14** showed a substantial correlation between the C–H protons on N3 and C2 that was not found for the N9-substituted compounds (data not shown).

When allyl bromide was used, only the N9-substituted product **8** was formed. The N9-substituents were chosen for their suitability for future radiolabelling. The propyl group was included to estimate the effect of $^3H_2$ reduction of the allyl's double bond. Furthermore, a benzyl group was introduced at this site as a template to verify whether the N9-position held enough space to accommodate an (iodinated) 4-iodobenzyl substituent.

Next, we explored substitution at the 8-position in an attempt to increase adenosine A_1 receptor affinity of these N-0840 derivatives (Fig. 1). The derivatives **6**, **8**, and **9** were dissolved in MeOH and refluxed in the presence of K*O*t*Bu, to synthesise their 8-methoxy analogues, resulting in high yields of products **15** and **16** and a low yield of derivative **17** (Scheme 3).[18] A similar procedure was carried out with 8-bromo-N^6-cyclopentyl-9-methyladenine (**6**) in the presence of ethanol, isopropanol, and *n*-propanol, giving good to high yields of the products **18**, **19**, and **20**. For comparison with **18**, N^6-cyclopentyl-8-thioethyl-9-methyladenine (**21**) was synthesised.

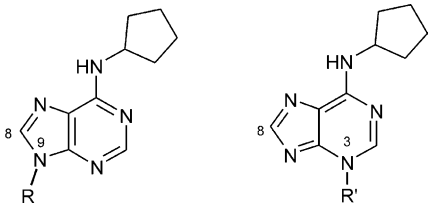
We also tried to introduce a hydroxyl group at the 8-position. During this reaction (Scheme 3) N^6-cyclopentyl-9-methyl-8-oxoadenine (**22**) was formed in a tautomeric equilibrium with the desired N^6-cyclopentyl-8-hydroxy-9-methyladenine.[19] However, high adenosine A_1 receptor affinity for this compound was not anticipated, since Martin et al.[10] reported a K_i value of 10 μM for N^6-endonorbornyl-8-hydroxy-9-methyladenine compared to 10 nM for the non-substituted analogue.

Additionally, in their study,[10] the authors adequately demonstrated the favourable presence of an amino group, and therefore in our study, amino substituents were investigated in more detail. In Scheme 4, the various methods to generate 8-amino substituted N^6-cyclopentyl-9-methyladenines (compounds **23**–**37**)[20,21] are summarised. Compounds **24**–**27** were synthesised from N^6-cyclopentyl-8-(*N*-methylamino)-9-methyladenine (**23**) under mild conditions at room temperature in DMF.[12,22] In addition, the other amines were capable of replacing the 8-bromine atom in **6** directly, resulting in the products **28**–**37**. For this route higher temperatures and a different solvent (dioxane) were required.

2.2. Biological evaluation

All compounds were tested in radioligand displacement experiments to determine their affinity for human adenosine A_1 and A_3 receptors expressed on CHO and HEK293 cells, respectively. The radioligand [^{3}H]DPCPX was used on adenosine A_1 receptors, while [^{125}I]-ABMECA [N^6-(4-amino-3-iodobenzyl)-5′-*N*-ethylcarboxamidoadenosine] was used on adenosine A_3 receptors. Tables 1 and 2 summarise the results from these ligand binding experiments. Except for derivatives **5** and **14**, the compounds had poor or negligible affinity for adenosine A_3 receptors. Moreover, these compounds

Table 1. Affinities of 3,8- and 8,9-disubstituted N^6-cyclopentyladenine analogues at human adenosine A_1 and A_3 receptors expressed as K_i values (in nM ± SEM, $n = 3$) or percentage displacement at 10 μM



Compd	C8	N9-R	N3-R′	K_i (nM) or % displacement at 10 μM		
				A_1 receptor[a]	A_3 receptor[b]	A_3/A_1
DPCPX	—	—	—	2.4 ± 0.1	1700 ± 170	708
N-0840	H	Methyl	—	852 ± 163	15%	
5	Br	H	—	2646 ± 623	1670 ± 630	0.6
6	Br	Methyl	—	43 ± 7	37%	
7[c]	Br	Methyl	—	467 ± 61	23%	
8	Br	Allyl	—	35 ± 9	5730 ± 1520	164
9	Br	Propyl	—	33 ± 1	7760 ± 170	235
10	Br	Benzyl	—	1220 ± 240	44%	
11	H	Benzyl	—	1810 ± 360	28%	
12	Br	—	Methyl	2760 ± 167	43%	
13	Br	—	Propyl	995 ± 160	10,600 ± 3100	11
14	Br	—	Benzyl	870 ± 140	1860 ± 420	2
15	OCH_3	Allyl	—	208 ± 36	32%	
16	OCH_3	Propyl	—	270 ± 20	38%	
17	OCH_3	Methyl	—	120 ± 7	21%	
18	OC_2H_5	Methyl	—	106 ± 10	46%	
19	$OCH(CH_3)_2$	Methyl	—	40 ± 7	5600 ± 730	140
20	OC_3H_7	Methyl	—	235 ± 56	9190 ± 2200	39
21	SC_2H_5	Methyl	—	224 ± 70	39%	
22	=O	Methyl	—	1610 ± 530	17%	

[a] Displacement of [^{3}H]DPCPX from CHO-$A_1^{+\,+}$ membranes.
[b] Displacement of [^{125}I]IBMECA from HEK293-A_3 membranes. K_i determined if displacement > 50%.
[c] No N^6-cyclopentyl substituent.

(**5** and **14**) along with derivatives **13** and **20** were only moderately A_1-selective, that is they possessed an A_3/A_1 ratio < 100. All other compounds, on the other hand, showed good selectivity for adenosine A_1 receptors.

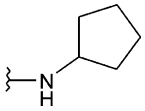
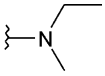
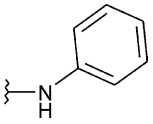
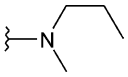
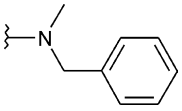
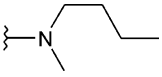
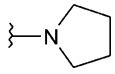
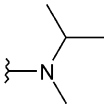
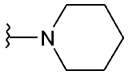
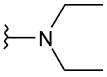
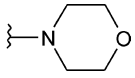
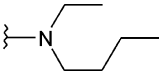
Furthermore, most of the novel derivatives (**6**–**9**, **15**–**21**, **23**–**26**, **28**, **31**, **33**, **35**, and **36**) had significantly higher affinity for the human adenosine A_1 receptor (7.7–467 nM) compared to the reference compound N-0840 (852 nM, Table 1).

Elongation of the 9-methyl group of **6** to a propyl group (derivative **9**) hardly affected adenosine A_1 receptor affinity, while 9-benzyl substitution (compound **10**) caused a strong decrease in affinity (43 and 33 nM vs 1220 nM, Table 1). The corresponding N3-substituted compounds (**12**–**14**) also had relatively low affinities. Thus, the most likely candidate for radiolabelling may be derivative **8**, in which the 9-allyl group can be reduced with $^3H_2$ to yield a radioligand analogue of product **9**. Along these lines, Thompson et al.[3] found the following rank order in affinities of 9-substituted N^6-cyclopentyladenines for rat adenosine A_1 receptor, 9-phenyl < 9-cyclopentyl < 9-methyl≈9-ethyl. However, a high affinity in the low nanomolar range is preferable for a radioligand, and 8-bromo-N^6-cyclopentyl-9-propyladenine did not meet this criterion with its affinity of 33 nM (Table 1).

With an appropriate substituent at the 8-position of N-0840 (Fig. 1) adenosine A_1 receptor affinity was increased (Tables 1 and 2). For example, elongation of the *O*-alkyl substituent (products **17**–**20**) was favourable for adenosine A_1 receptor binding, and product **19** (*O*-isopropoxy) was the best derivative in this series. Comparing derivatives **18** (ethoxy), **21** (thioethyl), and **28** (aminoethyl), a substituent with an oxygen atom gave the best results. However, Martin et al.[10] already argued that this C8-position does not tolerate hydrogen bonding substituents. Consequently, replacement of the free hydrogen in product **28** by a methyl group, as in derivative **25**, resulted in an almost 4-fold increased adenosine A_1 receptor affinity. In general, the secondary amines as substituents (compounds **23**, **28**–**30**) led to lower adenosine A_1 receptor affinities than the tertiary (compounds **24**–**27**, **31**, **33**, **34**) and cyclic (compounds **35**, **36**) amines. Moreover, lengthening of the methyl (**25**) to an ethyl group (derivative **33**), and 'ring closure' to a six-membered ring, as in derivative **36**, slightly improved affinity. Apparently there is sufficient space to accommodate bulkier substituents. Derivative **31** (LUF 5608) with a branched *N*-methyl-*N*-isopropylamino substituent on C8 had the best adenosine A_1 receptor affinity in this study, that is 7.7 nM (Table 2).

Next, the efficacy of the compounds was investigated with [^{35}S]GTPγS binding experiments (Table 3).

Table 2. Affinities of 8-substituted N^6-cyclopentyl-9-methyladenine (N-0840, see also Table 1) analogues at human adenosine A_1 and A_3 receptors expressed as K_i values (in nM ± SEM, $n = 3$) or percentage displacement at 10 μM (assays as in footnotes to Table 1)

Compd	C8 substituent	K_i (nM) or % displacement at 10 μM		Compd	C8 substituent	K_i (nM) or % displacement at 10 μM		
		A_1 receptor	A_3 receptor (%)			A_1 receptor	A_3 receptor (%)	A_3/A_1
N-0840	H	852 ± 163	15					
23	$NHCH_3$	206 ± 27	23	**24**		169 ± 28	20	
28	NHC_2H_5	344 ± 97	19					
29		2040 ± 200	54	**25**		89 ± 9	28	
30		3560 ± 1530	37	**26**		160 ± 23	31	
32		5900 ± 1300	32	**27**		1011 ± 125	n.d.	
35		403 ± 63	28	**31**		7.7 ± 1.4	14,200 ± 1960	1844
36		68 ± 6	40	**33**		75 ± 8	18	
37		2840 ± 307	11	**34**		706 ± 70	30	

[^{35}S]GTPγS binding was measured on membranes of CHO cells expressing the human adenosine A_1 receptor at high density (CHO-A_1^{++}, ~3 pmol/mg of protein). DPCPX was included as a reference adenosine A_1 inverse agonist and N-0840 as a neutral antagonist.[1] The high receptor expression was necessary to distinguish neutral antagonists from inverse agonists. Namely, DPCPX as well as some of the novel compounds (**5**–**9**, **18**–**22**, **28**, and **31**) were unable to modulate basal [^{35}S]GTPγS binding to membranes from CHO cells expressing a lower (~0.65 pmol/mg of protein) adenosine A_1 receptor number (data not shown).

Since agonists activate G proteins via receptor activation, basal [^{35}S]GTPγS binding (set to 100%) increases in the presence of an agonist, while inverse agonists show the opposite effect ([^{35}S]GTPγS binding < 100%). For example, DPCPX decreased basal [^{35}S]GTPγS binding to CHO-A_1^{++} membranes to 60% of control (Table 3). A neutral antagonist, such as N-0840, had no (significant) effect on basal [^{35}S]GTPγS binding (107%, Table 3).

Eight of the newly synthesised compounds (**5**, **14**, **22**, **23**, **25**, **26**, **33** and **34**) did not significantly change basal [^{35}S]GTPγS binding, thus behaving as neutral adenosine A_1 receptor antagonists. Moreover, four of these products (**23**, **25**, **26**, and **33**) showed a 4- to 10-fold increased adenosine A_1 receptor affinity compared to N-0840, while the others had similar (**14** and **34**) or lower (**5** and **22**) affinity (Tables 1 and 2). All other compounds, except for compounds **10**, **11** and **13**, significantly decreased basal [^{35}S]GTPγS binding to a varying extent, and acted as (partial) inverse agonists. Moreover, compound **31**, with the highest affinity for adenosine A_1 receptors, showed the largest decrease of basal [^{35}S]GTPγS binding, to 56% of control. This compound has an *N*-methyl, *N*-isopropylamino group on the C8 position, as is the case in WRC-0571 [8-(*N*-methylisopropylamino)-N^6-(5′-endohydroxy-endonorbornan-2-yl)-9-methyladenine], a partial inverse agonist.[1] Comparable compounds without a C8-substituent, WRC-0342 and N-0861, acted as neutral antagonists. Thus, the inverse agonistic behaviour of WRC-0571 may be caused by the 8-(*N*-methyl-*N*-isopropyl)-amino group, which is also present in compound **31**. The nature of 8-amino substitution appears to matter, since some 8-amino substituted N-0840 derivatives behaved as inverse agonists (e.g., **24**, **28**–**31**, **35**,

Table 3. Affinities of 3,8- and 8,9-disubstituted N^6-cyclopentyladenine analogues at human adenosine A_1 receptors expressed as K_i values (in nM ± SEM), and their effect on [^{35}S]GTPγS binding (% of basal)

Compd	% [^{35}S]GTPγS binding[a,b]	Compd	% [^{35}S]GTPγS binding[a,b]
DPCPX	60 ± 8	**21**	61 ± 4
N-0840	107 ± 10	**22**	104 ± 6
5	91 ± 4	**23** (LUF 5666)	91 ± 2
6	76 ± 4	**24**	85 ± 1
7	81 ± 4	**25** (LUF 5668)	90 ± 1
8	76 ± 4	**26** (LUF 5669)	90 ± 1
9	64 ± 3	**27**	n.d.
10	162 ± 13	**28**	72 ± 4
11	156 ± 6	**29**	76 ± 1
12	84 ± 1	**30**	73 ± 2
13	151 ± 6	**31** (LUF 5608)	56 ± 2
14	93 ± 4	**32**	n.d.
15	75 ± 1	**33** (LUF 5674)	92 ± 1
16	75 ± 1	**34**	99 ± 2
17	61 ± 2	**35**	77 ± 3
18	74 ± 4	**36**	80 ± 3
19	62 ± 4	**37**	n.d.
20	60 ± 3		

n.d., not determined.
[a] At $10 \times K_i$.
[b] CHO-A_1^{++} membranes (basal [^{35}S]GTPγS binding ~450 cpm/μg of protein).

36), while others showed neutral antagonistic behaviour (e.g., **23**, **25**, **26**, **33**, **34**).

Interestingly, compounds **10**, **11** and **13** increased basal [^{35}S]GTPγS binding to 151–162% of basal. These compounds seem to act as partial agonists, since the full adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA), showed an increase to 317% (data not shown). An explanation for these observations is not readily given. However, their increase in basal [^{35}S]GTPγS binding was limited, compared to the effect of the full agonist CPA (approximately 20% of CPA's effect). Thus, if these compounds are indeed partial agonists, they only have a very low intrinsic activity. The fact that their partial agonistic behaviour was not observed in cAMP determinations (data not shown) also indicates a very low intrinsic activity.

3. Conclusions

The 3,8- and 8,9-disubstituted N^6-cyclopentyladenine derivatives described in the present study were synthesised, starting from commercially available 6-chloropurine. It appeared that in the synthetic process N9-substitution was favoured over N3-substitution. Furthermore, elongation of the 9-methyl group in N-0840 to a 9-propyl was tolerated without loss of adenosine A_1 receptor affinity. A broad range of substituents was introduced at the 8-position. In general, tertiary aliphatic and cyclic amines represented derivatives with good adenosine A_1 receptor affinity. Compound **31**, with a branched tertiary amine, had a more than 100-fold higher affinity than the reference compound N-0840, 7.7 and 852 nM, respectively.

Next, we evaluated the intrinsic activity of all compounds. In [^{35}S]GTPγS binding experiments, eight derivatives (**5**, **14**, **22**, **23**, **25**, **26**, **33**, **34**) behaved as neutral antagonists, and four of these products (**23**, **25**, **26**, and **33**) showed a gain in adenosine A_1 receptor affinity compared to N-0840. On the other hand, derivative **31** behaved as full inverse agonist, decreasing basal [^{35}S]GTPγS binding to 56%.

In summary, we have synthesised various 3,8- and 8,9-disubstituted N^6-cyclopentyladenine derivatives with higher adenosine A_1 receptor affinity than the reference compound N-0840. Four of the newly synthesised compounds, LUF 5666 (**23**), LUF 5668 (**25**), LUF 5669 (**26**), and LUF 5674 (**33**), are classified as neutral antagonists for human adenosine A_1 receptors with substantially higher affinities than the lead compound N-0840. Derivative **31** (LUF 5608), with the highest adenosine A_1 receptor affinity of this series, acted as a full inverse agonist on this receptor.

4. Experimental

All chemicals and solvents used were commercially available and of analytical grade, unless stated otherwise. [^{3}H]DPCPX (111.6 Ci/mmol), [^{35}S]GTPγS (1250 Ci/mmol) and [^{3}H]cAMP (32.4 Ci/mmol) were purchased from NEN (Du Pont Nemours, 's-Hertogenbosch, The Netherlands). CPA, DPCPX, and N-0840 were obtained from Research Biochemicals Inc. (Natick, USA).

4.1. Chromatography

Thin-layer chromatography was carried out using aluminium sheets with silica gel F_{254} from Merck. Spots were visualised under UV light (254 nm). Preparative column chromatography was performed on silica gel (230–400 mesh, ASTM).

4.2. Instruments and analyses

Elemental analyses were performed for C, H, and 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 Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. NOESY experiments were obtained with a Bruker DMX-600 instrument (t_{mix}: 1 s).

All high-resolution mass spectra were measured on a Finnigan MAT 900 mass spectrometer equipped with a direct insertion probe for EI experiments (70 eV with resolution 1000). Melting points were determined in a Büchi capillary melting point apparatus and are uncorrected.

4.2.1. 6-Chloro-9-methyladenine (2). To a suspension of 6-chloropurine (**1**, 3.0 g, 19.4 mmol) in DMF (95 mL), NaH (60% in mineral oil, 0.776 g, 19.4 mmol) was added in 20 min. After stirring for 1 h, the reaction mixture was cooled in an ice bath while methyl iodide (1.21 mL, 19.4 mmol) was added. After stirring this reaction mixture for 16 h at room temperature, it was neutralised with acetic acid, and the DMF was evaporated. The product was purified with column chromatography (ethyl acetate) and crystallised from EtOH. Yield 1.77 g (54%). Mp 135–137 °C. ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H, CH_3), 8.40 (s, 1H, *H*-2), 8.54 (s, 1H, *H*-8).

4.2.2. N^6-Cyclopentyl-9-methyladenine (3) (N-0840). 6-Chloro-9-methyladenine (**2**, 1.77 g, 10.5 mmol), cyclopentylamine (1.04 mL, 10.5 mmol), triethylamine (2.92 mL, 21.0 mmol), and *n*-butanol (5 mL) were added to a pressure tube and heated at 120 °CC for 16 h. Purification was performed using column chromatography (5% MeOH/ethyl acetate). Yield 1.77 g (78%). Mp 102–106 °C (lit. 109 °C[3]). ^{1}H NMR ($CDCl_3$) δ 1.41–2.10 (m, 8H, 4×CH_2), 3.49 (s, 3H, CH_3), 4.64 (bs, 1H, C*H*), 6.00 (d, 1H, N^6*H*), 7.72 (s, 1H, *H*-2), 8.41 (s, 1H, *H*-8).

4.2.3. N^6-Cyclopentyladenine (4). N^6-Cyclopentyladenine (**4**) was prepared as described for **3** starting from 6-chloropurine (**1**, 3 g, 19.4 mmol). Yield 3.43 g (87%). ^{1}H NMR ($CDCl_3$) δ 1.57–2.19 (m, 8H, 4×CH_2), 8.00 (s, 1H, *H*-2), 8.45 (s, 1H, *H*-8).

4.2.4. 8-Bromo-N^6-cyclopentyladenine (5). A freshly prepared Na_2HPO_4 solution (10% w/v, pH = 7, 220 mL) saturated with bromine was added to N^6-cyclopentyladenine (**4**, 4.20 g, 20.7 mmol) dissolved in dioxane (220 mL). After stirring the solution overnight, 1.8 M $NaHSO_3$ was added dropwise until the solution turned light yellow. After evaporation of the solvent, the solid residue was dissolved in acetone, and the remaining salts were filtered off. Purification was performed using column chromatography (5% MeOH/CH_2Cl_2). Yield 3.68 g (63%). Mp 210 °C (dec). ^{1}H NMR ($CDCl_3$) δ 1.14–1.64 (m, 8H, 4×CH_2), 2.96 (m, 1H, C*H*), 4.13 (d, 1H, N^6*H*), 7.45 (m, 1H, N-9-*H*), 7.82 (s, 1H, *H*-2). MS: *m*/*e* 282 (MH^+). Anal. ($C_{10}H_{12}BrN_5$) C, H, N.

4.2.5. 8-Bromo-N^6-cyclopentyl-9-methyladenine (6) and 8-bromo-9-methyladenine (7). 8-Bromo-N^6-cyclopentyl-9-methyladenine (**6**) was prepared in a similar manner as **5**, starting from N^6-cyclopentyl-9-methyladenine (**3**, 1.41 g, 6.49 mmol). Two organic products were formed and extracted with ethyl acetate (100 mL, three times). Separation and purification was performed by chromatography (ethyl acetate).

6. Yield 0.88 g (46%). Mp 105–108 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–2.17 (m, 8H, 4×CH_2), 3.76 (s, 3H, CH_3), 4.58 (m, 1H, C*H*), 5.58 (d, 1H, N^6*H*), 8.36 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{11}H_{15}N_5Br$ $[M+H]^+$: found, 296.0533; calcd, 296.051. Anal. ($C_{11}H_{14}N_5Br$·0.4DMF) C, H, N.

4.2.5.2. 7. Yield 0.46 g (31%). Mp 260 °C (dec). ^{1}H NMR (DMSO-d_6) δ 3.64 (s, 3H, CH_3), 7.35 (s, 2H, NH_2), 8.12 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_6H_7N_5Br$ $[M+H]^+$: found, 227.9807; calcd, 227.9884. Anal. ($C_6H_6N_5Br$) C, H, N.

4.3. General procedure for the synthesis of N3- and N9-substituted N^6-cyclopentyladenine (8–14): method A

The appropriate alkyl halide (5 equiv), 8-bromo-N^6-cyclopentyladenine (**5**, 1 equiv), K_2CO_3 (2 equiv), and DMF were mixed and stirred for 1 h. After filtration, the filtrate was evaporated and purified by column chromatography (ethyl acetate/PE 40-60).

4.3.1. 9-Allyl-8-bromo-N^6-cyclopentyladenine (8). This was prepared according to method A, starting from allyl bromide in a yield of 23%. Mp 62–63 °C. ^{1}H NMR ($CDCl_3$) δ 1.52–2.17 (m, 8H, 4×CH_2), 4.60 (m, 1H, C*H*), 4.82 (m, 2H, CH_2CH=CH_2), 5.16 (m, 2H, CH=CH_2), 5.67 (d, 1H, N^6*H*), 5.95 (m, 1H, C*H*=CH_2), 8.36 (s, 1H, *H*-2). Anal. ($C_{13}H_{16}BrN_5$) C, H, N.

4.3.2. 8-Bromo-N^6-cyclopentyl-9-propyladenine (9) and 8-bromo-N^6-cyclopentyl-3-propyl adenine (13). These were prepared according to method A, starting from propyl iodide in a yield of 41 and 28%, respectively. Separation was performed by column chromatography (ethyl acetate/PE 40-60).

9. Mp 94–95 °C. ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, CH_3), 1.50–2.16 (m, 10H, $CH_2CH_2CH_3$, 4×CH_2), 4.16 (t, 2H, $CH_2CH_2CH_3$), 4.61 (m, 1H, C*H*), 5.72 (d, 1H, N^6*H*), 8.35 (s, 1H, *H*-2). Anal. ($C_{13}H_{18}BrN_5$) C, H, N.

13. Mp 138–140 °C. ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, CH_3), 1.59-2.08 (m, 10H, $CH_2CH_2CH_3$, 4×CH_2), 4.28 (t, 2H, $CH_2CH_2CH_3$), 4.55 (m, 1H, C*H*), 6.23 (m, 1H, N^6*H*), 7.95 (s, 1H, *H*-2). Anal. ($C_{13}H_{18}BrN_5$) C, H, N.

4.3.3. 9-Benzyl-8-bromo-N^6-cyclopentyladenine (10) and 3-benzyl-8-bromo-N^6-cyclopentyl adenine (14). These were prepared according to method A, starting from benzyl bromide in a yield of 35 and 23%, respectively. Separation was performed by column chromatography (ethyl acetate/PE 40-60).

10. Mp 128–129 °C. ^{1}H NMR ($CDCl_3$) δ 1.45–2.19 (m, 8H, 4×CH_2), 4.63 (m, 1H, C*H*), 5.42 (s, 2H, CH_2), 5.74 (d, 1H, N^6*H*), 7.35 (m, 5H, CH_{phenyl}), 8.41 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{17}H_{19}N_5Br$ $[M+H]^+$: found, 372.0803; calcd, 372.0823. Anal. ($C_{17}H_{18}N_5Br$·0.5MeOH) C, H, N.

14. Mp 104–106 °C. ^{1}H NMR ($CDCl_3$) δ 1.54–2.17 (m, 8H, 4×CH_2), 4.54 (m, 1H, C*H*), 5.48 (s, 2H, CH_2), 6.50 (d, 1H, N^6*H*), 7.36 (m, 5H, CH_{phenyl}), 7.97 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{17}H_{19}N_5Br$ $[M+H]^+$: found, 372.0788; calcd, 372.0823.

4.3.4. 9-Benzyl-N^6-cyclopentyladenine (11). This was prepared according to method A, starting from N^6-cyclopentyladenine (**4**, 0.2 g, 1 mmol) and benzyl bromide

(0.12 mL, 1 mmol) in a yield of 27%. The product was purified with hexane. Mp 122–123 °C. ^{1}H NMR ($CDCl_3$) δ 1.52–2.28 (m, 8H, 4×CH_2), 4.65 (bs, 1H, C*H*), 5.35 (s, 2H, CH_2), 5.99 (d, 1H, N^6*H*), 7.30 (m, 5H, CH_{phenyl}), 7.72 (s, 1H, *H*-2), 8.42 (s, 1H, *H*-8). HRMS (ESI-MS) for $C_{17}H_{20}N_5$ $[M+H]^+$: found, 294.1663; calcd, 294.1719.

4.3.5. 8-Bromo-*N*^{6}-cyclopentyl-3-methyladenine (12). This was prepared according to method A, starting from methyl iodide. The mixture was separated by flash chromatography. One compound was identical to 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**). Compound **12** was isolated in a yield of 23%. Mp 175–177 °C. ^{1}H NMR ($CDCl_3$) δ 1.51–2.15 (m, 8H, 4×CH_2), 3.98 (s, 3H, CH_3), 4.53 (m, 1H, C*H*), 6.31 (d, 1H, N^6*H*), 7.97 (s, 1H, *H*-2). Anal. ($C_{11}H_{14}BrN_5$) C, H, N.

4.4. General procedure for the synthesis of 9-alkyl-*N*^{6}-cyclopentyl-8-oxyadenines (15–20): method B

The appropriate 8-bromo-*N*^{6}-cyclopentyl-9-alkyladenine (**6**, 1 equiv), the corresponding alcohol, and KO*t*Bu (1 equiv) was mixed and refluxed for 2–64 h. The reaction mixture was then neutralised with acetic acid, and the products were purified with column chromatography.

4.4.1. 9-Allyl-*N*^{6}-cyclopentyl-8-methoxyadenine (15). This was prepared according to method B, starting from 9-allyl-8-bromo-*N*^{6}-cyclopentyladenine (**8**) and MeOH in a yield of 91%. Purification was performed with column chromatography (ethyl acetate/PE 40-60). Mp 50–52 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–2.18 (m, 8H, 4×CH_2), 4.14 (s, 3H, OCH_3), 4.60 (m, 3H, CH_2CH=CH$_2$, C*H*), 5.10 (m, 2H, CH=CH_2), 5.40 (d, 1H, N^6*H*), 5.92 (m, 1H, C*H*=CH$_2$), 8.30 (s, 1H, *H*-2). Anal. ($C_{14}H_{19}N_5O$) C, H, N.

4.4.2. N^6-Cyclopentyl-8-methoxy-9-propyladenine (16). This was prepared according to method B, starting from 8-bromo-*N*^{6}-cyclopentyl-9-propyladenine (**9**) and MeOH in a yield of 88%. Purification was performed with column chromatography (ethyl acetate/PE 40-60). Mp 58–60 °C. ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, CH_3), 1.50–2.14 (m, 10H, CH$_2$CH_2CH$_3$, 4×CH_2), 3.95 (t, 2H, CH_2CH$_2$CH$_3$), 4.14 (s, 3H, OCH_3), 4.60 (m, 1H, C*H*), 5.39 (d, 1H, N^6*H*), 8.31 (s, 1H, *H*-2). Anal. ($C_{14}H_{21}N_5O$) C, H, N.

4.4.3. N^6-Cyclopentyl-8-methoxy-9-methyladenine (17). This was prepared according to method B, starting from 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**) and MeOH in a yield of 19% (16 mg). Purification was performed with column chromatography (MeOH/ethyl acetate). Mp 80–90 °C. ^{1}H NMR ($CDCl_3$) δ 1.59–2.18 (m, 8H, 4×CH_2), 3.55 (s, 3H, CH_3), 4.15 (s, 3H, OCH_3), 4.42 (m, 1H, C*H*), 5.25 (d, 1H, N^6*H*), 8.30 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{12}H_{18}N_5O$ $[M+H]^+$: found, 284.1586; calcd, 248.1511.

4.4.4. N^6-Cyclopentyl-8-ethoxy-9-methyladenine (18). This was prepared according to method B, starting from 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**) and abs EtOH (10 mL) in a yield of 67% (0.20 g). Purification was performed with column chromatography (ethyl acetate) and crystallisation with CH_2Cl_2/hexane. Mp 93–97 °C. ^{1}H NMR ($CDCl_3$) δ 1.48 (t, 3H, CH$_2$CH_3), 1.52–2.20 (m, 8H, 4×CH_2), 3.54 (s, 3H, CH_3), 4.55 (m, 3H, CH_2CH$_3$, C*H*), 5.75 (d, 1H, N^6*H*), 8.29 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{13}H_{20}N_5O$ $[M+H]^+$: found, 262.1731; calcd, 262.1668. Anal. ($C_{13}H_{19}N_5O\cdot1.3H_2O$) C, H, N.

4.4.5. N^6-Cyclopentyl-8-isopropoxy-9-methyladenine (19). This was prepared according to method B, starting from 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**) and 2-propanol (10 mL) in a yield of 84% (0.26 g). Purification was performed with column chromatography (MeOH/CH_2Cl_2). Mp 75–83 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 6H, CH(CH_3)$_2$), 1.50–2.22 (m, 8H, 4×CH_2), 3.52 (s, 3H, CH_3), 4.64 (m, 1H, C*H*), 5.19–5.38 (m, 1H, C*H*(CH$_3$)$_2$), 5.88 (d, 1H, N^6*H*), 8.26 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{14}H_{22}N_5O$ $[M+H]^+$: found, 276.1827; calcd, 276.1824.

4.4.6. N^6-Cyclopentyl-9-methyl-8-propoxy-adenine (20). This was prepared according to method B, starting from 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**) and *n*-propanol (10 mL) in a yield of 99% (0.31 g). Purification was performed with column chromatography (ethyl acetate). Mp 77–79 °C. ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3H, CH$_2$CH$_2$CH_3), 1.45–2.24 (m, 10H, CH$_2$CH_2CH$_3$, 4××CH_2), 3.55 (s, 3H, CH_3), 4.43 (t, 2H, CH_2CH$_2$CH$_3$), 4.61 (m, 1H, C*H*), 5.63 (d, 1H, N^6*H*), 8.28 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{14}H_{22}N_5O$ $[M+H]^+$: found, 276.1812; calcd, 276.1824.

4.4.7. ^{6}N-Cyclopentyl-8-(ethylthio)-9-methyladenine (21). A mixture of 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**, 0.34 g, 1.13 mmol), ethanethiol (0.17 mL, 2.26 mmol), KO*t*Bu (0.25 g, 2.26 mmol), and EtOH (7 mL) was added in a pressure tube and stirred at 50° CC overnight. Purification was performed with column chromatography (ethyl acetate) and crystallisation with hexane. Yield 0.29 g (93%). Mp 94–96 °C C. ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, CH$_2$CH_3), 1.49–2.22 (m, 8H, 4××CH_2), 3.30 (q, 2H, CH_2CH$_3$), 3.67 (s, 3H, CH_3), 4.62 (m, 1H, C*H*), 5.59 (d, 1H, N^6*H*), 8.32 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{13}H_{20}N_5S$ $[M+H]^+$: found, 278.1443; calcd, 278.1439. Anal. ($C_{13}H_{19}N_5S\cdot0.9H_2O$) C, H, N.

4.4.8. *N*^{6}-Cyclopentyl-9-methyl-8-oxo-adenine (22). 8-Bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**, 0.22 g, 0.73 mmol) was refluxed for 3 h in a 1 N NaOH solution (9.42 mL). The reaction mixture was neutralised with a 10% HCl solution, and chilled on ice. Purification was performed with column chromatography (ethyl acetate). Yield 0.12 g (70%). Mp 188–190 °C C. ^{1}H NMR ($CDCl_3$) δ 1.48–2.14 (m, 8H, 4××CH_2), 3.37 (s, 3H, CH_3), 4.47 (m, 1H, C*H*), 5.81 (d, 1H, N^6*H*), 8.30 (s, 1H, *H*-2), 10.56 (s, 1H, N-7-*H*). HRMS (ESI-MS) for $C_{11}H_{16}N_5O$ $[M+H]^+$: found, 234.1349; calcd, 234.1355.

4.4.9. ^{6}-Cyclopentyl-8-(*N*-methylamino)-9-methyladenine (23, LUF 5666). 8-Bromo-*N*^{6}-cyclopentyladenine ((**5**, 501 mg, 1.69 mmol) was dissolved in a 40% w/v aqueous methylamine solution (150 mL, excess) and

stirred for 16 h at room temperature. The reaction mixture was then concentrated in vacuo and the product was crystallised from water. Yield 423 mg (99%). Mp 131–135 °C C. ^{1}H NMR ($CDCl_3$) δ 1.57–2.14 (m, 8H, 4××CCH_2), 3.17 (d, 3H, N^8-CH_3), 3.53 (s, 3H, N-9-CH_3), 4.39 (q, 1H, N^8*H*), 4.62 (m, 1H, C*H*), 5.79 (d, 1H, N^6*H*), 8.25 (s, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 23.69 (2C, $CH_2CH_2CH_2$, cyclopentyl), 29.63 (*C*H_3, N-9), 33.37 (2C, CH*C*H_2CH_2, cyclopentyl), 41.52 (*C*H_3, methylamine), 52.32 (*C*H, cyclopentyl), 116.91 (C-8), 151.02 (C-2), 150.36, 152.24, 155.94 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{12}H_{19}N_6$ $[M+H]^+$: found, 247.1572; calcd, 247.1671.

4.4.10. N^6-Cyclopentyl-8-(*N*,*N*-dimethylamino)-9-methyladenine (24). *N*^{6}-Cyclopentyl-8-(*N*-methylamino)-9-methyladenine (**23**, 76 mg, 0.31 mmol) was dissolved in 1.5 mL DMF and NaH (60% in mineral oil, 13.5 mg, 0.31 mmol), and stirred for 15 min. Then methyl iodide was added (53 mg, 0.34 mmol) an the reaction mixture was stirred for 16 h at room temperature. Afterwards, 10 mL of water was added to the mixture and the organic product was extracted with CH_2Cl_2 (two times, 4 mL). Purification was performed with column chromatography (MeOH/CH_2Cl_2) and the product was collected as oil. Yield 34 mg (43%). ^{1}H NMR ($CDCl_3$) δ 1.50–2.17 (m, 8H, 4×CH_2), 2.93 (s, 3H, N^8-CH_3), 3.60 (s, 3H, N-9-CH_3), 4.64 (m, 1H, C*H*), 5.49 (d, 1H, N^6*H*), 8.26 (s, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 23.69 (2C, $CH_2CH_2CH_2$, cyclopentyl), 29.66 (*C*H_3, N-9), 33.39 (2C, CH*C*H_2CH_2, cyclopentyl), 41.52 (2C, *C*H_3, dimethylamine), 52.29 (*C*H, cyclopentyl), 116.79 (C-8), 151.09 (C-2), 150.24, 152.24, 155.88 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{13}H_{21}N_6$ $[M+H]^+$: found, 261.1856; calcd, 261.1828.

4.4.11. N^6-Cyclopentyl-8-(*N*-methyl-*N*-ethylamino)-9-methyladenine (25, LUF 5668). This was prepared as **24**, starting with *N*^{6}-cyclopentyl-8-(*N*-methylamino)-9-methyladenine (**23**, 61 mg, 0.25 mmol) in DMF/NaH and ethyl iodide (10.3 mg, 0.25 mmol). Yield 24 mg (36%, oil). ^{1}H NMR ($CDCl_3$) δ 1.11–1.48 (t, 3H, CH_2CH_3), 1.52–2.16 (m, 8H, 4×CH_2), 2.92 (s, 3H, N^8-CH_3), 3.41–3.22 (q, 2H, CH_2CH$_3$), 3.59 (s, 3H, N-9-CH_3), 4.65 (m, 1H, C*H*), 5.50 (d, 1H, N^6*H*), 8.27 (s, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 12.50 (*C*H_3, ethyl), 23.69 (2C, $CH_2CH_2CH_2$, cyclopentyl), 29.57 (*C*H_3, N-9), 33.42 (2C, CH*C*H_2CH_2, cyclopentyl), 38.15 (*C*H_3, methylamine), 48.34 (*C*H_2N, ethyl), 52.38 (*C*H, cyclopentyl), 116.91 (C-8), 151.06 (C-2), 150.18, 152.27, 155.51 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{14}H_{23}N_6$ $[M+H]^+$: found, 275.1961; calcd, 275.1984.

4.4.12. N^6-Cyclopentyl-8-(*N*-methyl-*N*-propylamino)-9-methyladenine (26, LUF 5669). This was prepared as **24**, starting with *N*^{6}-cyclopentyl-8-(*N*-methylamino)-9-methyladenine (**23**, 62 mg, 0.25 mmol) in DMF/NaH and ethyl iodide (49.3 mg, 0.29 mmol). Yield 26 mg (36%, oil). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, $CH_2CH_2CH_3$), 1.40–2.19 (m, 10H, $CH_2CH_2CH_3$, 8H, 4×CH_2), 2.93 (s, 3H, N^8-CH_3), 3.17 (t, 2H, N^8-CH_2), 3.60 (s, 3H, N-9-CH_3), 4.57 (m, 1H, C*H*), 5.43 (d, 1H, N^6*H*), 8.27 (s, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 11.25 (*C*H_3, propyl), 20.56 (*C*H_2CH$_2$N, propyl), 23.72 (2C, $CH_2CH_2CH_2$, cyclopentyl), 29.66 (*C*H_3, N-9), 33.45 (2C, CH*C*H_2CH_2, cyclopentyl), 38.85 (*C*H_3, methylamine), 52.32 (*C*H, cyclopentyl), 55.53 (*C*H_2N, propyl), 116.94 (C-8), 150.99 (C-2), 150.18, 152.17, 155.72 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{15}H_{25}N_6$ $[M+H]^+$: found, 289.2156; calcd, 289.2141.

4.4.13. N^6-Cyclopentyl-8-(*N*-butyl-*N*-methylamino)-9-methyladenine (27). This was prepared as **24**, starting with *N*^{6}-cyclopentyl-8-(*N*-methylamino)-9-methyladenine (**23**, 39 mg, 0.16 mmol) in DMF/NaH and butyl iodide (49 mg, 0.29 mmol). Yield 12 mg (26%, oil). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, $CH_2CH_2CH_2CH_3$), 1.19–2.20 (m, 12H, $CH_2CH_2CH_2CH_3$, butyl, 4×CH_2), 2.94 (s, 3H, N^8-CH_3), 3.21 (m, 2H, N^8-CH_2), 3.61 (s, 3H, N-9-CH_3), 4.59 (m, 1H, C*H*), 5.44 (d, 1H, N^6*H*), 8.29 (s, 1H, *H*-2). ^{13}C NMR ($CDCl_3$): δ 13.82 (*C*H_3, butyl), 20.19 (*C*H_2CH$_2$CH$_2$N, butyl), 23.70 (2C, $CH_2CH_2CH_2$, cyclopentyl), 29.38 (*C*H_2CH$_2$N, butyl), 29.68 (*C*H_3, N-9), 33.43 (2C, CH*C*H_2CH_2, cyclopentyl), 38.85 (*C*H_3, methylamine), 50.67 (*C*H_2N, butyl), 52.31 (*C*H, cyclopentyl), 116.97 (C-8), 151.02 (C-2), 149.99, 152.25, 154.67 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{16}H_{27}N_6$ $[M+H]^+$: found, 303.2245; calcd, 303.2297.

4.5. General procedure for the synthesis of 8-aminosubstituted *N*^{6}-cyclopentyl-9-methyl adenines (28–33, 35–37): method C

The appropriate amine was added in excess to a solution of 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**) in dioxane. The reaction mixture was stirred in a pressure tube at 80–120 °C for 16–120 h, and the resulting products were purified with column chromatography.

4.5.1. N^6-Cyclopentyl-8-(*N*-ethylamino)-9-methyladenine (28). This was prepared according to method C, starting from a 70% w/v ethylamine solution and stirred at 80 °C for 48 h. Purification was performed with column chromatography (MeOH/ethyl acetate). Yield 0.29 g (99%). Mp 77–81 °C. ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, CH_2CH_3), 1.50–2.17 (m, 8H, 4×CH_2), 3.50 (s, 3H, CH_3), 3.56 (m, 2H, CH_2CH$_3$), 4.05 (bs, 1H, N^8*H*), 4.59 (m, 1H, C*H*), 5.37 (d, 1H, N^6*H*), 8.23 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{13}H_{21}N_6$ $[M+H]^+$: found, 261.1906; calcd, 261.1828. Anal. ($C_{13}H_{20}N_6$·0.4SiO_2) C, H, N.

4.5.2. N^6-Cyclopentyl-8-(*N*-cyclopentylamino)-9-methyladenine (29). This was prepared according to method C, starting from cyclopentylamine solution and stirred at 120 °C for 120 h. Purification was performed with column chromatography (MeOH/ethyl acetate). Yield 0.17 g (53%). Mp 62–65 °C. ^{1}H NMR ($CDCl_3$) δ 1.46–2.19 (m, 16H, 8×CH_2), 3.48 (s, 3H, N-9-CH_3), 4.18–4.32 (m, 1H, N^{8}C*H*), 4.52 (d, 1H, N^8*H*), 4.46–4.64 (m, 1H, C*H*), 5.78 (d, 1H, N^6*H*), 8.21 (s, 1H, *H*-2). HRMS (ESI-MS) for $C_{16}H_{25}N_6$ $[M+H]^+$: found, 301.2152; calcd, 301.2141.

4.5.3. N^6-cyclopentyl-8-(*N*-phenylamino)-9-methyladenine (30). This was prepared according to method C, starting from a 70% w/v aniline solution and stirred at 100 °C for 32 h. Purification was performed with col-

umn chromatography (ethyl acetate) and crystallisation with CH_2Cl_2/hexane. Yield 0.12 g (53%). Mp 191–195 °C. ^{1}H NMR ($CDCl_3$) δ 1.38–2.28 (m, 8H, 4×CH_2), 3.59 (s, 3H, N-9-CH_3), 4.60 (m, 1H, CH), 5.45 (d, 1H, N^6H), 6.80 (bs, 1H, N^8H), 7.02 (t, 1H, CH_{phenyl}), 7.31 (t, 2H, CH_{phenyl}), 7.50 (d, 2H, CH_{phenyl}), 8.31 (s, 1H, H-2). HRMS (ESI-MS) for $C_{18}H_{21}N_6$ $[M+H]^+$: found, 309.1795; calcd, 309.1828.

4.5.4. N^6-Cyclopentyl-8-(*N*-methylisopropylamino)-9-methyladenine (31, LUF 5608). This was prepared according to method C, starting from a 70% w/v aqueous *N*-methylisopropylamine solution and stirred at 80 °C for 48 h. Purification was performed with column chromatography (ethyl acetate) and crystallisation with hexane. Yield 0.12 g (37%). Mp 59–62 °C. ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 6H, CH(CH_3)$_2$), 1.49–2.19 (m, 8H, 4×CH_2), 2.81 (m, 3H, N^{8}CH_3), 3.60 (s, 3H, N-9-CH_3), 3.73 (m, 1H, CH(CH$_3$)$_2$), 4.57 (m, 1H, CH), 5.89 (m, 1H, N^6H), 8.29 (s, 1H, H-2). HRMS (ESI-MS) for $C_{15}H_{25}N_6$ $[M+H]^+$: found, 289.2114; calcd, 289.2141.

4.5.5. N^6-Cyclopentyl-8-(*N*-methyl-*N*-benzylamino)-9-methyladenine (32). This was prepared according to method C, starting from a 70% w/v aqueous *N*-methylbenzylamine solution and stirred at 80 °C for 66 h, and subsequently at 100 °C for 48 h. Purification was performed with column chromatography (MeOH/CH_2Cl_2). Yield 52 mg (46%, oil). ^{1}H NMR ($CDCl_3$): δ 1.55-2.17 (m, 8H, 4×CH_2), 2.88 (m, 3H, N^{8}CH_3), 3.67 (s, 3H, N-9-CH_3), 4.43 (s, 2H, CH_2), 4.64 (m, 1H, CH), 5.60 (d, 1H, N^6H), 7.35–7.39 (m, 5H, CH_{phenyl}), 8.30 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$): δ 23.67 (2C, CH$_2$CH_2CH$_2$, cyclopentyl), 29.60 (CH_3, N-9), 33.39 (2C, CHCH_2CH$_2$, cyclopentyl), 38.73 (CH_3, methylamine), 52.44 (CH, cyclopentyl), 57.56 (CH_2, benzyl), 116.73 (C-8), 127-136 (6C, phenyl), 151.02 (C-2), 150.17, 152.24, 155.42 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{19}H_{25}N_6$ $[M+H]^+$: found, 337.2113; calcd, 337.2141.

4.5.6. N^6-Cyclopentyl-8-(*N*,*N*-diethylamino)-9-methyladenine (33, LUF 5674). 8-Bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**, 80 mg, 0.27 mmol) and diethylamine (1.0 mL, 10 mmol) were dissolved in 2 mL dioxane and 1 mL water. The mixture was transferred into a pressure tube, and heated in a boiling water bath for 40 h. Purification was performed with column chromatography (acetone/hexane) and the product was collected as oil. Yield 26 mg (35%). ^{1}H NMR ($CDCl_3$) δ 1.10–1.23 (t, 3H, CH$_2$CH_3), 1.52–2.11 (m, 8H, 4×CH_2), 3.21–3.32 (q, 2H, CH_2CH$_3$), 3.58 (s, 3H, N-9-CH_3), 4.61 (m, 1H, CH), 5.73 (d, 1H, N^6H), 8.27 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$): δ 12.89 (CH_3, ethylamine), 23.72 (2C, CH$_2$CH_2CH$_2$, cyclopentyl), 29.33 (CH_3, N-9), 33.42 (2C, CHCH_2CH$_2$, cyclopentyl), 45.43 (CH_2N, ethyl), 52.38 (CH, cyclopentyl), 117.09 (C-8), 151.09 (C-2), 149.99, 152.33, 154.48 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{15}H_{25}N_6$ $[M+H]^+$: found, 289.2172; calcd, 289.2141.

4.5.7. N^6-Cyclopentyl-8-(*N*-ethyl-*N*-butylamino)-9-methyladenine (34). This was prepared as **33**, starting with 8-bromo-*N*^{6}-cyclopentyl-9-methyladenine (**6**, 72 mg, 0.24 mmol) and *N*-ethylbutylamine (1 mL, 14 mmol). Purification was performed with column chromatography (acetone/hexane). Yield 23 mg (30%, oil). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, CH$_2$CH$_2$CH$_2$CH_3), 1.15 (t, 3H, CH$_2$CH_3), 1.21–2.18 (m, 12H, CH$_2$CH_2CH_2CH$_3$, butyl, 4×CH_2), 3.20–3.35 (m, 4H, CH_2CH$_3$, N^8-CH_2), 3.59 (s, 3H, N-9-CH_3), 4.58 (m, 1H, CH), 5.65 (d, 1H, N^6H), 8.28 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$): δ 12.87 (CH_3, ethyl), 13.87 (CH_3, butyl), 20.18 (CH_2CH$_2$CH$_2$N, butyl), 23.73 (2C, CH$_2$CH_2CH$_2$, cyclopentyl), 29.40 (CH_2CH$_2$N, butyl), 29.82 (CH_3, N-9), 33.46 (2C, CHCH_2CH$_2$, cyclopentyl), 46.19 (CH_3, methylamine), 50.62 (CH_2N, butyl), 52.41 (CH, cyclopentyl), 117.08 (C-8), 151.01 (C-2), 149.98, 152.29, 154.65 (C-6, C-5, C-4). HRMS (ESI-MS) for $C_{17}H_{29}N_6$ $[M+H]^+$: found, 317.2451; calcd, 317.2454.

4.5.8. N^6-Cyclopentyl-8-(*N*-pyrrolidino)-9-methyladenine (35). This was prepared according to method C, starting from a 70% w/v pyrrolidine solution and stirred at 100 °C for 16 h. Purification was performed with column chromatography (ethyl acetate). Yield 0.13 g (62%). Mp 95–96 °C. ^{1}H NMR ($CDCl_3$) δ 1.35–2.23 (m, 12H, 4×CH_2, N^8-CH$_2$CH_2), 3.58 (m, 4H, N^8-CH_2), 3.68 (s, 3H, N-9-CH_3), 4.57 (m, 1H, CH), 5.75 (d, 1H, N^6H), 8.25 (s, 1H, H-2). HRMS (ESI-MS) for $C_{15}H_{23}N_6$ $[M+H]^+$: found, 287.1982; calcd, 287.1984. Anal. ($C_{15}H_{22}N_6{\cdot}0.3SiO_2$) C, H, N.

4.5.9. N^6-Cyclopentyl-8-(*N*-piperidino)-9-methyladenine (36). This was prepared according to method C, starting from a 70% w/v aqueous piperidine solution and stirred at 100 °C for 16 h. Purification was performed with column chromatography (ethyl acetate) and crystallisation with hexane. Yield 0.21 g (95%). Mp 137–139 °C. ^{1}H NMR ($CDCl_3$) δ 1.37–2.26 (m, 14H, 4×CH_2, 3×CH_2), 3.19 (m, 4H, N^8-CH_2), 3.60 (s, 3H, N-9-CH_3), 4.59 (m, 1H, CH), 5.61 (d, 1H, N^6H), 8.30 (s, 1H, H-2). Anal. ($C_{16}H_{24}N_6$) C, H, N.

4.5.10. N^6-Cyclopentyl-8-morpholino-9-methyladenine (37). This was prepared according to method C, starting from a 70% w/v aqueous morpholine solution and stirred at 120 °C for 66 h. Purification was performed with column chromatography (MeOH/CH_2Cl_2). Yield 83 mg (95%). Mp 118–122 °C. ^{1}H NMR ($CDCl_3$) δ 1.4-2.2 (m, 8H, 4×CH_2), 3.25–3.30 (t, 4H, CH_2OCH_2), 3.63 (s, 3H, N-9-CH_3), 3.92–3.87 (t, 4H, CH_2NCH_2), 4.59 (m, 1H, CH), 5.45 (d, 1H, N^6H), 8.32 (s, 1H, H-2). HRMS (ESI-MS) for $C_{15}H_{23}N_6O$ $[M+H]^+$: found, 303.1916; calcd, 303.1933.

4.6. Radioligand displacement experiments

The adenosine A_1 receptor binding assays were carried out on membranes of CHO cells that have a high adenosine A_1 receptor density (~3 pmol/mg of protein, CHO-A_1^{++}). Membrane aliquots, containing 6 μg of protein and increasing concentrations of the compound, were incubated in 400 μL of 50 mM Tris–HCl, pH 7.4 at 25 °C for 60 min in the presence of ~1.6 nM [^{3}H]DPCPX. Non-specific binding was measured in the presence of 10 μM CPA. Incubations were stopped by dilution with the above-mentioned buffer, and bound

radioligand was separated by rapid filtration through Whatman GF/B filters using a Brandel harvester. Filters were subsequently washed three times with the same ice-cold buffer. Bound radioactivity was measured by scintillation spectrometry after the addition of 3.5 mL of Packard Emulsifier Safe. Adenosine A_3 binding experiments were done with membranes of HEK293 cells expressing the human adenosine A_3 receptor (HEK293-A_3). Briefly, membrane aliquots (40 μg of protein) were incubated at 37 °C for 60 min with ~0.1 nM of [^{125}I]AB-MECA ([^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-ethylcarboxamidoadenosine), and a fixed concentration of all compounds (10 μM) or range of concentrations for selected compounds (**5**, **8**, **9**, **13**, **14**, **19**, **20**, and **32**), in a final volume of 200 μL of 50 mM Tris/10 mM $MgCl_2$/1 mM EDTA (ethylenediaminetetra-acetic acid)/0.01% CHAPS [3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate] buffer. Nonspecific binding was measured in the presence of 100 μM *R*-PIA [(*R*)-N^6-phenylisopropyladenosine]. Binding reactions were terminated by dilution with ice-cold buffer. Samples were then filtered through Whatman GF/B glass-fiber filters using a Brandel cell harvester, and filters were washed three times. Bound radioactivity was measured in a Beckman 5500B γ-counter.

4.7. [^{35}S]GTPγS binding

The modulation of [^{35}S]GTPγS binding was determined according to the method of Lorenzen et al.[23] with minor modifications. Incubations were performed at 25 °C for 90 min with 4–5 μg of membrane protein. The GDP and NaCl concentrations were 3 μM and 100 mM, respectively. Basal [^{35}S]GTPγS binding was set to 100%. Under these conditions, CPA behaved as a full agonist stimulating basal [^{35}S]GTPγS binding to 317%.

4.8. Data analysis

K_i values were calculated using a non-linear regression curve fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). The K_d value of [^{3}H]DPCPX at CHO-A_1^{++} membranes used was 1.6 nM. The intrinsic activities of the compounds are reported as the percentage of basal [^{35}S]GTPγS binding (set at 100%) remaining in the presence of concentrations of $10\times K_i$ determined from receptor binding experiments.

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