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Modulation of adenosine receptor affinity and intrinsic efficacy in adenine nucleosides substituted at the 2-position

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Abstract—We studied the structural determinants of binding affinity and efficacy of adenosine receptor (AR) agonists. Substituents at the 2-position of adenosine were combined with N^6-substitutions known to enhance human A_3AR affinity. Selectivity of binding of the analogues and their functional effects on cAMP production were studied using recombinant human A_1, A_{2A}, A_{2B}, and A_3ARs. Mainly sterically small substituents at the 2-position modulated both the affinity and intrinsic efficacy at all subtypes. The 2-cyano group decreased hA_3AR affinity and efficacy in the cases of N^6-(3-iodobenzyl) and N^6-(*trans*-2-phenyl-1-cyclopropyl), for which a full A_3AR agonist was converted into a selective antagonist; the 2-cyano-N^6-methyl analogue was a full A_3AR agonist. The combination of N^6-benzyl and various 2-substitutions (chloro, trifluoromethyl, and cyano) resulted in reduced efficacy at the A_1AR. The environment surrounding the 2-position within the putative A_3AR binding site was explored using rhodopsin-based homology modeling and ligand docking.

1. Introduction

Receptors for the extracellular local modulator adenosine consist of four subtypes: A_1, A_{2A}, A_{2B}, and A_3.[1] The selective activation of the A_3AR (adenosine receptor) is both cardioprotective and cerebroprotective in a variety of ischemic models.[2,3] The activation of this receptor subtype has also been associated with a cytostatic, anticancer effect in several tumor models.[4] Thus, selective A_3AR agonists have therapeutic potential.

Previous medicinal chemical studies demonstrated that an adenosine derivative's ability to activate the A_3AR is more structure sensitive than at other AR subtypes.[5–7] Changes in various regions of the adenosine molecule have been shown to reduce A_3AR efficacy, which leads to nucleoside analogues that are partial agonists or antagonists. A structure–efficacy relationship at this subtype, separate from the structure–affinity relationship derived from receptor binding experiments, was analyzed.[7] Substitution with N^6-benzyl groups, 2-chloro substitution of the adenine moiety, and conformational constraint of the ribose moiety all contribute to a reduction of efficacy. In the present study, we evaluated the binding affinity and functional properties of adenosine derivatives modified at the N^6-position to achieve high A_3AR affinity and at the 2-position with simple substitutions, such as cyano, carbonyl, aminomethyl, and trifluoromethyl. Previously, the effects of such substitutions at AR subtypes had not been fully evaluated.[8]

2. Results and discussion

2.1. Chemistry

Adenosine agonists **2**–**17** and **19**–**21** were prepared (Schemes 1–3) to study the effects of 2-position substitution in interactions with ARs. Key synthetic intermediates **24** and **25** contained the 2-iodo and 2-cyano group, respectively, in combination with 6-chloro substitution.

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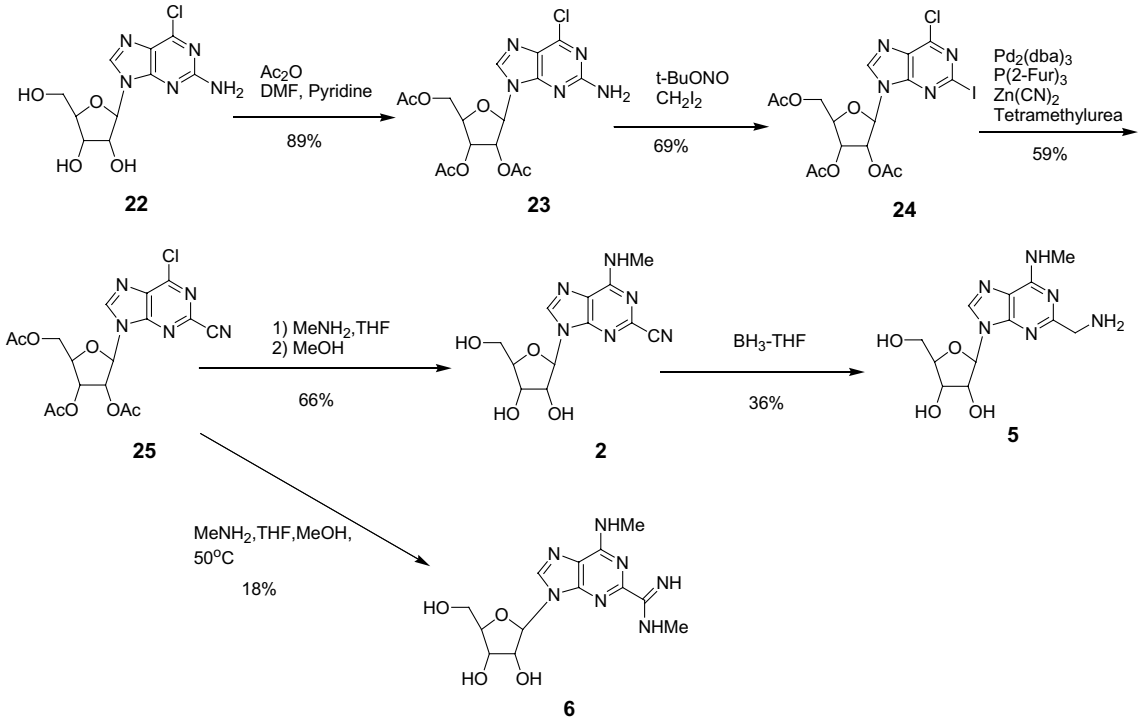
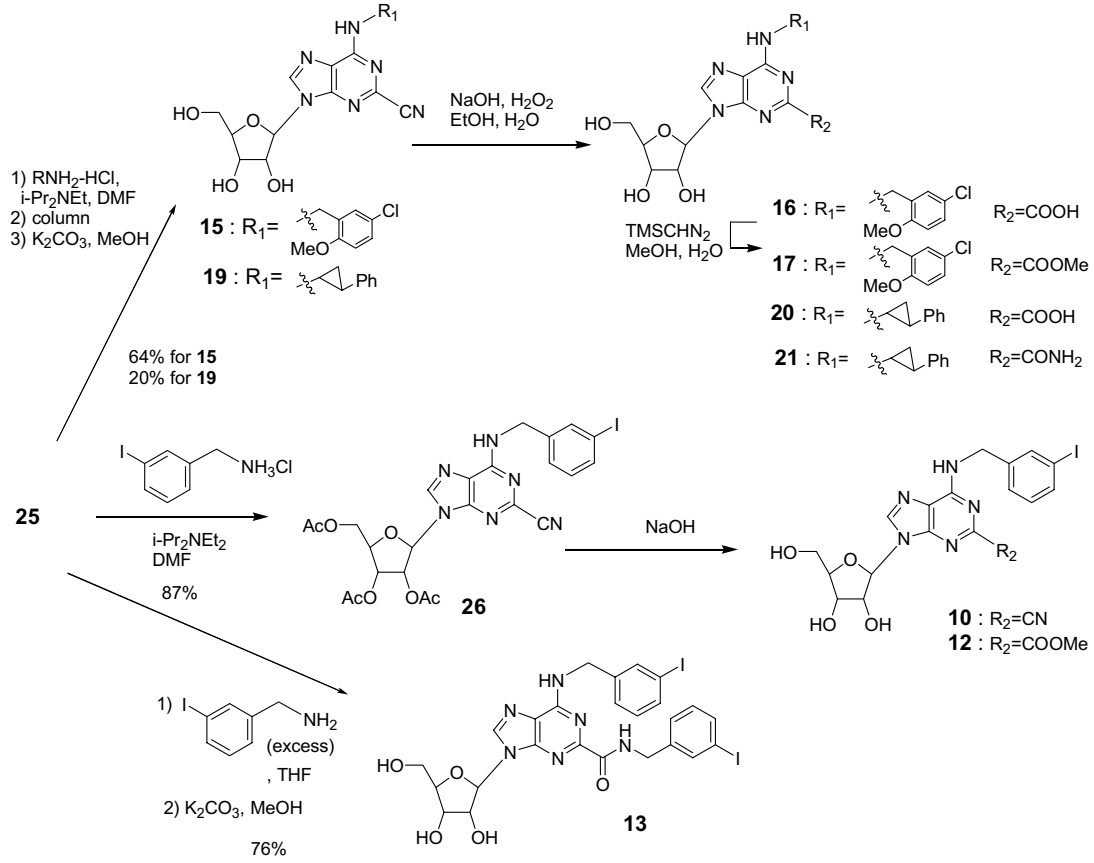
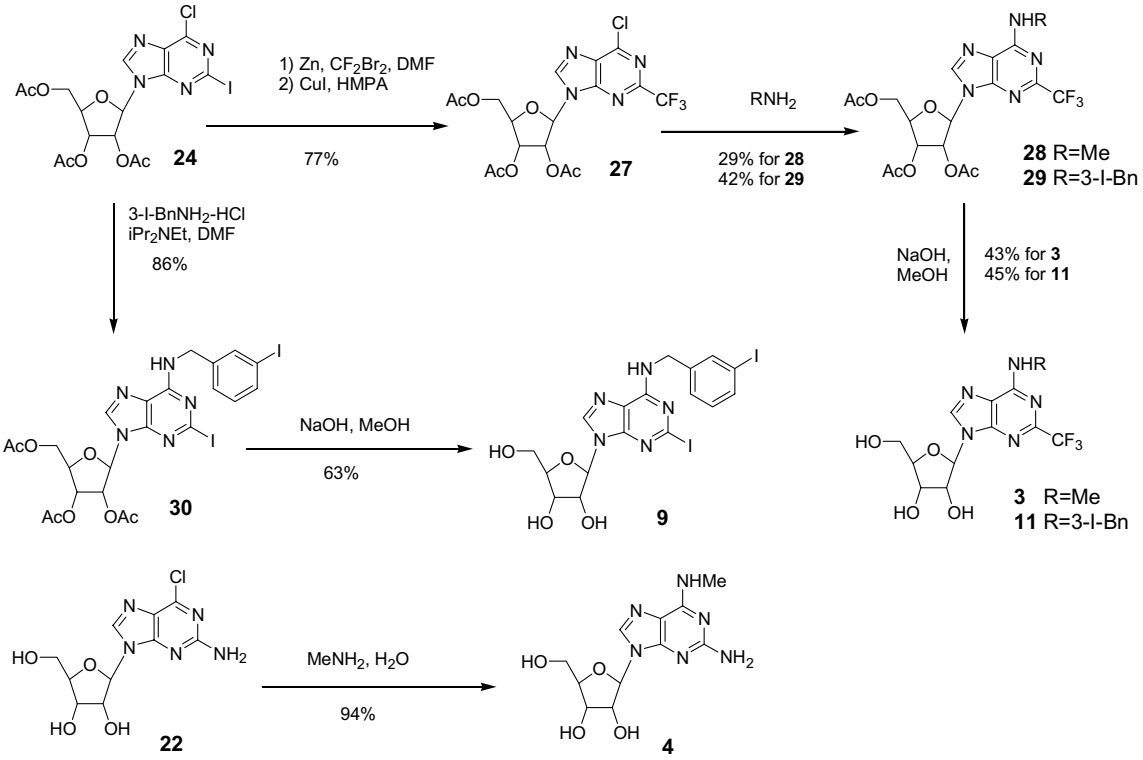


Scheme 1.



Scheme 2.

The introduction of the 2-cyano group was carried out by the reaction of zinc cyanide on the corresponding 2-iodo analogue with palladium chemistry (Scheme 1). We chose the protected 2-amino nucleoside **23** as the



Scheme 3.

synthetic intermediate because of the simplicity of its synthesis from (−)-2-amino-6-chloropurine riboside **22**. An attempted Sandmeyer-type cyanation (*t*-BuONO, CuCN, MeCN, 65 °C) on **23** resulted in decomposition without isolation of the desired 2-CN compound. We then applied Pd chemistry to this cyanation. The 2-amino group of **23** was converted to 2-iodo using a neutral purinyl radical, which was generated transiently from the thermal homolysis of the 2-diazonium intermediate.[9] Stille-type conditions ($PdCl_2(PPh_3)_2$, Bu_3SnCN) or use of potassium cyanide ($PdCl_2(PPh_3)_2$, KCN)[10] on the iodo derivative **24** resulted in decomposition. Only use of zinc cyanide and a Pd(0) complex generated in situ from tris-2-furylphosphine and $Pd_2(dba)_3$ (tris(dibenzylideneacetone)dipalladium(0) chloroform adduct)[11] effected cyanation of **24**. Use of tetramethylurea as solvent gave a higher yield than *N*-methyl-2-pyrrolidinone, which was recommended in the literature.

Intermediate **25** was coupled with the selected amines (methylamine, 5-chloro-2-methoxybenzylamine, or *trans*-2-phenyl-1-cyclopropylamine) at room temperature, and the acetyl groups were hydrolyzed to give the final target compounds **2**, **15**, and **19** (Schemes 1 and 2). The 2-cyano group could be reduced with BH_3 to give the 2-aminomethyl derivative **5** (Scheme 1) and in other cases was hydrolyzed to give two 2-carboxylic acid derivatives **16** and **20** (Scheme 2). In the case of the N^6-methyl derivative **2**, the 2-cyano group also reacted with methylamine at elevated temperature and provided a *N*-methyl-carboxyamidine (C(=NH)NHMe), which was hydrolyzed to give **6**. In the case of the 3-iodobenzylamine derivative **26**, the methanol solvent reacted with the 2-cyano group and upon hydrolysis produced the 2-methoxycarbonyl derivative **12**. In methanol-free hydrolysis conditions, the desired N^6-(3-iodobenzyl) derivative **10** was obtained. Adding excess 3-iodobenzylamine to **25** and heating resulted in amine addition at the 2-cyano group to give the 2-(3-iodobenzylamide) derivative **13** upon hydrolysis.

Trifluoromethylation was performed on the intermediate **24** by treatment with 'CF_3Cu' species, which was generated in situ from CF_3ZnBr and CuI (Scheme 3).[12,13] Intermediate **27** was coupled with methylamine or 3-iodobenzylamine, and the acetyl groups were hydrolyzed to give the final target compounds **3** and **11**.

A 2-iodoadenosine derivative **9** was also synthesized from **24** by treatment with 3-iodobenzylamine followed by hydrolysis. A 2-amino-N^6-methyl derivative **4** was synthesized by treatment of **22** with methylamine without the use of a protecting group. Each compound obtained was purified by column chromatography, preparative thin-layer chromatography, or HPLC.

2.2. Biological activity

We measured the binding affinities of the adenosine derivatives examined in this study (**1**–**21**) at hA_1, hA_{2A}, and hA_3 (human) ARs and at the rA_3AR (rat) and their degree of activation at the four hAR subtypes (Table 1). The efficacy of each of these adenosine derivatives in activation of the hARs was evaluated at a fixed concentration of 10 μM. Four different N^6-substitutions were included: methyl, 3-iodobenzyl, 5-chloro-2-methyloxybenzyl, and *trans*-2-phenyl-1-cyclopropyl. The

choice of these four N^6-substituents was based on either their prior use as groups that enhance hA_3AR affinity or selectivity[6,7,14] or structural similarity to such a moiety.[15] Stereoselectivity of the N^6-functional group of adenosine derivatives in receptor binding has been established at A_3ARs.[7] Although the *trans*-2-phenyl-1-cyclopropyl analogues **18**–**21** shown in Table 1 are diastereomeric mixtures, it was previously established that the principal biological activity at the A_3AR for this series is associated with the (1*S*,2*R*) isomer.[7] These N^6-substitutions were combined with various 2-modifications, including chloro, iodo, cyano, trifluoromethyl, carboxylic acid, amide, ester, or aminomethyl groups.

The codependence of N^6 and C-2 regions in AR binding and activation was evident. With a small group at the N^6 position (methyl), the affinity at the hA_3AR was reduced by some groups at C-2 (trifluoromethyl **3**, amino **4**, aminomethyl **5**, and *N*-methyl-carboxamidine **6**) and enhanced by another group, cyano **2**. In the case of N^6-methyl derivatives, the 2-cyano, 2-trifluoromethyl, and 2-amino analogues **2**–**4** were full agonists, and **2** also showed moderate hA_3AR selectivity ($A_1AR/A_3AR = 20$-fold, $A_{2A}AR/A_3AR > 1000$-fold). Aminomethyl **5** and *N*-methyl-carboxamidine **6** analogues, being positively charged at physiological pH, decreased the affinity at ARs significantly. Also, by virtue of a charged group, **5** and **6** may prove to be neoligands for engineered ARs.[16]

In contrast to N^6-methyl, with a larger N^6-substituent, the 2-cyano substitution did not enhance the affinity, but rather resulted in a reduction in the ability to activate the receptor. For example, for N^6-(5-chloro-2-methoxybenzyl), the efficacy of the 2-cyano analogue **15** was somewhat reduced compared to the 2-H analogue **14** of similar A_3AR affinity. Nevertheless, both were partial agonists. Similarly, a loss of efficacy was seen for the corresponding N^6-(3-iodobenzyl) analogues, that is **10** compared with **7**. The 2-cyano analogue **15** showed improved hA_3AR selectivity ($A_1AR/A_3AR = 23$-fold, $A_{2A}AR/A_3AR = 457$-fold); **14** behaved as an A_1AR full agonist and an A_3AR partial agonist. For a pair of *trans*-2-phenyl-1-cyclopropyl derivatives, at the hA_3AR the addition of the 2-cyano group transformed a selective full agonist **18** into a selective full antagonist **19**.

Other 2-substitutions had varied effects. Curiously, when substituted with 2-COOMe, the high affinity of these N^6-substituted adenosine analogues at the hA_3AR was maintained or even increased (**12** compared with **7** with N^6-(3-iodobenzyl)). Also, **12** showed favorable hA_3AR selectivity ($A_1AR/A_3AR = 23$-fold, $A_{2A}AR/A_3AR = 130$-fold). Compound **12** appeared to be an A_3AR antagonist and an A_1AR weak, partial agonist. Another 2-COOMe derivative, **17**, was a highly selective A_3AR partial agonist.

At the hA_1AR, our compounds showed variable efficacy. In a previous report, 2-halo analogues showed full agonist activity at the A_1AR.[17] In our results, **14**, **18**, and **21** showed full agonism, and **9**–**11** were antagonists. Compounds **2**, **7**, **8**, **12**, and **15** appeared to be partial

Table 1. Binding affinities and maximal agonist effects of adenosine derivatives (upon structural variation at N^6 and C2 positions) at human A_1, A_{2A}, A_{2B}, and A_3ARs and at rat A_3ARs expressed in CHO (Chinese hamster ovary) cells[a]

#	N^6-R_1	C-2	K_i (hA_1AR)[a] or % displ.	% Act. (hA_1AR)[b]	K_i ($hA_{2A}AR$)[a] or % displ.	% Act. ($hA_{2A}AR$)[b]	% Act. ($hA_{2B}AR$)[b]	K_i (hA_3AR)[a]	% Act. (hA_3AR)[b]	K_i (rA_3AR)[a]
1	CH_3	H	5970 ± 2030	38 ± 10	17%	5 ± 5	16 ± 3	9.3 ± 0.4	96 ± 3	6390
2	CH_3	CN	69.8 ± 4.4	60 ± 7	23%	0	0	3.4 ± 0.8	101 ± 7	>10,000
3	CH_3	CF_3	4650 ± 580	4 ± 3	9%	20 ± 3	0	64.5 ± 3.8	93 ± 3	>10,000
4	CH_3	NH_2	484 ± 22	13 ± 2	15%	29 ± 6	11 ± 2	39.0 ± 2.4	98 ± 3	>10,000
5	CH_3	CH_2NH_2	27%	32 ± 4	25%	30 ± 3	4 ± 2	719 ± 37	57 ± 4	>10,000
6	CH_3	CH(=NH)–$NHCH_3$	10%	8 ± 5	9%	12 ± 3	13 ± 8	2730 ± 268	7 ± 1	ND
7	IB	H	7.4 ± 1.7	78 ± 6	135 ± 22	90 ± 7	58 ± 1	5.8 ± 0.4	46 ± 8	9.5 ± 1.4[c]
8	IB	Cl	16.8 ± 2.2	8 ± 3	197 ± 34	99 ± 2	16 ± 3	1.8 ± 0.1	0	2.7 ± 1.2
9	IB	I	191 ± 12	0	1910 ± 320	92 ± 8	9 ± 6	24.3 ± 3.1	0	ND
10	IB	CN	1750 ± 290	1 ± 1	25%	32 ± 5	0	119 ± 19	0	256 ± 30
11	IB	CF_3	612 ± 131	0	35%	61 ± 3	15 ± 2	138 ± 7	0	257 ± 19
12	IB	CO_2CH_3	73.1 ± 22.9	38 ± 4	390 ± 160	91 ± 9	18 ± 2	3.21 ± 0.17	0	6.05 ± 0.16
13	IB	CONH(3-IB)	500 ± 160	66 ± 16	3990 ± 150	52 ± 18	5 ± 2	133 ± 19	0	ND
14	CMB	H	9.2 ± 0.5	98 ± 8	399 ± 6	93 ± 6	56 ± 4	1.31 ± 0.16	53 ± 3	ND
15	CMB	CN	63.2 ± 16.9	36 ± 13	1260 ± 190	90 ± 15	16 ± 3	2.76 ± 0.51	29 ± 6	12.8 ± 2.3
16	CMB	COOH	41%	33 ± 10	9%	31 ± 6	6 ± 7	211 ± 36	0	698 ± 100
17	CMB	CO_2CH_3	6200 ± 790	30 ± 4	35%	74 ± 3	6 ± 4	8.04 ± 0.4	35 ± 4	ND
18	PC	H	124 ± 30	101 ± 13	2530 ± 720	88 ± 23	20 ± 15	0.86 ± 0.09	101 ± 5	399 ± 28
19	PC	CN	1750 ± 280	28 ± 14	5640 ± 780	51 ± 8	6 ± 4	8.7 ± 2.1	0	ND
20	PC	COOH	30%	6 ± 2	0%	5 ± 5	3 ± 1	1730 ± 540	28 ± 1	ND
21	PC	$CONH_2$	295 ± 25	98 ± 7	41%	94 ± 4	16 ± 3	19.7 ± 0.4	74 ± 6	ND

[a] All AR binding experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human or rat ARs (unit: nM). Radioligands: 2 nM [^{3}H]*R*-PIA (A_1), 15 nM [^{3}H]CGS21680 (A_{2A}), and 0.5 nM [^{125}I]I-AB-MECA (A_3). % displacement at 10 μM.

[b] Percent activity at 10 μM, relative to 10 μM CPA (A_1), 10 μM NECA (A_{2A}, A_{2B}), or 10 μM Cl-IB-MECA (A_3). Values are expressed as mean ± sem, $n = 3$–5.

[c] Data from Kim et al.[25] ND, not determined. IB, 3-iodobenzyl; CMB, 5-chloro-2-methyloxybenzyl; PC, *trans*-2-phenyl-1-cyclopropyl.

agonists at the hA_1AR. $hA_{2A}AR$ affinities were moderate to weak, and there was no indication of partial agonism. In a previous report, 2-halo substituents reduced the efficacy at the $A_{2A}AR$.[17] At the $hA_{2A}AR$, 2-Cl (**8**) and 2-I (**9**) analogues showed full agonism. Compounds **7**, **12**, **14**, **15**, and **21** were also full $A_{2A}AR$ agonists. At the $hA_{2B}AR$, only **7** and **14** substantially activated at 10 μM.

The loss of A_3AR affinity of **13**, which was substituted with a 2-CONHBn group, could be explained by the bulkiness of this group. The less bulky 2-iodo analogue **9** was four-fold less potent in binding to the hA_3AR than the 2-H, while the corresponding methyl ester **12** was twice as potent. Another electron withdrawing group, 2-CF_3, was similar to 2-CN in its effect on A_3AR affinity (**11** compared with **10**) when N^6-(3-iodobenzyl) was present. However, with N^6-methyl there was a 20-fold reduction (**3** compared with **2**). 2-COOH, which is also charged at physiological pH, decreased the affinity at several ARs significantly (**16** compared with **14** and **20** compared with **18**). However, 2-$CONH_2$ **21** retained A_3AR affinity. Although compound **21** fully activated the A_1AR, it was only a partial agonist at the A_3AR.

The usefulness of the compounds may also be limited by species differences. For example, with the N^6-methyl group, the affinity at the rA_3AR was dramatically reduced (e.g., **1** and **2**); this is similar to previous observations.[7,18] Species differences at the A_3ARs were also observed for larger N^6-substituent derivatives, for example, **18**. However, for substituted N^6-benzyl derivatives, binding affinity at the rA_3AR tended to be preserved. For example, **12** displayed a K_i value of 6 nM at the rA_3AR, which was almost the same as its hA_3AR affinity. Compound **12** also displayed substantial A_3AR selectivity. Thus, **12** may prove to be an A_3AR-selective antagonist in the study of rat models. In general, for N^6-(3-iodobenzyl)–derivatized compounds, the affinity at the rA_3AR was no more than three-fold weaker than at hA_3AR, making these compounds attractive for testing in nonprimate animals.

2.3. Molecular modeling

To locate energetically favorable binding orientations of 2-H and 2-CN derivatives **1**, **2**, **7**, **10**, **14**, and **15**, the previously reported Cl-IB-MECA hA_3AR complex was used as the starting geometry for the ribose-binding position.[6] For the optimal interaction, various t_0 angles for the N^6-substituent and χ_1 angles for the adenine ring were generated, and the resulting conformations were compared energetically in the putative binding site.

The hA_3AR docking result of the high-affinity nucleoside **15** is shown in Figure 1. Residues in the putative binding site that were within 5-Å proximity to the ligand were L91 (3.33), T94 (3.36), H95 (3.37), Q167 (EL2), F168 (EL2), S181 (5.42), M177 (5.38), V178 (5.39), F182 (5.43), W243 (6.48), L246 (6.51), S247 (6.52), N250 (6.55), I268 (7.39), S271 (7.42), and H272 (7.43). Molecular modeling results were correlated with results of point mutation in TMs (transmembrane helical domains) 3, 6, 7, and EL2.[6,19–21]

The purine ring was surrounded by a hydrophobic pocket, which was defined by L91 (3.33) and L246 (6.51). In addition, H-bonds formed between the exocyclic amine and the hydroxyl group of S247 (6.52), which was also in proximity to N250 (6.55), and between the purine N^3 atom and the side chain of Q167 (EL2) (Fig. 1A). The 2′-OH group of the ribose moiety formed an H bond with the backbone carbonyl group of I268 (7.39), and the 3′-OH group H bonded with the backbone carbonyl group of S271 (7.42) and with the side chain of H272 (7.43), consistent with our A_3 neoceptor model.[19] The

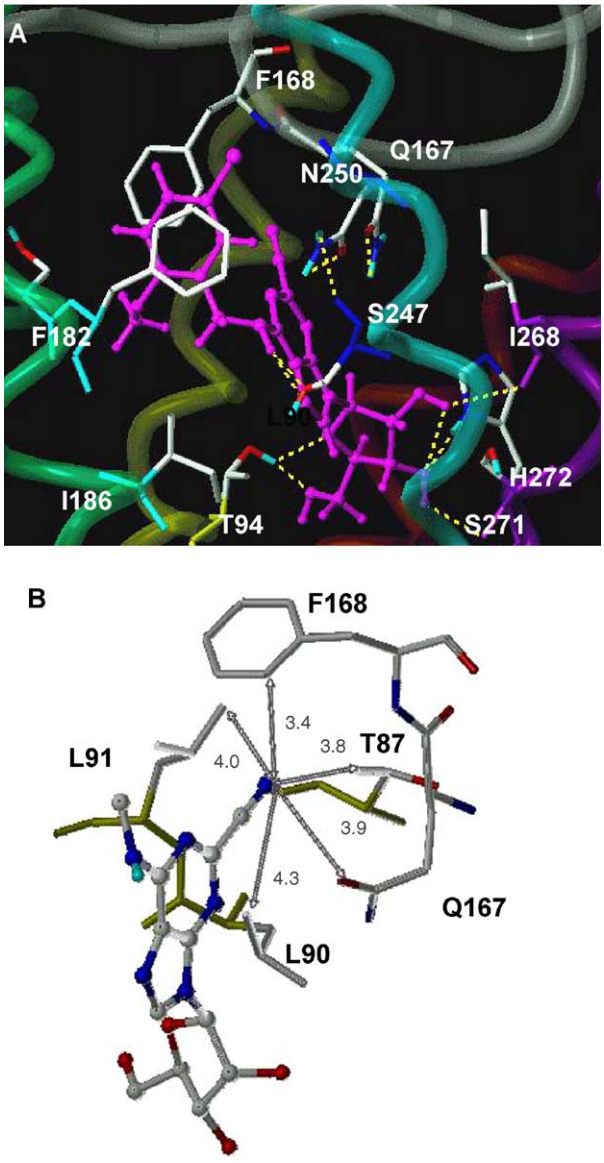


Figure 1. The complexes of the A_3AR with 2-cyano ligands (A) the overall binding site of **15** and (B) the 2-cyano binding site of **2** with distances in Å. All ligands are displayed as ball-and-stick models in magenta color, and the side chains of the hA_3AR are shown as stick models. The H bonding between the ligand and the hA_3AR is displayed in yellow. The A_3AR is represented by a tube model, with a different color for each TM (TM3 in yellow, TM5 in green, TM6 in cyano, TM7 in purple).

5′-hydroxyl group also formed an H bond with T94 (3.36). The phenyl moiety showed an additional hydrophobic interaction with F168 (EL2), and the methyl of the methoxy group was stabilized through hydrophobic interaction with L91 (3.33) and I186 (5.47), consistent with its higher binding affinity.

In comparison to N^6-methyl adenosine **1**, the more potent 2-cyano-N^6-methyl derivative **2** showed a more favorable nonbonding van der Waals interaction between the cyano group and the side chains of L90 (3.32) and L91 (3.33) and between the methyl group of the side chain of T87 (3.29) and the aromatic ring of F168 of EL2 (Fig. 1B). The distances between the N atom of the cyano group and the closest carbon atoms of the surrounding hydrophobic residues were 3.4–4.3 Å. The C-2 substituent was also proximal to the hydrophilic residue Q167 (EL2), with a distance of only 3.9 Å between the N atom of the cyano group and the side chain carbonyl O atom of Q167. There are some variable amino acids surrounding the binding site of the 2-cyano group. The amino acid corresponding to L90 (3.32) was Val in other ARs. T87 (3.29) was Ala in other human AR subtypes and Ser for the rA_3AR. Q167 was a highly variable residue among the different subtypes and species, corresponding to Glu for A_1AR, Leu for A_{2A} and $A_{2B}AR$, and His for rat A_3AR. Thus, the hA_3AR preference of 2-cyanoadenosine derivatives might be explained by an optimal van der Waals interaction. Another possibility is that a different binding preference for χ_1 angle at each AR subtype would vary the residues of interaction with the C-2 substituents.

The preferred t_0 and χ_1 angles of the energetically favorable bound conformation, derived from hA_3AR docking of various compounds, depended on the particular N^6 and C-2 substituents. In the lowest-energy complexes of **1** and **2**, the t_0 and χ_1 angles were $-134.7°$ and $-102.1°$ and $-162.7°$ and $-97.3°$, respectively. For larger N^6 groups, such as 3-I-benzyl, the preferred t_0 and χ_1 angles were $-113.8°$ and $-111.5°$ for **7** and $-117.5°$ and $-108.3°$ for **10**. Compared to **2**, the binding of the 2-cyano group in **10** was directed more toward the methyl group of T87 (3.29), resulting in unfavorable van der Waals interactions, consistent with decreased binding affinity. The difference in 2-CN orientation between **2** and **10** was a consequence of additional hydrophobic interaction of the phenyl ring of the N^6-substituent. Thus, the modeling has demonstrated how the interactions at the N^6 and C-2 regions of the hA_3AR might be interdependent.[22]

3. Conclusions

In previous studies, it was demonstrated that 2-chloro and other 2-substitutions of the adenine ring may increase the affinity and decrease the efficacy of the adenosine derivatives for the hA_3AR.[5–7] The 2-chloro modification has been incorporated into many of the more highly potent AR probes. At the A_1ARs, 2-chloro has also been observed to enhance affinity of selective agents. Here we further demonstrated that the 2-substitution with the cyano group and other small groups has variable effects.

Although the SAR (structure–activity relationship) of the many 2-substitutions of adenosine at ARs has been thoroughly explored,[23–26] the cyano, trifluoromethyl, simple ester, and other groups included here have not been among them. However, various 2-alkyne derivatives,[18,27] such as 2-hexynyladenosine-5′-*N*-ethyluronamide, have been found to bind potently to both A_{2A} and A_3ARs. The 2-iodo group was found previously to reduce efficacy at the $A_{2A}AR$ and retain efficacy at the A_1AR.[17] In our study, 2-iodo analogue **9** showed full agonism at the $hA_{2A}AR$ and antagonism at the hA_1AR.

We have studied the effects of mainly sterically small groups substituted at the 2-position on the affinity and intrinsic efficacy at A_1, A_{2A}, A_{2B}, and A_3 receptors. The previously reported compound **8** appears to be a mixed A_1/A_3AR antagonist. Compound **12** appears to be an A_3AR antagonist and an A_1AR weak, partial agonist. Compound **14** behaves as an A_1AR full agonist and an A_3AR partial agonist, and compound **17** is a highly selective A_3AR partial agonist. Compound **19** is a potent A_3AR antagonist, and the related compound **21** is an A_1AR full agonist and an A_3AR partial agonist. Thus, we have modulated both the AR affinity and efficacy upon substitution at the 2-position in combination with various N^6-substituents. This has resulted in a collection of adenosine analogues having widely differing pharmacological activities, both quantitatively and qualitatively. The dependence of intrinsic efficacy at the A_3AR has recently been emphasized,[5–7] and with this study we extend the analysis to other AR subtypes.

In conclusion, we have expanded the range of structures suitable as nucleoside antagonists of the A_3AR. Such compounds include **12** and **16**. A_3AR antagonists are of interest for treatment of glaucoma and possibly asthma.[1] Partial A_1AR agonists, such as **2**, **12**, and **15**, may be of interest for treatment of cardiac arrhythmias.[26] Whether the presence of A_3AR agonism or antagonism in these compounds might provide a therapeutic advantage is unknown. Compound **2** as a selective hA_3AR agonist could be examined for activity as a cardioprotective and cerebroprotective agent.[2,3,28] These compounds may also be useful as receptor probes for mutagenesis and for the design of neoceptors (e.g., moderately potent ligands that have either negative, for example, **16**, or positive, for example, **5**, charges near the 2-position).[16,19–21] The expanded analysis of the SAR of nucleoside binding at the A_3AR may now be applied to other agonists series, to probe the additivity with ribose ring modifications.[6,7]

4. Experimental materials

[^{125}I]N^6-(4-Amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide ([^{125}I]I-AB-MECA; 2000 Ci/mmol), N^6-[(*R*)-phenylisopropyl]adenosine ([^{3}H]*R*-PIA; 34 Ci/mmol), and [^{3}H]cyclic AMP (40 Ci/mmol) were obtained from Amersham Pharmacia Biotech (Piscataway, NJ). 2-[*p*-

(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamido-adenosine ([^{3}H]CGS21680; 47 Ci/mmol) was supplied by Perkin–Elmer Life Sciences (Boston, MA). Several N^6-substituted adenosine derivatives **1**, **7**, **8**, and **18** were prepared as reported (Ref. 7 and references therein). All other chemicals were of analytical grade from standard commercial sources.

4.1. Chemistry

Nucleosides and synthetic reagents were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich (Milwaukee, WI). ^{1}H NMR spectra were obtained with a Varian Gemini-300 spectrometer (300 MHz) with D_2O, $CDCl_3$, CD_3OD, and DMSO-d_6 as a solvent. Purity of the nucleosides was checked using a Hewlett–Packard 1100 HPLC equipped with a Luna 5 μ RP-C18(2) analytical column (250×4.6 mm; Phenomenex, Torrance, CA). System A: linear gradient solvent system: H_2O/CH_3CN/AcOH from 90/10/0.05 to 50/50/0.05 in 20 min and 10/90/0.05 in 50 min, flow rate 0.5 mL/min. System B: linear gradient solvent system: H_2O/CH_3CN/AcOH from 90/10/0.05 to 50/50/0.05 in 40 min and 0/100/0.05 in 60 min, flow rate 0.5 mL/min. System C: linear gradient solvent system: CH_3CN/TBAP from 5/95 to 80/20 in 20 min, flow rate 1.0 mL/min. System D: linear gradient solvent system: H_2O/CH_3CN/AcOH from 80/20/0.05 to 60/40/0.05 in 5 min and 30/70/0.05 in 20 min, flow rate 1.0 mL/min. System E: linear gradient solvent system: H_2O/CH_3CN from 95/5 to 0/100 in 30 min, flow rate 1.0 mL/min. Peaks were detected by UV absorption with a diode array detector. All derivatives tested for biological activity showed ⩾97% purity in the HPLC systems. Low-resolution mass spectra were measured with a Finnigan–Thermoquest LCQ with APCI (Atmospheric Pressure Chemical Ionization) interface. Vaporizer heater was 480 °C interfaced with HP1100 LC apparatus (System A). Low-resolution and high-resolution FAB (fast atom bombardment) mass spectrometry was performed with a JEOL SX102 spectrometer with 6-kV Xe atoms following desorption from a glycerol matrix.

4.2. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Cyano-6-methylamino-purin-9-yl)-5-hydroxy-methyl-tetrahydrofuran-3,4-diol (2)

A solution of 2.0 M $MeNH_2$ in THF (2.0 mL) was added to a solution of **25** (42 mg, 0.096 mmol) in THF (0.80 mL), and the mixture was stirred at rt (room temperature) for 26 h. MeOH (2.0 mL) was added, and the reaction mixture was stirred at rt for 3 h. The solvent was removed under reduced pressure and the residue was purified by preparative TLC (silica gel, thickness 0.25 mm, solvent: $CHCl_3$/MeOH = 10/1) to give **2** (19 mg, 66%). ^{1}H NMR (DMSO-d_6) δ 8.62 (s, 1H), 8.48 (br, 1H), 5.90 (d, 1H, $J = 5.7$ Hz), 5.52 (d, 1H, $J = 6.1$ Hz), 5.23 (d, 1H, $J = 5.1$ Hz), 5.05 (t, 1H, $J = 5.7$ Hz), 4.52 (m, 1H), 4.15 (m, 1H), 3.95 (m, 1H), 3.75–3.60 (m, 1H), 3.60–3.50 (m, 1H), 2.97 (d, 3H, $J = 4.2$ Hz); MS (*m*/*e*) (CI) 307 $(M+H)^+$, HRMS (positive-FAB) calcd for $C_{12}H_{15}N_6O_4$ $(M+H)^+$ 307.1155, found 307.1158; HPLC (System B) 24.6 min (99%) (System C), 9.9 min (99%).

4.3. (2*R*, 3*S*, 4*S*, 5*R*)-2-(6-Methylamino-2-trifluoromethyl-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (3)

To a solution of **28** (2.6 mg, 0.0055 mmol) in MeOH (2.0 mL) was added 3 N NaOH (0.02 mL) and the mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure and the residue was purified by preparative TLC (silica gel, thickness 0.25 mm, solvent AcOEt), to give **3** (0.8 mg, 43%). ^{1}H NMR (CD_3OD) δ 8.38 (s, 1H), 6.00 (d, 1H, $J = 6.0$ Hz), 4,72 (t, 1H, $J = 5.4$ Hz), 4.34 (dd, 1H, $J = 3.3$, 5.1 Hz), 4.14 (dd, 1H, $J = 3.3$, 6.3 Hz), 3.88 (dd, 1H, $J = 3.0$, 12.3 Hz), 3.75 (dd, 1H, $J = 3.6$, 12.3 Hz), 3.13 (br s, 3H); MS (*m*/*e*) (positive-FAB) 350 $(M+H)^+$, 372 $(M+Na)^+$. HRMS (positive-FAB) calcd for $C_{12}H_{15}N_5O_4F_3$ $(M+H)^+$ 350.1076, found 350.1069; HPLC (System C) 11.8 min (99%) (System E), 4.3 min (99%).

4.4. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Amino-6-methylamino-purin-9-yl)-5-hydroxy-methyl-tetrahydrofuran-3,4-diol (4)

A solution of **22** (19 mg, 0.063 mmol) in 30% methylamine in H_2O (2.0 mL) was stirred at 60 °C for 7 h. The solvent was removed under reduced pressure and the residue was purified by C18 reverse-phase silica gel chromatography (eluted by a linear gradient of H_2O/MeCN = 100/0 to 0/100), to give **4** (18 mg, 94%). ^{1}H NMR (DMSO-d_6) δ 7.94 (s, 1H), 5.90 (d, 1H, $J = 6.6$ Hz), 4.77–4.72 (m, 1H), 4.41 (dd, 1H, $J = 3.0$, 5.4 Hz), 4.27 (dd, 1H, $J = 3.0$, 6.0 Hz), 3.91 (dd, 1H, $J = 2.7$, 12.9 Hz), 3.82 (dd, 1H, $J = 3.3$, 12.9 Hz), 3.04 (s, 3H); MS (*m*/*e*) (positive-FAB) 297 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{11}H_{17}N_6O_4$ $(M+H)^+$ 297.1311, found 297.1317; HPLC (System C) 5.5 min (99%) (System E), 3.3 min (99%).

4.5. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Aminomethyl-6-methylamino-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (5)

To a solution of **2** (2.4 mg, 0.0079 mmol) in THF (0.50 mL) was added 1.0 M BH_3–THF complex THF solution (1.5 mL), and the mixture was stirred at 60 °C for 20 h. MeOH (2.0 mL) was added to the reaction mixture, and the solvent was removed under reduced pressure. The residue was purified on an Amberlite CG-50 column (eluted by a linear gradient of ammonium bicarbonate 0–1.0 M) and HPLC (Luna 5 μ RP-C18(2) column, eluted by a linear gradient of H_2O/MeCN/AcOH = 100/0/0.05 to 80/20/0.05 in 15 min), to give **5** as acetic acid salt (1.1 mg, 36%). ^{1}H NMR (D_2O) δ 8.23 (s, 1H), 6.10 (d, 1H, $J = 3.9$ Hz), 4.56 (m, 1H), 4.44 (m, 1H), 4.26 (m, 1H), 4.20–4.12 (br, 2H), 3.92–3.73 (m, 2H), 3.12 (br s, 3H) 1.90 (s, 3H); MS (*m*/*e*) (CI) 311 $(M+H)^+$ (positive-FAB), 311 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{12}H_{19}N_6O_4$ $(M+H)^+$ 311.1468, found 311.1464; HPLC (System B) 5.1 min (99%) (System C), 2.4 min (99%).

4.6. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-methylamino-9H-purine-2-carboxamidine (6)

To a solution of **25** (21 mg, 0.047 mmol) was added to a 2.0 M $MeNH_2$ THF solution (2.0 mL), and the mixture was stirred at rt for 2 h. MeOH (0.5 mL) was added, and the reaction mixture was stirred at 50 °C for 2 h. The solvent was removed under reduced pressure and the residue was purified on a short column (silica gel 2.0 mL, eluent: $CHCl_3$/MeOH = 10/1) and an Amberlite CG-50 column (eluted by linear gradient of ammonium bicarbonate 0–1.0 M), to give **6** (2.8 mg, 18%). ^{1}H NMR (D_2O) δ 8.61 (s, 1H), 6.20 (d, 1H, $J = 6.9$ Hz), 4.99 (t, 1H, $J = 6.5$ Hz), 4.49 (m, 1H), 4.21 (m, 1H) 3.82 (d, 2H, $J = 3.6$ Hz), 3.17 (s, 3H), 2.87 (s, 3H); MS (*m*/*e*) (CI) 338 $(M+H)^+$ (positive-FAB), 338 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{13}H_{20}N_7O_4$ $(M+H)^+$ 338.1577, found 338.1571; HPLC (System B) 12.1 min (99%) (System C), 2.1 min (99%).

4.7. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Iodo-6-(3-iodo-benzylamino)-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (9)

To a solution of **24** (18.5 mg, 0.034 mmol) in DMF (0.10 mL) was added 3-iodobenzylamine (25 mg) and *i*-Pr_2NEt (0.10 mL), and the mixture was stirred at rt for 20 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, eluent: AcOEt/petroleum ether = 2/1), to give triacetate (21 mg). To a solution of this triacetate (21 mg) in MeOH (1.0 mL) was added 3 N NaOH (0.10 mL), and the mixture was stirred at rt for 3 h. The solvent was removed under reduced pressure, and the residue was purified by short-column chromatography (silica gel, eluent: AcOEt) and preparative TLC (silica gel, thickness 0.25 mm, solvent AcOEt), to give **9** (11 mg, 53%). ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.79 (s, 1H), 7.60 (d, 1H, $J = 7.8$ Hz), 7.39 (d, 1H, $J = 7.8$ Hz), 7.09 (t, 1H, $J = 7.8$ Hz), 5.89 (d, 1H, $J = 6.3$ Hz), 4.65 (m, 1H), 4.30 (dd, 1H, $J = 3.3$, 5.1 Hz), 4.13 (m, 1H), 3.88 (dd, 1H, $J = 2.7$, 12.6 Hz), 3.74 (dd, 1H, $J = 3.3$, 12.6 Hz); MS (*m*/*e*) (positive-FAB) 610 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{17}H_{18}N_5O_4I_2$ $(M+H)^+$ 609.9448, found 609.9424; HPLC (System C) 16.0 min (99%) (System E), 6.9 min (99%).

4.8. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Cyano-6-(3-iodo-benzylamino)-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (10)

To a solution of **26** (7.4 mg, 0.012 mmol) in MeCN (1.00 mL) and H_2O (0.030 mL) was added 3 N aqueous NaOH (0.030 mL), and the mixture was stirred at rt for 6 h. Aqueous citric acid (5%, 0.050 mL) was added to neutralize the reaction and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, eluent: AcOEt then $CHCl_3$/MeOH = 5/1) first and then purified again by HPLC (Luna 5 μ RP-C18(2) column, eluted by a linear gradient of H_2O/MeCN/AcOH = 70/30/0.05), to give **10** (1.2 mg, 20%). ^{1}H NMR (DMSO-d_6) δ 8.51 (s, 1H), 7.34 (br s, 1H), 7.68 (m, 1H), 7.60 (m, 1H), 7.35 (m, 1H), 7.12 (m, 1H), 5.95 (m, 1H), 4.65 (br, 2H), 4.51 (m, 1H), 4.13–4.10 (m, 2H), 3.92 (m, 2H); MS (*m*/*e*) (CI) 509 $(M+H)^+$, HRMS (positive-FAB) calcd for $C_{18}H_{18}N_6O_4I$ $(M+H)^+$ 509.0434, found 509.0429; HPLC (System B) 49.2 min (99%) (System C), 16.1 min (99%) (System D), 11.6 min (99%).

4.9. (2*R*, 3*S*, 4*S*, 5*R*)-2-(6-(3-Iodo-benzylamino)-2-trifluoromethyl-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (11)

To a solution of **29** (9.2 mg, 0.014 mmol) in MeOH (1.0 mL) was added potassium carbonate (18.5 mg, 0.13 mmol), and the mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, eluent: $CHCl_3$/MeOH = 10/1) and preparative TLC (silica gel, thickness 0.25 mm, solvent AcOEt), to give **11** (3.4 mg, 45%). ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H), 7.76 (s, 1H), 7.63 (d, 1H, $J = 8.1$ Hz), 7.37 (d, 1H, $J = 7.2$ Hz), 7.08 (t, 1H, $J = 7.8$ Hz), 6.65 (br s, 1H), 5.84 (d, 1H, $J = 6.9$ Hz), 4.99 (m, 1H), 4.80 (m, 2H), 4.49 (d, 1H, $J = 4.5$ Hz), 4.35 (s, 1H), 3.95 (dd, 1H, $J = 1.8$, 12.6 Hz), 3.77 (d, 1H, $J = 12.6$ Hz); MS (*m*/*e*) (positive-FAB) 552 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{18}H_{18}N_5O_4F_3I$ $(M+H)^+$ 552.0356, found 552.0336; HPLC (System C) 17.8 min (99%) (System E), 8.2 min (99%).

4.10. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Methoxycarbonyl-6-(3-iodo-benzylamino)-purin-9-yl)-5-hydroxymethyl-tetrahydrofuran-3,4-diol (12)

To a solution of **26** (10.5 mg, 0.017 mmol) in *i*-PrOH (0.60 mL), MeOH (0.05 mL) and THF (0.20 mL) was added 3 N aqueous NaOH (0.040 mL), and the mixture was stirred at rt for 5 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, eluent: $CHCl_3$/MeOH = 10/1), to give **12** (1.3 mg, 15%). ^{1}H NMR (DMSO-d_6) δ 8.74 (br s, 1H), 8.56 (s, 1H), 7.82 (br s, 1H), 7.58 (m, 1H), 7.39 (m, 1H), 7.10 (m, 1H), 5.93 (d, 1H, $J = 6.0$ Hz), 5.46 (d, 1H, $J = 6.3$ Hz), 5.22 (d, 1H, $J = 4.5$ Hz), 5.03 (t, 1H, $J = 4.5$ Hz), 4.65 (m, 1H), 4.58 (br, 2H), 4.12 (m, 1H), 3.94 (m, 1H), 3.85 (s, 3H), 3.65 (m, 2H); MS (*m*/*e*) (CI) 542 $(M+H)^+$, HRMS (positive-FAB) calcd for $C_{19}H_{21}N_5O_6I$ $(M+H)^+$ 542.0537, found 542.0540; HPLC (System A) 28.6 min (99%) (System C), 14.5 min (99%).

4.11. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(3-iodo-benzylamino)-9H-purine-2-carboxylic acid 3-iodo-benzylamide (13)

To a solution of **25** (9 mg, 0.020 mmol) in THF (1.50 mL) was added 3-iodobenzylamine (19 mg, 0.082 mmol), and the mixture was stirred at 50 °C for

21.5 h. MeOH (2.0 mL) and potassium carbonate (24 mg, 0.174 mmol) were added, and stirring continued at rt for 3 h. The solvent was removed under reduced pressure and the residue was purified by preparative TLC (silica gel, thickness 0.25 mm, solvent $CHCl_3$/MeOH = 5/1), to give **13** (11 mg, 76%). ^{1}H NMR (DMSO-d_6) δ 9.05 (br s, 1H), 8.84 (s, 1H), 7.80 (d, 2H, $J = 5.4$ Hz), 7.58 (d, 2H, $J = 8.4$ Hz), 7.39 (d, 2H, $J = 8.7$ Hz), 7.09 (t, 2H, $J = 8.0$ Hz), 6.10 (d, 1H, $J = 5.4$ Hz), 5.49 (d, 1H, $J = 6.0$ Hz), 5.24 (d, 1H, $J = 4.2$ Hz), 4.89 (s, 2H), 4.76 (s, 2H), 4.55 (m, 1H), 4.17 (m, 1H), 3.98 (m, 1H), 3.65–3.50 (m, 2H); MS (*m*/*e*) (positive-FAB) 743 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{25}H_{25}N_6O_5I_2$ 742.9976, found 742.9972; HPLC (System A) 24.6 min (99%) (System B), 35.0 min (99%) (System C), 12.9 min (99%).

4.12. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(5-chloro-2-methoxybenzylamino)-9H-purine (14)

Compound **14** (240 mg, 0.57 mmol) was prepared in one step[19] from 6-chloropurine riboside (180 mg, 0.64 mmol), 5-chloro-2-methoxy-benzylamine hydrochloride[29] (200 mg, 0.96 mmol) and triethylamine (120 μL, 0.83 mmol) in 88% yield. ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H), 8.35 (br s, 1H), 8.18 (s, 1H), 7.24 (dd, 1H, $J = 2.5$, 8.7 Hz), 7.04 (s, 1H), 7.00 (d, 1H), 5.89 (d, 1H, $J = 5.9$ Hz), 5.37 (t, 1H, $J = 5.6$ Hz), 5.20 (d, 1H, $J = 4.7$ Hz), 4.63 (br, 3H), 4.14 (d, 1H, $J = 2.9$ Hz), 3.95 (d, 1H, $J = 2.9$ Hz), 3.83 (s, 3H), 3.66 (m, 2H), 3.54 (m, 2H); HRMS (ESI) calcd for $C_{18}H_{21}ClN_5O_5$ $(M+H)^+$: 422.1231, found 422.1223; Anal. ($C_{18}H_{20}ClN_5O_5$) C, H, N.

4.13. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(5-chloro-2-methoxybenzylamino)-9H-purine-2-carbonitrile (15)

To a solution of triacetate **25** (6.2 mg, 0.014 mmol) in DMF (0.10 mL) was added 5-chloro-2-methoxybenzylamine hydrochloride (7.0 mg, 0.034 mmol) and *i*-Pr_2NEt (0.10 mL), and the mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure, and the residue was purified by short-column chromatography (silica gel, eluent: AcOEt). The obtained material was dissolved in MeOH (1.0 mL) and added potassium carbonate (15 mg, 0.108 mmol), and the mixture was stirred at rt for 6 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, eluent: $CHCl_3$/MeOH = 10/1 then 5/1), to give **15** (5.2 mg, 82%). ^{1}H NMR (DMSO-d_6) δ 8.49 (s, 1H), 7.28 (d, 1H, $J = 2.7$ Hz), 7.23 (dd, 1H, $J = 2.7$, 8.5 Hz), 6.97 (d, 1H, $J = 8.5$ Hz), 6.01 (d, 1H), 4.65 (t, 1H, $J = 5.4$ Hz), 4.32 (t, 1H, $J = 4.7$ Hz), 4.13 (s, 2H), 3.88 (m, 3H), 3.63 (s, 3H); MS (*m*/*e*) (CI) 447, 449 $(M+H)^+$ (relative peak height ratio was 3:1), HRMS (positive-FAB) calcd for $C_{19}H_{20}N_6O_5Cl$ $(M+H)^+$ 447.1184, found 447.1188; HPLC (System A) 31.2 min (99%) (System C), 15.6 min (99%).

4.14. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(5-chloro-2-methoxybenzylamino)-9H-purine-2-carboxylic acid (16)

To a solution of **15** (3.1 mg, 0.0069 mmol) in EtOH (0.50 mL) was added 3 N NaOH (0.100 mL) and 30% H_2O_2 (0.040 mL), and the mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure, and the residue was purified by Sephadex column chromatography (eluted by a linear gradient of ammonium bicarbonate 0–0.5 M), to give **16** (1.1 mg, 33%). ^{1}H NMR (D_2O) δ 8.32 (s, 1H), 7.40 (d, 1H, $J = 3.0$ Hz), 7.33 (dd, 1H, $J = 3.0$, 9.0 Hz), 7.04 (d, 1H, $J = 9.0$ Hz), 6.12 (d, 1H, $J = 5.7$ Hz), 4.88 (m, 1H), 4.73 (s, 2H), 4.44 (m, 1H), 4.30 (m, 1H), 3.93 (dd, 1H, $J = 2.7$, 12.9 Hz), 3.88 (s, 3H), 3.83 (dd, 1H, $J = 2.7$, 12.9 Hz); MS (*m*/*e*) (CI) 466, 468 $(M+H)^+$ (relative peak height ratio was 3:1) (negative-FAB), 464, 466 $(M-H)^+$ (relative peak height ratio was 3:1). HRMS (negative-FAB) calcd for $C_{19}H_{19}N_5O_7Cl$ $(M-H)^+$ 464.0973, found 464.0969; HPLC (System B) 36.0 min (99%) (System C), 13.1 min (99%).

4.15. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(5-chloro-2-methoxybenzylamino)-9H-purine-2-carboxylic acid methyl ester (17)

To a solution of **16** (1.0 mg, 0.0021 mmol) in MeOH (0.30 mL) and H_2O (0.10 mL) was added (trimethylsilyl)diazomethane (0.100 mL, 2.0 M in ether), and the mixture was stirred at rt for 5 h. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC (silica gel, thickness 0.25 mm, solvent $CHCl_3$/MeOH = 10/1), to give **17** (0.5 mg, 50%). ^{1}H NMR (CD_3OD) δ 8.41 (s, 1H), 7.54 (br s, 1H), 7.21 (dd, 1H, $J = 3.0$, 8.7 Hz), 6.95 (d, 1H, $J = 8.7$ Hz), 6.01 (d, 1H, $J = 6.0$ Hz), 4.70 (m, 1H), 4.58 (s, 2H), 4.33 (m, 1H), 4.16 (m, 1H), 3.98 (s, 3H), 3.88 (s, 3H), 3.75 (m, 1H), 3.55 (m, 1H); MS (*m*/*e*) (CI) 480, 482 $(M+H)^+$ (relative peak height ratio was 3/1). HRMS (positive-FAB) calcd for $C_{20}H_{23}N_5O_7Cl$ $(M+H)^+$ 480.1286, found 480.1279; HPLC (System A) 25.5 min (99%) (System C), 14.1 min (99%).

4.16. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(2-phenyl-cyclopropylamino)-9H-purine-2-carbonitrile (19)

To a solution of **25** (8.1 mg, 0.019 mmol) in DMF (0.10 mL) was added *trans*-2-phenyl cyclopropylamine hydrochloride (10.0 mg, 0.059 mmol) and *i*-Pr_2NEt (0.10 mL), and the mixture was stirred at rt for 26 h. The solvent was removed under reduced pressure, and the residue was purified by short-column chromatography (silica gel, eluent: AcOEt). The obtained material (5.7 mg) was dissolved in MeCN (1.00 mL) and H_2O (0.030 mL), and the mixture was treated with 3 N NaOHaq (0.030 mL) and was stirred at rt for 4 h. 5% citric acid aq (0.050 mL) was added to neutralize the

reaction, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, eluent: AcOEt), to give **19** (1.2 mg, 15%). ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.34–7.02 (m, 5H), 6.44 (br s, 1H), 5.85 (d, 1H, $J = 6.9$ Hz), 4.95 (m, 1H), 4.70 (m, 1H), 4.52 (m, 1H), 4.35 (m, 1H), 3.99 (m, 1H), 3.83 (m, 1H), 3.44 (br, 1H), 3.15 (br, 1H), 2.87 (m, 1H), 2.22 (m, 1H), 0.88 (m, 2H); (DMSO-d_6) δ 8.93 (br s, 1H), 8.64 (s, 1H), 7.32–7.10 (m, 5H), 5.90 (d, 1H, $J = 5.7$ Hz), 5.52 (d, 1H, $J = 5.7$ Hz), 5.23 (d, 1H, $J = 5.4$ Hz), 5.04 (t, 1H, $J = 5.1$ Hz), 4.50 (m, 1H), 4.14 (m, 1H), 3.95 (m, 1H), 3.65 (m, 1H), 3.57 (m, 1H), 3.10 (m, 1H), 2.16 (m, 1H), 1.45–1.30 (m, 2H); MS (*m*/*e*) (CI) 409 $(M+H)^+$, HRMS (positive-FAB) calcd for $C_{20}H_{21}N_6O_4$ $(M+H)^+$ 409.1624, found 409.1627; HPLC (System A) 30.2 min (99%) (System B), 46.7 min (99%) (System C), 15.3 min (99%).

4.17. (2*R*, 3*S*, 4*S*, 5*R*)-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(2-phenyl-cyclopropyl-amino)-9H-purine-2-carboxylic acid (20) and 9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydrofuran-2-yl)-6-(2-phenyl-cyclopropyl-amino)-9H-purine-2-carboxamide (21)

To a solution of **19** (4.4 mg, 0.0108 mmol) in EtOH (0.50 mL) was added 3 N NaOH (0.100 mL) and 30% H_2O_2 (0.050 mL), and the mixture was stirred at rt for 28 h. The solvent was removed under reduced pressure, and the residue was purified by Sephadex column chromatography (eluted by a linear gradient of ammonium bicarbonate 0–0.5 M), to give **20** (1.0 mg, 21%) and **21** (1.2 mg, 26%). For **20**: ^{1}H NMR (D_2O) δ 8.31 (s, 1H), 7.45–7.32 (m, 5H), 6.13 (d, 1H, $J = 5.7$ Hz), 5.05–5.00 (m, 1H), 4.43 (t, 1H, $J = 4.8$ Hz), 4.30 (m, 1H), 3.96–3.81 (m, 2H) 3.35 (m, 1H), 2.34 (m, 1H), 1.51–1.41 (m, 2H); MS (*m*/*e*) (CI) 428 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{20}H_{22}N_5O_6$ $(M+H)^+$ 428.1570, found 428.1571; HPLC (System B) 34.4 min (99%) (System C), 13.5 min (99%). For **21**: ^{1}H NMR (D_2O) δ 8.36 (s, 1H), 7.43–7.23 (m, 5H), 6.14 (d, 1H, $J = 5.7$ Hz), 5.00–4.95 (m, 1H), 4.44 (t, 1H, $J = 4.5$ Hz), 4.29 (m, 1H), 3.96–3.80 (m, 2H) 3.07 (m, 1H), 2.15 (m, 1H), 1.47 (m, 2H); MS (*m*/*e*) (CI) 427 $(M+H)^+$. HRMS (positive-FAB) calcd for $C_{20}H_{23}N_6O_5$ $(M+H)^+$ 427.1730, found 427.1733; HPLC (System B) 35.0 min (99%) (System C), 12.1 min (99%).

4.18. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Amino-6-chloro-purin-9-yl)-3,4-diacetoxy-5-acetoxymethyl-tetrahydrofuran (23)

To a solution of (−)-2-amino-6-chloropurine riboside **22** (995 mg, 3.30 mmol) in DMF (15 mL) was added pyridine (2.0 mL, 25 mmol) and acetic anhydride (2.0 mL, 21 mmol), and the mixture was stirred at 80 °C for 2 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent: AcOEt/petroleum ether = 1/1 then 1/0), to give **23** (1.26 g, 89%). ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1H), 6.01 (d, 1H, $J = 4.8$ Hz), 5.96 (t, 1H, $J = 5.2$ Hz), 5.75 (t, 1H, $J = 5.0$ Hz), 5.19 (br s, 2H), 4.49–4.36 (m, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H); MS (*m*/*e*) (positive-FAB) 428, 430 $(M+H)^+$ (relative peak height ratio was 3:1).

4.19. (2*R*, 3*S*, 4*S*, 5*R*)-2-(6-Chloro-2-iodo-purin-9-yl)-3,4-diacetoxy-5-acetoxymethyl-tetrahydrofuran (24)

To a solution of **23** (206 mg, 0.48 mmol) in MeCN (0.50 mL) and CH_2I_2 (2.0 mL) was added *t*-butyl nitrite (0.200 mL, 2.22 mmol), and the mixture was stirred at 80 °C for 3 h. The reaction mixture was directly purified by silica gel column chromatography (eluent: chloroform then AcOEt/petroleum ether = 2/1), to give 2-iodo compound **24** (178 mg, 69%). ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 6.21 (d, 1H, $J = 5.4$ Hz), 5.78 (t, 1H, $J = 5.6$ Hz), 5.59 (t, 1H, $J = 5.0$ Hz), 4.48 (t, 1H, $J = 3.9$ Hz), 4.42 (m, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H); MS (*m*/*e*) (positive-FAB) 539, 541 $(M+H)^+$ (relative peak height ratio was 3:1).

4.20. (2*R*, 3*S*, 4*S*, 5*R*)-2-(6-Chloro-2-cyano-purin-9-yl)-3,4-diacetoxy-5-acetoxymethyl-tetrahydrofuran (25)

A solution of Pd_2 (dba)$_3$–$CHCl_3$ (50 mg, 0.048 mmol) and tri-2-furylphosphine (150 mg, 0.646 mmol) in tetramethylurea (0.50 mL) was stirred at 80 °C for 30 min. The solution was cooled to rt and treated with **24** (110 mg, 0.204 mmol) and $Zn(CN)_2$ (125 mg, 1.06 mmol) in tetramethyl urea (3.50 mL) and stirred at 80 °C for 22 h. The reaction mixture was passed through a short column (SiO_2 3.0 mL and eluent AcOEt), and the eluted fraction was evaporated to dryness. The residue was purified twice by silica gel column chromatography (first column eluent: AcOEt/petroleum ether = 2/1, second column eluent: AcOEt/petroleum ether = 1/1), to give 2-cyano compound **25** (52 mg, 59%). ^{1}H NMR ($CDCl_3$) δ 8.51 (s, 1H), 6.28 (d, 1H, $J = 5.7$ Hz), 5.77 (t, 1H, $J = 5.7$ Hz), 5.54 (dd, 1H, $J = 5.7$, 3.9 Hz), 4.53 (dd, 1H, $J = 3.9$, 7.5 Hz), 4.44 (m, 2H), 2.19 (s, 3H) 2.17 (s, 3H), 2.11 (s, 3H); MS (*m*/*e*) (CI) 438 $(M+H)^+$.

4.21. (2*R*, 3*S*, 4*S*, 5*R*)-2-(2-Cyano-6-(3-iodo-benzyl-amino)-purin-9-yl)-3,4-diacetoxy-5-acetoxymethyl-tetrahydrofuran (26)

To a solution of **25** (8.3 mg, 0.019 mmol) in DMF (0.10 mL) was added 3-iodobenzylamine hydrochloride (9.0 mg, 0.033 mmol) and *i*-Pr_2NEt (0.10 mL), and the mixture was stirred at rt for 25 h. The solvent was removed under reduced pressure, and the residue was purified by short-column chromatography (silica gel, eluent: AcOEt), to give triacetate **26** (10.5 mg, 87%). ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.73 (s, 1H), 7.64 (d, 1H, $J = 7.8$ Hz), 7.36 (d, 1H, $J = 7.8$ Hz), 7.09 (t, 1H, $J = 7.8$ Hz), 6.36 (br s, 1H), 6.18 (d, 1H, $J = 5.7$ Hz), 5.78 (t, 1H, $J = 5.6$ Hz), 5.57 (t, 1H, $J = 5.1$ Hz), 4.80 (br, 2H), 4.45 (m, 1H), 4.40 (m, 2H),

2.17 (s, 3H) 2.16 (s, 3H), 2.10 (s, 3H); MS (*m*/*e*) (CI) 635 $(M+H)^+$.

4.22. (2*R*, 3*S*, 4*S*, 5*R*)-Acetic acid 4-acetoxy-2-acetoxymethyl-5-(6-chloro-2-trifluoromethyl-purin-9-yl)-tetrahydrofuran-3-yl ester (27)

To a suspension of activated Zn powder (140 mg, 2.20 mmol) in DMF (0.50 mL) was added CF_2Br_2 (0.10 mL, 1.08 mmol), and the mixture was stirred at rt for 2.5 h. HMPA (0.40 mL) was added to the reaction mixture, and it was cooled to 0 °C. CuI (76 mg) was added to the reaction mixture. A solution of **24** (76 mg, 0.141 mmol) in DMF (0.50 mL) was added at 0 °C. The reaction mixture was stirred at 50 °C for 26.5 h and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, solvent AcOEt/petroleum ether = 2/1), to give **27** (52 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 9.09 (br s, 1H), 6.34 (d, 1H, $J = 5.4$ Hz), 5.84 (t, 1H, $J = 5.4$ Hz), 5.62 (t, 1H, $J = 5.0$ Hz), 4.55 (m, 1H), 4.45 (m, 2H), 2.20 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H); MS (*m*/*e*) (positive-FAB) 481 $(M+H)^+$.

4.23. (2*R*, 3*S*, 4*S*, 5*R*)-Acetic acid 4-acetoxy-2-acetoxymethyl-5-(6-methylamino-2-trifluoromethyl-purin-9-yl)-tetrahydrofuran-3-yl ester (28)

A solution of **27** (8.8 mg, 0.019 mmol) in 2 N methylamine in THF (2.0 mL) was stirred at rt for 20 h and at 70 °C for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, eluent: $CHCl_3$/MeOH = 5/1) and preparative TLC (silica gel, thickness 0.25 mm, solvent AcOEt/petroleum ether = 1/1), to give **28** (2.6 mg, 29%). ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H), 6.16 (d, 1H, $J = 4.8$ Hz), 6.04 (br s, 1H), 5.83 (t, 1H, $J = 5.3$ Hz), 5.72 (t, 1H, $J = 5.1$ Hz), 4.50–4.35 (m, 3H), 3.22 (s, 3H), 2.17 (s, 3H), 2.10(s, 3H), 2.09 (s, 3H); MS (*m*/*e*) (positive-FAB) 476 $(M+H)^+$, 498 $(M+Na)^+$.

4.24. (2*R*, 3*S*, 4*S*, 5*R*)-Acetic acid 4-acetoxy-2-acetoxymethyl-5-(6-(3-iodo-benzylamino)-2-trifluoromethyl-purin-9-yl)-tetrahydrofuran-3-yl ester (29)

To a solution of **27** (16.2 mg, 0.034 mmol) in DMF (0.40 mL) was added 3-iodobenzylamine hydrochloride (30 mg, 0.111 mmol) and *i*-Pr_2NEt (0.10 mL), and the mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, solvent AcOEt) and preparative TLC (silica gel, thickness 0.25 mm, solvent AcOEt/petroleum ether = 1/1), to give **29** (9.5 mg, 42%). ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.78 (s, 1H), 7.63 (d, 1H, $J = 8.0$ Hz), 7.38 (d, 1H, $J = 8.0$ Hz), 7.08 (t, 1H, $J = 8.0$ Hz), 6.31 (br s, 1H), 6.16 (d, 1H, $J = 5.1$ Hz), 5.84 (t, 1H, $J = 5.3$ Hz), 5.71 (t, 1H, $J = 5.1$ Hz), 4.80 (br, 2H), 4.50–4.30 (m, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H); MS (*m*/*e*) (positive-FAB) 678 $(M+H)^+$, 700 $(M+Na)^+$.

4.25. Biological assays

4.25.1. Cell culture and membrane preparation. CHO cells expressing recombinant human and rat A_3ARs were cultured in DMEM (Dulbecco's modified Eagle's medium) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 μmol/mL glutamine, and 800 μg/mL geneticin. After harvest and homogenization, the cells were centrifuged at 500*g* for 10 min. The pellet was resuspended in 50 mM Tris–HCl buffer (pH 8.0) containing 10 mM $MgCl_2$ and 1 mM EDTA. The suspension was homogenized with an electric homogenizer for 10 s, and was then recentrifuged at 20,000*g* for 20 min at 4 °C. The resulting pellets were resuspended in buffer containing 3 units/mL of adenosine deaminase, and the suspension was stored at −80 °C prior to the binding experiments. The rat A_3AR was expressed recombinantly via transfection in CHO cells, and the procedure was the same as for the human subtype. The protein concentration was measured using the Bradford assay.[30]

4.25.2. Binding assay. For the A_3AR binding experiments, the procedures used were similar to those previously described.[6] Briefly, each tube contained 100 μL of membrane suspension, 50 μL of [^{125}I]I-AB-MECA (final concentration 0.5 nM), and 50 μL of increasing concentrations of compounds in Tris–HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10 μM NECA (5′-*N*-ethyluronamidoadenosine). The mixtures were incubated at 25 °C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandel, Gaithersburg, MD). Filters were washed three times with ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter. The binding of [^{3}H]*R*-PIA to the recombinant hA_1AR and the binding of [^{3}H]CGS21680 to the recombinant hA_{2A}AR was performed as previously described.[7,31]

4.25.3. Cyclic AMP accumulation assay. Intracellular cyclic AMP levels were measured with a competitive protein binding method.[32] CHO cells expressing recombinant human[33] and rat[34] ARs were harvested by trypsinization. After resuspension in the medium, cells were plated in 24-well plates in 0.5 mL medium/well. After 24 h, the medium was removed and cells were washed three times with 1 mL/well of DMEM, containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4. Cells were then treated with agonists and/or test compounds in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL) and incubated at 37 °C. For A_{2A} and A_{2B}ARs, incubation was carried out for 1 h. For A_1 and A_3ARs, after 45 min forskolin (10 μM) was added to the medium, and incubation was continued for an additional 15 min. The reaction was terminated upon removal of the medium, and the cells were lysed with 200 μL/well of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at −20 °C. For determination of cyclic AMP production,

protein kinase A (PKA) was incubated with [^{3}H]cyclic AMP (2 nM) in K_2HPO_4/EDTA buffer (K_2HPO_4, 150 mM; EDTA, 10 mM), 20 μL of the cell lysate, and 30 μL 0.1 M HCl. Bound radioactivity was separated by rapid filtration through Whatman GF/C filters under reduced pressure and washed once with cold buffer. Bound radioactivity was subsequently measured by liquid scintillation spectrometry.

4.26. Statistical analysis

Binding and functional parameters were estimated with GraphPAD Prism software (GraphPAD, San Diego, CA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng–Prusoff equation.[35] Data were expressed as mean ± standard error.

4.27. Molecular modeling

All calculations were performed on a Silicon Graphics Octane workstation (300 MHz MIPS R12000 (IP30) processor). All ligand structures were modified from the lowest-energy conformation of Cl-IB-MECA[6] with the Sketch Molecule of SYBYL 6.9.[36] In all cases, MMFF force field[37] and charge were applied using distance-dependent dielectric constants and conjugate gradient method until the gradient reached 0.05 kcal mol^{-1} Å^{-1}.

A hA_3AR model (PDB code: 1o74) constructed by homology to the X-ray structure of bovine rhodopsin with 2.8-Å resolution[38] was used for the docking study. Compounds **1**, **2**, **7**, **10**, **14**, and **15** in Table 1 were docked within the hA_3AR model. The atom types of all ligands were manually assigned with Amber all-atom force field,[39] and their charges were calculated before docking. The starting geometry of ligand conformation was chosen from the hA_3AR complex model with Cl-IB-MECA, which was already validated by point mutation.[6] The ribose-binding position of this series was fixed, using an atom-by-atom fitting method for the carbon atoms of the ribose ring. To determine the binding region of N^6 derivatives and C-2 substituents, the flexible bonds, χ_1 (O–$C_{1'}$–N_9–C_4) and t_0 (C_5–C_6–N_6–C) angles were variously searched in the putative binding cavity. χ_1 was rotated by −60°, −110°, and −160° within the *anti*-conformational range and was rotated in 30° increments. Several conformations without any steric bump were selected for further optimization. The initial structures of all complexes were optimized using the Amber force field with fixed dielectric constant of 4.0 and terminating gradient of 0.1 kcal mol^{-1} Å^{-1}.

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