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Discovery of A New Human A_{2A} Adenosine Receptor Agonist, Truncated 2-Hexynyl-4′-thioadenosine

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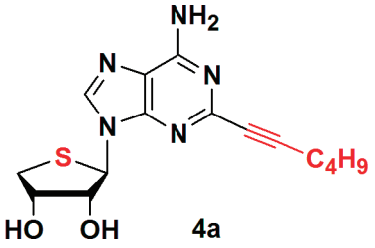
ABSTRACT The truncated C2- and C8-substituted 4′-thioadenosine derivatives **4a**−**d** were synthesized from D-mannose, using palladium-catalyzed cross-coupling reactions as key steps. In this study, an A_3 adenosine receptor (AR) antagonist, truncated 4′-thioadenosine derivative **3**, was successfully converted into a potent A_{2A} AR agonist **4a** (K_i = 7.19 ± 0.6 nM) by appending a 2-hexynyl group at the C2-position of a derivative of **3** that was N^6-substituted. However, C8-substitution greatly reduced binding affinity at the human A_{2A} AR. All synthesized compounds **4a**−**d** maintained their affinity at the human A_3 AR, but **4a** was found to be a competitive A_3 AR antagonist/A_{2A} AR agonist in cyclic AMP assays. This study indicates that the truncated C2-substituted 4′-thioadenosine derivatives **4a** and **4b** can serve as novel templates for the development of new A_{2A} AR ligands.

KEYWORDS A_{2A} adenosine receptor agonists, truncated 2-hexynyl-4′-thioadenosine, palladium-catalyzed cross-coupling reactions, binding mode



The endogenous cytoprotective modulator adenosine (**1**) exerts its pharmacological effects against hypoxia, ischemia, and inflammation through binding to four subtypes (A_1, A_{2A}, A_{2B}, and A_3) of adenosine receptors (ARs), members of the G protein-coupled receptor (GPCR) family.[1] Among these, activation of A_{2A} AR has been known to play a role in the suppression of immune and inflammatory responses and in vascular responses to adenosine.[2−4] It is also highly localized within the central nervous system (CNS), and selective antagonists have become an attractive target for the treatment of Parkinson's disease.[5] The A_3 AR is the most recently identified subtype and is found in the cardiovascular system, CNS, immune cells, lung, and liver.[6,7] The activation of A_3 AR is beneficial in models of myocardiac and cerebral ischemia and cancer, while its antagonism is of interest for treating asthma, inflammation, and glaucoma.[8]

Thio-Cl-IB-MECA (**2**),[9,10] which is bioisosteric with 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (Cl-IB-MECA),[11] was discovered as a potent and selective A_3 AR agonist (K_i = 0.38 nM). This compound showed a potent anticancer activity by inhibiting the Wnt signaling pathway.[12] On the basis of rational design, truncated 4′-thioadenosine derivative **3** lacking the 5′-uronamide of **2** essential for the receptor activation was discovered as a potent, selective, and species-independent A_3 AR antagonist (K_i = 1.66 nM).[13,14] This compound and other related A_3 AR antagonists with nucleoside skeletons are expected to be suitable for evaluation in small animal models and for further development as drugs (Figure 1).

On the basis of the observation that truncation resulting in the 4′-thioadenosine antagonist derivative **3** preserved AR affinity and selectivity, we designed and synthesized the truncated C2- and C8-substituted 4′-thioadenosine derivatives **4a**−**d** as potential new ligands for the A_{2A} AR. This expectation was supported by reports that C2- or C8-substitution sometimes leads to substantial enhancement in the binding affinity or selectivity at the A_{2A} AR or other AR subtype.[15−17] C2- or C8-substitution was readily achieved through Sonogashira[18] and Suzuki[19] cross-coupling reactions. From this study, a C2-alkynyl derivative was found to be potent, mixed A_{2A} AR agonist and A_3 AR antagonist, which is an excellent combination for the antiasthmatic activity. Herein, we describe the synthesis and pharmacological activity of novel C2- and C8-substituted 4′-thioadenosine derivatives **4a**−**d** from D-mannose.

D-Mannose was converted to the glycosyl donor **5** according to our previously published procedure.[13,14] The glycosyl donor **5** was condensed with 2-amino-6-chloropurine in the presence of TMSOTf as a Lewis acid to give the β-anomer **6** (30%) as a single stereoisomer (Scheme 1). The anomeric assignment was easily accomplished in a ^{1}H NMR experiment that showed a nuclear Overhauser effect between H-8 and

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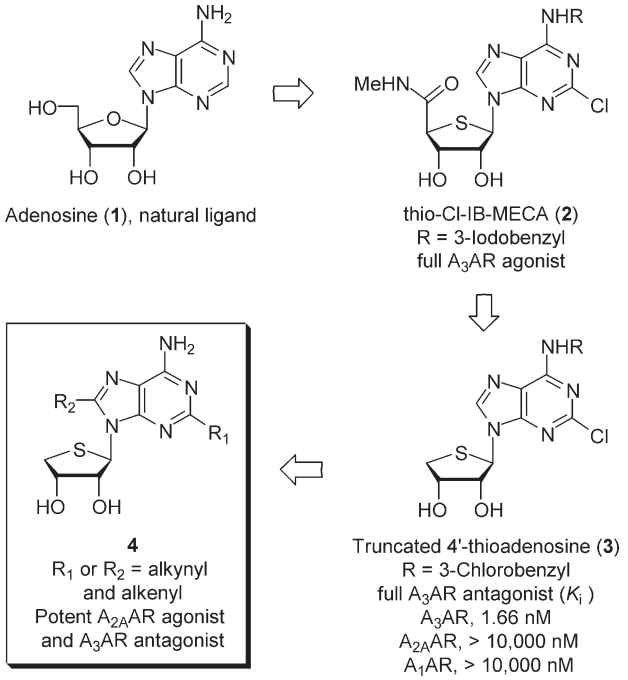


Figure 1. Rationale for the design of A_{2A} AR agonists.

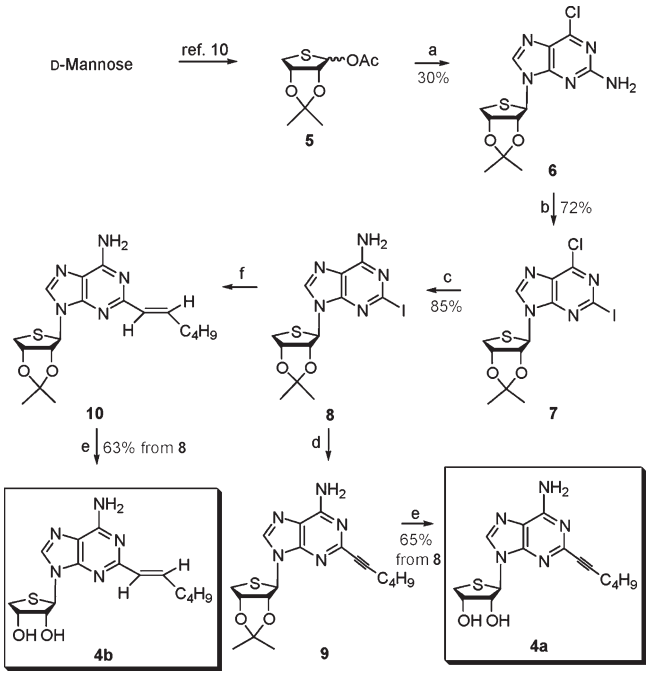
3′-H. Treatment of 2-amino-6-chloro derivative **6** with isoamyl nitrite, iodine, and methylene iodide in the presence of CuI afforded the 2-iodo-6-chloro derivative **7**, which was converted to the 2-iodo-6-amino derivative **8** upon treatment with methanolic ammonia. A Sonogashira[18] coupling reaction of **8** with 1-hexyne in the presence of bis(triphenylphosphine)palladium dichloride yielded the 2-hexynyl derivative **9**. Finally, removal of the isopropylidene of **9** with 1 N HCl produced the final 2-hexynyl-4′-thioadenosine derivative **4a**. Suzuki[19] coupling reaction of the 2-iodo derivative **8** with (*E*)-1-catecholboranylhexene,[20] prepared by treating with 1-hexyne and catecholborane, in the presence of tetrakis(triphenylphosphine)palladium(0) afforded the 2-hexenyl derivative **10**. Removal of the acetonide of **10** with 1 N HCl gave the 2-hexenyl-4′-thioadenosine derivative **4b**.

Using a strategy similar to Scheme 1, 8-substituted adenosine derivatives **4c** and **4d** were synthesized from the glycosyl donor **5** (Scheme 2). Condensation of **5** with 8-bromoadenine[21] under Lewis acid conditions afforded the 8-bromo derivative **11**. Coupling of **11** with 1-hexyne under Sonogashira conditions gave 8-hexynyl derivative **12**, which was treated with 1 N HCl to yield the final 8-hexynyl-4′-thioadenosine derivative **4c**.

The 8-bromo derivative **11** was condensed with (*E*)-1-catecholboranylhexene[20] under Suzuki conditions[19] to give the 2-hexenyl derivative **13**. Removal of the isopropylidene group of **13** under acidic conditions afforded the final 8-hexenyl-4′-thio adenosine derivative **4d**.

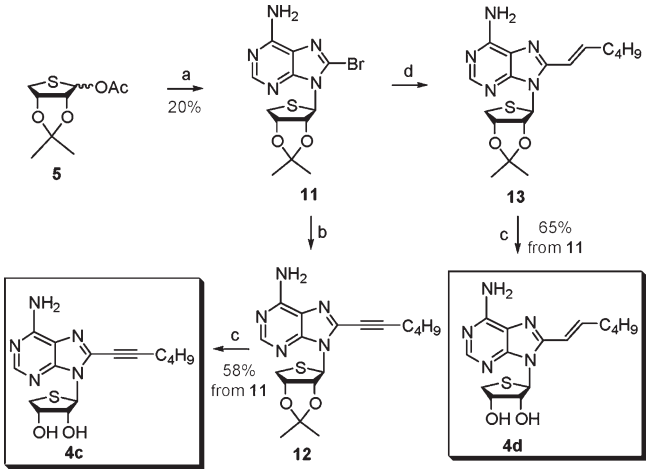
Binding assays were carried out using standard radioligands and membrane preparations from Chinese hamster ovary (CHO) cells stably expressing the human (h) A_1 or A_3 AR or human embryonic kidney cells (HEK-293) expressing the hA_{2A} AR.[22−25] Unlike the parent N^6-substituted compound **3** that only weakly bound to the A_{2A} AR, C2-substituted

Scheme 1. Synthesis of the 2-Substituted Derivatives **4a** and **4b**[a]



[a] Reagents and conditions: (a) Silylated 2-amino-6-chloropurine, TMSOTf, DCE, room temperature to 80 °C, 3 h. (b) CuI, isoamyl nitrite, I_2, CH_2I_2, THF, 110 °C, 45 min. (c) NH_3/MeOH, 80 °C, 2 h. (d) 1-Hexyne, CuI, TEA, DMF, bis(triphenylphosphine)palladium dichloride, room temperature, 3 h. (e) 1 N HCl, THF, room temperature, 15 h. (f) (*E*)-1-catecholboranylhexene, then tetrakis(triphenylphosphine)palladium-(0), Na_2CO_3, DMF, H_2O, 90 °C, 15 h.

Scheme 2. Synthesis of the 8-Substituted Derivatives **4c** and **4d**[a]



[a] Reagents and conditions: (a) Silylated 8-bromoadenine, TMSOTf, DCE, room temperature to 90 °C, 2 h. (b) 1-Hexyne, CuI, TEA, DMF, bis(triphenylphosphine) palladium dichloride, room temperature, 3 h. (c) 1 N HCl, THF, room temperature, 15 h. (d) (*E*)-1-catecholboranylhexene, then tetrakis-(triphenylphosphine) palladium(0), Na_2CO_3, DMF, H_2O, 90 °C, 15 h.

variations of compound **3** led to a dramatic increase in the binding affinity (K_i = 7.19 ± 0.6 nM for **4a** and 72.0 ± 19.1 nM for **4b**) at the hA_{2A} AR, while maintaining high binding

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Table 1. Binding Affinities of Known A_3 AR Antagonist **3** and Truncated 2- and 8-Substituted 4′-Thioadenosine Derivatives **4a**−**d** at Three Subtypes of hARs

	affinity[a]		
compounds	hA_1 (% inhibition)	hA_{2A} (% inhibition)	hA_3 (K_i, nM)
3	37.9	17.7	1.66 ± 0.90
4a	38.9 ± 9.9	97.2 ± 4.1	11.8 ± 1.3
4b	16.2 ± 8.4	95.9 ± 8.7	13.2 ± 0.8
4c	49.3 ± 4.9	46.5 ± 4.3	20.0 ± 4.0
4d	3.7 ± 2.9	22.8 ± 6.4	259 ± 10

[a] All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR (A_1 AR and A_3 AR in CHO cells and A_{2A} AR in HEK-293 cells). Binding was carried out using 1 nM [^{3}H]CCPA, 10 nM [^{3}H]CGS21680, 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 means ± SEMs, n = 3−4 (outliers eliminated) and normalized against a nonspecific binder, 5′-*N*-ethylcarboxamidoadenosine (NECA, 10 μM). Values expressed as a percentage refer to the percent inhibition of specific radioligand binding at 10 μM, with nonspecific binding defined using 10 μM NECA.

affinity (K_i = 11.8 ± 1.3 nM for **4a** and 13.2 ± 0.8 nM for **4b**) at the hA_3 AR (Table 1).

These results indicate that bulky hydrophobic pockets exist in the binding sites of A_{2A} AR and A_3 AR, allowing the C2-substituent to form favorable hydrophobic interactions. The 2-alkynyl derivative **4a** showed a better binding affinity than the 2-alkenyl derivative **4b**. Interestingly, C8-substitution on **3** abolished the binding affinity at the hA_{2A} AR, but the binding affinity at the hA_3 AR was maintained although decreased. These findings suggest that a bulky hydrophobic group at position 8 could be tolerated at the binding site of the hA_3 but not hA_{2A} AR. The ability to enhance affinity at the A_{2A} AR in the truncated series by extending an unsaturated carbon chain at the 2-position implies a mode of receptor binding in common with the riboside series.[15−17] All compounds showed very weak binding affinity at the hA_1 AR.

Compounds **4a** and **4b** were found to be potent and full antagonists in a cyclic AMP functional assay at the hA_3 AR. In this assay, **4a** dose dependently shifted the concentration−response curve for agonist Cl-IB-MECA to the right as an antagonist, corresponding to a K_B value of 1.69 nM calculated by Schild analysis (Figure 2). This is consistent with previous studies in which truncated N^6-substituted 4′-thioadenosine derivatives have generally displayed A_3 AR antagonist activity.[13,14] However, in a cyclic AMP functional assay at the hA_{2A} AR expressed in CHO cells, compound **4a** behaved as a full agonist as compared to the standard 2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680) and displayed an EC_{50} of 12 nM. At the hA_{2B} AR expressed in CHO cells, **4a** was a weak partial agonist in cyclic AMP accumulation (EC_{50} ∼10 μM). The finding that compound **4a** is both a potent and a selective agonist at the hA_{2A} AR and a competitive antagonist at the hA_3 AR is similar to the pharmacological profile of a more heavily 2,5′-substituted adenosine derivative,[26] which inhibited both formation of reactive oxygen species and eosinophils degranulation for the antiasthmatic activity.

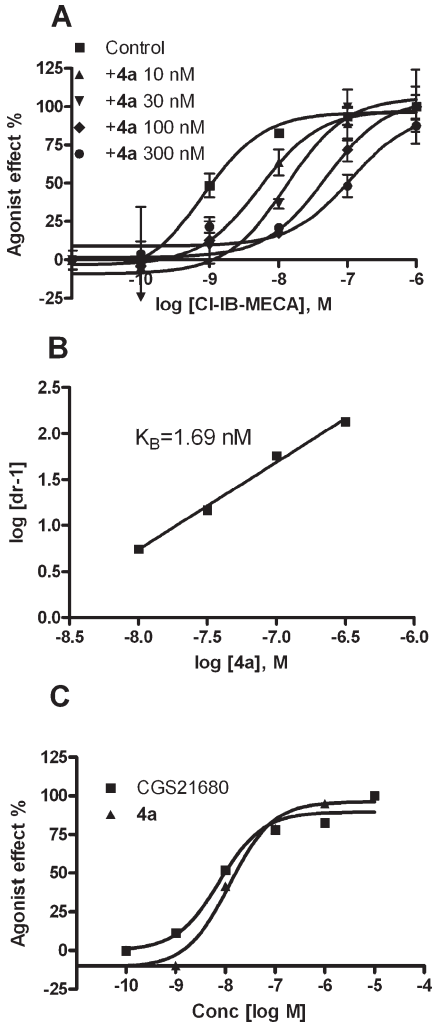

Figure 2. Parallel right shifts induced by compound **4a** on the concentration−response curve of a full agonist in the inhibition of cyclic AMP production at the hA_3 AR expressed in CHO cells (A), the corresponding Schild plot (B), and the activity of **4a** as a full agonist at the hA_{2A} AR expressed in CHO cells, as compared to CGS21680 (C).

To investigate the binding mode, we performed a study of docking the C2- and C8-substituted 4′-thioadenosine derivatives **4a**−**d** in the hA_{2A} AR X-ray crystallographic structure (PDB code: 3EML)[27] using GOLD software,[28] considering the flexibility of the binding site residues. As shown in Figure 3, the C2-substituted 4′-thioadenosine derivatives **4a** and **4b**, whose binding affinities are in the nanomolar range, occupied the binding site very well. Their bulky and rigid C2-substituents oriented toward the extracellular region, forming hydrophobic interactions. The adenine moieties appeared to form three H-bonds with Glu169 and Asn253, and the ring systems were in $\pi-\pi$ stacking with Phe168. Also, the thio-sugar rings were located deep inside the binding pocket, and the 3′-OH groups were able to donate a H-bond to Ser277. On the basis of this result, the C8-substituents were expected to be oriented in a hydrophobic pocket deep inside the binding site, which was not occupied

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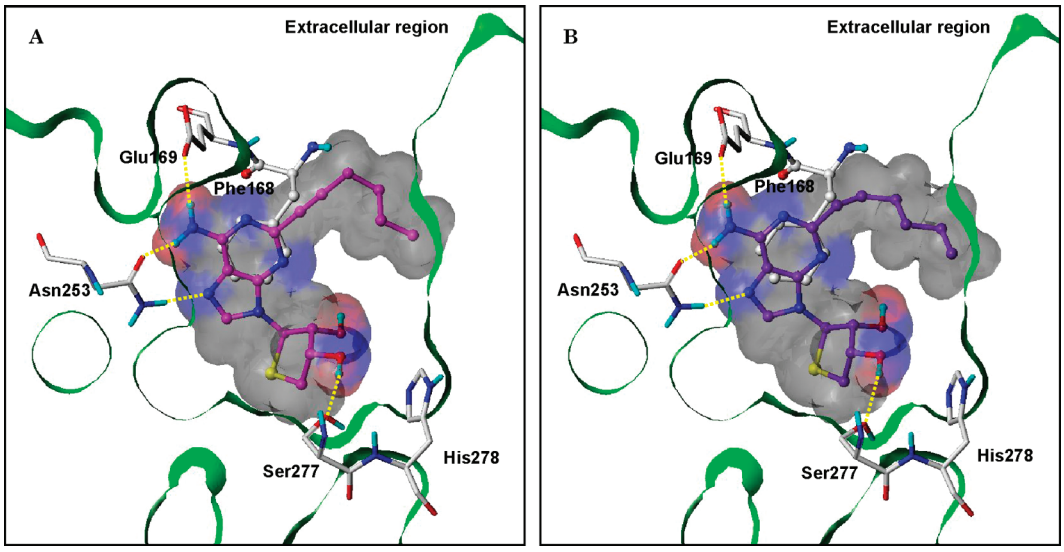

Figure 3. Predicted binding modes of (A) **4a** and (B) **4b** docked in the hA_{2A} AR crystal structure. The key interacting residues are marked and displayed in capped-stick, except Phe168 in ball-and-stick, with carbon atoms in white. The ligands are depicted as ball-and-stick with carbon atoms in magenta (**4a**) and purple (**4b**). Hydrogen bonds are shown in yellow dashed lines. The van der Waals surfaces of the ligands were generated by MOLCAD and colored by hydrogen-bonding property (red, H-bond donating regions; blue, H-bond accepting regions). The fast Connolly surface of the protein is Z-clipped, and nonpolar hydrogens are undisplayed for clarity.

by **4a** and **4b** in Figure 3. However, the C8-substitued derivatives **4c** and **4d** showed various binding modes (data not shown). In addition to the expected binding mode, the C8-substituents alternately pointed toward the extracellular region through a rotation of the bond between the adenine and the thio-sugar rings. It might be due to spatial restriction of the long and rigid C8-substituents in the hydrophobic pocket inside the binding site, which would cause the nucleosides to lose some H-bonding and/or $\pi-\pi$ stacking interactions that were shown for the C2-substituted derivatives. These results might explain why the A_{2A} AR affinities of **4c** and **4d** were reduced.

In conclusion, we synthesized the truncated C2- and C8-substituted 4′-thioadenosine derivatives **4a**−**d**, starting from D-mannose, using palladium-catalyzed cross-coupling reactions as key steps. Although the antagonist activity of various truncated 4′-thionucleosides at the A_3 AR was well explored previously, this is the first characterization of the functional activity of such derivatives at the A_{2A} AR. From this study, we successfully identified potent and sterically compact A_{2A} AR agonists, **4a** and **4b**, by placing extended hydrophobic 2-hexynyl or 2-hexenyl groups on truncated and N^6-unsubstituted 4′-thioadenosine derivatives. This observation was supported by molecular modeling that placed the chain at the 2-position in a hydrophobic region of the A_{2A} AR. However, C8-substitution greatly reduced binding affinity at the hA_{2A} AR. Thus, the absence of a 5′-uronamide of typical A_{2A} AR agonists[2−4] or the native $-CH_2OH$ of adenosine did not preclude potent binding and full activation of the hA_{2A} AR. This suggested a major difference between the A_{2A} AR and the A_3 AR in the pathway of receptor activation. All synthesized compounds **4a**−**d** maintained their binding affinity at the human A_3 AR, and as for the 2-H or 2-Cl analogues that were N^6-substituted, competitive A_3 AR antagonism was demonstrated. This study establishes that the truncated C2-substituted 4′-thioadenosine derivatives **4a** and **4b** can serve as a novel template for the development of new A_{2A} AR ligands, although they still may interact at the A_3 AR. This mixed activity as A_{2A} AR agonist/A_3 AR antagonist might also be advantageous in disease models such as asthma.[26] Thorough elucidation of the structure−activity relationship of this series is in progress in our laboratory.

SUPPORTING INFORMATION AVAILABLE

Complete experimental procedures and characterization data and ^1H and ^{13}C NMR copies of **4a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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