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Letters

Discovery of a New Nucleoside Template for Human A_3 Adenosine Receptor Ligands: D-4′-Thioadenosine Derivatives without 4′-Hydroxymethyl Group as Highly Potent and Selective Antagonists

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Abstract: Truncated D-4′-thioadenosine derivatives lacking the 4′-hydroxymethylene moiety were synthesized starting from D-mannose, using cyclization to the 4-thiosugar and one-step conversion of the diol to the acetate as key steps. At the human A_3 adenosine receptor (AR), N^6-substituted purine analogues bound potently and selectively and acted as antagonists in a cyclic AMP functional assay. An N^6-(3-chlorobenzyl)purine analogue **9b** displayed a K_i value of 1.66 nM at the human A_3 AR. Thus, truncated D-4′-thioadenosine is an excellent template for the design of novel A_3 AR antagonists to act at both human and murine species.

Adenosine (**1**), a natural mediator, regulates cell signaling through specific adenosine receptors (AR), of which four subtypes (A_1, A_{2A}, A_{2B}, and A_3) have so far been identified. The A_3 AR is the most recently identified and may be a good therapeutic target for the development of clinically efficacious drug candidates.[1,2] For example, A_3 AR agonists may be useful for the treatment of myocardiac and cerebral ischemia and cancer,[3−5] and A_3 AR antagonists are being investigated as anti-asthma, anti-glaucoma, and anti-inflammatory agents.[6−9]

Extensive structural modifications of adenosine (**1**) have been carried out at the N^6 position and/or 4′-hydroxymethyl group, in search of potent and selective A_3 AR ligands. Most of these adenosine analogues displayed agonism at the A_3 AR, consistent with the structural similarity to adenosine. For example, compounds **2** and **3** were optimized to fully activate the A_3 AR with high binding affinity and selectivity (Chart 1). Nevertheless, a few nucleoside analogues[7,10] have been reported to show A_3 AR antagonism, for example, compound **4**, a pure antagonist of the A_3 AR. However, the most potent and selective antagonists of the human (h) A_3 AR possess nonpurine heterocyclic skeletons rather than nucleoside skeletons.[11]

Strikingly, nearly all of the nonpurine heterocyclic hA_3 AR antagonists were found to be weak or ineffective at the rat A_3 AR and were thereby unsuitable for evaluation in small animal models and, thus, for further development as drugs. Therefore, A_3 AR antagonists having high affinity and selectivity independent of species may be considered as preferred drug candidates. Because certain potent and selective nucleoside agonists of the A_3 AR, such as **2** and **3**, tend to bind to the receptor with minimal species dependence in comparison to nonpurine heterocyclic antagonists,[12] it has been highly desirable to develop species-independent A_3 AR antagonists starting from nucleoside templates.

Molecular modeling studies of the A_3 AR predicted that flexibility and H-bonding ability of the 5′-uronamide in the adenosine derivative correlated with putative conformational changes of the receptor associated with activation.[10,13] Those findings were indirectly supported by the *N*,*N*-dimethyluronamide derivative **5** (K_i = 29 nM at the hA_3 AR) of compound **3**, in which the H-bond-donating ability of the 5′-uronamide of **3** was removed, resulting in the inability to activate the A_3 AR.[14]

Recently, we have reported 4′-thionucleosides, including the 4′-thio analogue **6** of compound **3** and its N^6-methyl analogue **7**, which showed enhanced binding affinity to the A_3 AR and pure agonist activity, suggesting that they could potentially serve as excellent, novel templates for the design of hA_3 AR agonists (Chart 2).[15−17] The *N*,*N*-dimethyluronamide derivative **8** of compound **6** was also found to exhibit pure antagonist activity (binding K_i = 15 nM) at the hA_3 AR but with higher and more

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Chart 1. Structures of A_3 AR Agonists and Antagonists

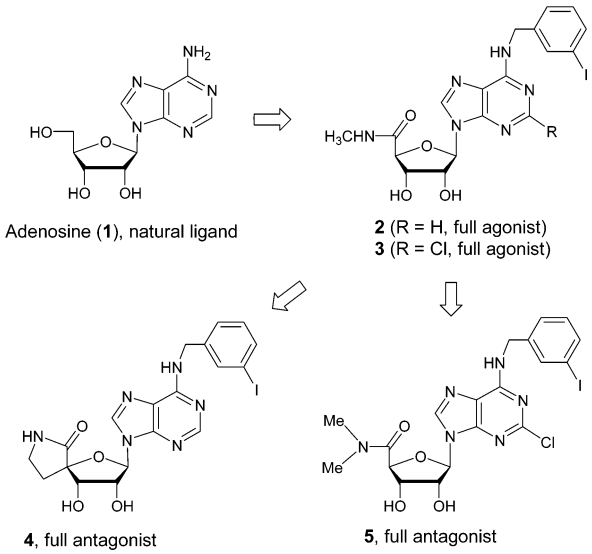
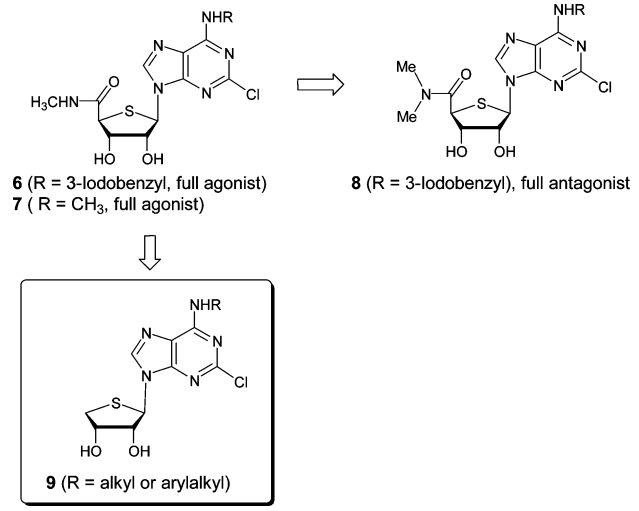


Chart 2. Rationale for the Design of Human A_3 AR Antagonists



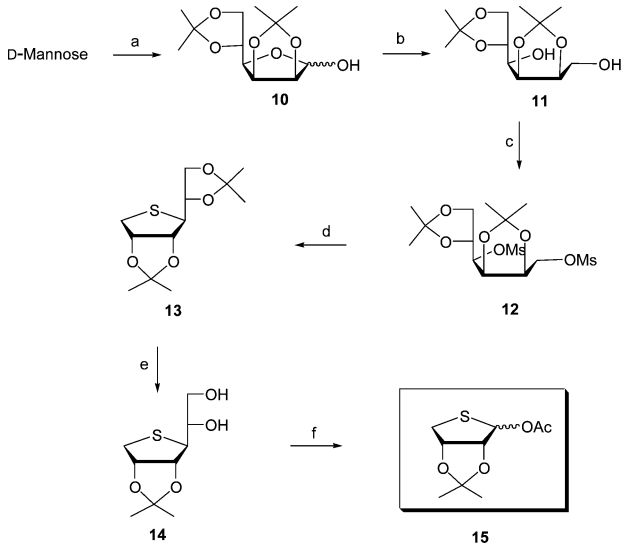
selective binding affinity than **5**, implying that 4′-thionucleosides might be better templates than the corresponding 4′-oxonucleosides for the design of hA_3 AR antagonists.[14]

To discover more potent hA_3 AR antagonists than **8**, we designed and synthesized compound **9** by completely removing the 5′-uronamide groups of compounds **6** and **7**, which putatively form multiple H-bonds in the binding site, thus, impeding the conformational change needed for receptor activation. All synthesized compounds exhibited excellent binding affinity and selectivity at the A_3 AR. Herein, we report the derivatization and optimization of the truncated D-4′-thioadenosine derivative **9**, that is, lacking the 4′-hydroxymethylene moiety, as a novel template for potent and selective A_3 AR antagonists.

The synthetic strategy to the desired nucleosides was to synthesize the glycosyl donor and then to condense with a purine base. The synthesis of the glycosyl donor **15**, starting from D-mannose, is shown in Scheme 1.

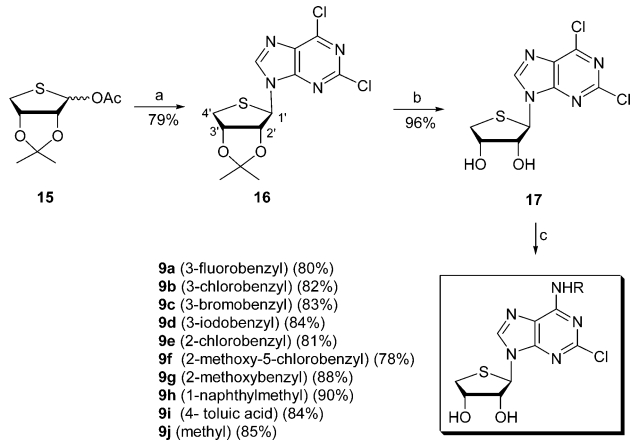
D-Mannose was converted to the diacetonide **10** under standard conditions. Reduction of **10** with sodium borohydride followed by mesylation of the resulting diol **11** afforded the dimesylate **12**. Cyclization of **12** with anhydrous sodium sulfide

Scheme 1. Synthesis of the Glycosyl Donor **15**[a]



[a] Reagents and conditions: (a) 2,2-dimethoxypropane, camphorsulfonic acid, CH_3COCH_3, rt, 15 h; (b) $NaBH_4$, EtOH, rt, 2 h; (c) MsCl, Et_3N, CH_2Cl_2, rt, 1 h; (d) Na_2S, DMF, 80 °C, 15 h; (e) 60% AcOH, rt, 2 h; (f) $Pb(OAc)_4$, EtOAc, rt, overnight.

Scheme 2. Synthesis of the Target Nucleosides **9a**−**j**[a]



[a] Reagents and conditions: (a) 2,6-dichloropurine, ammonium sulfate, HMDS, 170 °C, 15 h, then TMSOTf, DCE, rt to 80 °C, 3 h; (b) 2 N HCl, THF, rt, 15 h; (c) RNH_2, Et_3N, EtOH, rt, 1−3 d.

in DMF yielded the thiosugar **13**. Selective hydrolysis of the 5,6-acetonide of **13** in the presence of the 2,3-acetonide was achieved using 60% aqueous acetic acid to give diol **14** in 81% yield, based on the recovered starting material. A one-step conversion of diol **14** into the acetate **15** was achieved by treatment with excess lead tetraacetate at room temperature for 15 h.[18]

Synthesis of the final nucleosides **9a**−**j** is shown in Scheme 2. The glycosyl donor **15** was condensed with 2,6-dichloropurine in the presence of trimethylsilyl trifluoromethansulfonate (TMSOTf) as a Lewis acid to give the β-anomer **16** (79%) as a single stereoisomer. The anomeric assignment was easily achieved by ^{1}H NOE[a] experiments. Strong NOE effects between H-8 and 2′-H and between H-8 and 3′-H were observed, confirming that compound **16** had β-orientation. The initially formed *N*-3 isomer was completely converted into the desired *N*-9 isomer upon heating to 80 °C for 2 h. Removal of the acetonide in **16** with 2 *N* HCl gave diol **17**. Treatment of **17**

Table 1. Binding Affinities of Known A_3 AR Agonists (**3** and **6**) and Antagonists (**4**, **5**, and **8**) and Truncated 4′-Thioadenosine Derivatives **9a**−**j** at Three Subtypes of Human ARs

	affinity, K_i, nM ± SEM (or % inhibition)[a,b]		
compound	hA_1	hA_{2A}	hA_3[c]
3 (Cl-IB-MECA)	222 ± 22	5360 ± 2470	1.4 ± 0.3
4	12 100	29 800	29.3
5	5870 ± 930	>10 000	29.0 ± 4.9
6	193 ± 46	223 ± 36	0.38 ± 0.07
8	6220 ± 640	>10 000	15.5 ± 3.1
9a	(20%)	(48%)	7.4 ± 1.3
9b	(38%)	(18%)	1.66 ± 0.90
9c	(34%)	(18%)	8.99 ± 5.17
9d[d]	2490 ± 940	341 ± 75	4.16 ± 0.50
9e	(13%)	1600 ± 135	25.8 ± 6.3
9f	(24%)	4020 ± 1750	12.7 ± 3.7
9g	(9%)	(18%)	19.9 ± 7.1
9h	(22%)	(−8%)	24.8 ± 8.1
9i	(13%)	(0%)	(42%)
9j	55.4 ± 1.8	45.0 ± 1.4	3.69 ± 0.25

[a] All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate human AR (A_1 AR and A_3 AR in CHO cells and A_{2A} AR in human embryonic kidney (HEK)-293 cells). Binding was carried out using 1 nM [^{3}H]CCPA, 10 nM [^{3}H]CGS-21680, or 0.5 nM [^{125}I]I-AB-MECA as radioligands for A_1, A_{2A}, and A_3 ARs, respectively. Values are expressed as mean ± sem, $n = 3-4$ (outliers eliminated), and are normalized against a nonspecific binder, NECA (10 μM). [b] When value is expressed as a percentage, it refers to the percent inhibition of a specific radioligand binding at 10 μM, with nonspecific binding defined using 10 μM NECA. [c] A functional assay was also carried out at this subtype: percent inhibition at 10 μM forskolin-stimulated cyclic AMP production in CHO cells expressing the hA_3 AR as a mean percentage of the response of the full agonist **3** ($n = 1-3$). None of the analogues activated the hA_3 AR by this criterion. [d] Compound **9d** at 10 μM displayed <10% of the full stimulation of cyclic AMP production in CHO cells expressing the hA_{2B} AR, in comparison to 10 μM NECA; no inhibition of the stimulatory effect of 150 nM NECA was observed.

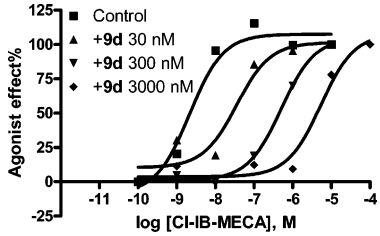


Figure 1. Effects of compound **9d** on the concentration−response curve of a full agonist in the inhibition of cyclic AMP production at the hA_3 AR expressed in CHO cells.

with various alkyl or arylalkylamines afforded 10 different N^6-substituted D-4′-thionucleosides **9a**−**j**.[19]

Binding assays (Supporting Information) were carried out using standard radioligands and membrane preparations from Chinese hamster ovary (CHO) cells stably expressing a hAR subtype. Most of the synthesized compounds exhibited high binding affinity at the hA_3 AR with extremely high selectivity over other subtypes, A_1 and A_{2A} ARs (Table 1). Among compounds tested, compound **9b** (R = 3-chlorobenzyl) showed the highest binding affinity ($K_i = 1.66 \pm 0.90$ nM) at the hA_3 AR with extremely low binding affinities to A_1 and A_{2A} ARs. Although compounds **9j** (R = Me; $K_i = 3.69 \pm 0.25$ nM) and **9d** (R = 3-iodobenzyl; $K_i = 4.16 \pm 0.50$ nM) exhibited high binding affinity at the hA_3 AR, these compounds were less selective than **9b** at the A_1 and A_{2A} ARs. In the halobenzyl series, binding affinity was in the order Cl > I > F > Br, and the 3-chlorobenzyl analogue **9b** exhibited a higher binding affinity at the hA_3 AR than 2-chlorobenzyl **9e** ($K_i = 25.8 \pm 6.3$ nM). 3-Substitution on the aromatic ring appeared to be preferred over 2- or 4-substitution or 2,5-disubstitution with respect to binding affinity at the hA_3 AR. Compound **9i** substituted with a polar carboxylic acid (COOH) on the aromatic ring was totally devoid of binding affinity at the hA_3 AR, indicating that the hydrophobic N^6-substituent was essential for binding interaction.

Compound **9d** also bound with high affinity at the rat A_3 AR expressed in CHO cells ($K_i = 3.89 \pm 1.15$ nM), indicating that it is suitable for evaluation in small animal models or for further drug development and was inactive as agonist or antagonist in a cyclic AMP functional assay[20] at the hA_{2B} AR, from which relatively minor structural changes (such as replacement of the iodo group with another halo group) are not expected to produce an active compound at the hA_{2B} AR.

All compounds tested were found to be full antagonists in a cyclic AMP functional assay at the hA_3 AR. In this assay, **9d** dose-dependently shifted the concentration−response curve for agonist **3** to the right as an antagonist, corresponding to a K_B value of 1.92 nM calculated by Schild analysis (Figure 1).

In conclusion, we synthesized the D-4′-thioadenosine derivatives **9** without a 4′-hydroxymethyl group, starting from D-mannose, using as key steps a cyclization to the 4-thiosugar and a one-step conversion of the diol to the acetate. The novel N^6-substituted analogues bound to the hA_3 AR with high potency and selectivity binding affinity and acted as pure antagonists in a functional assay of cyclic AMP effects mediated by this receptor. A 3-chlorobenzyl analogue **9b** is the most potent and selective analogue with a nucleoside structure at the A_3 AR yet reported. To our best knowledge, the truncated 4′-thioadenosine derivative **9** is the optimal nucleoside-derived template for hA_3 AR antagonists and the resultant derivatives are expected to be suitable for evaluation in small animal models or for further development as drugs. Extensive in vivo studies are being conducted in models of glaucoma and will be reported separately.

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Supporting Information Available: Complete experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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[a] Abbreviations: CCPA, 2-chloro-N^6-cyclopentyladenosine; CGS-21680, 2-[*p*-(2-carboxyethyl]phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; I-AB-MECA, N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine; NECA, 5′-*N*-ethylcarboxamidoadenosine; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl) adenosine-5′-*N*-methylcarbamoyladenosine; NOE, nuclear Overhauser effect.

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