N^6-Substituted D-4′-Thioadenosine-5′-methyluronamides: Potent and Selective Agonists at the Human A_3 Adenosine Receptor

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Received May 10, 2003

Abstract: 4′-Thio analogues **3**−**5** of Cl-IB-MECA (**2**) (K_i = 1.0 ± 0.2 nM at the human A_3 adenosine receptor) were synthesized from D-gulono-γ-lactone via 4-thioribosyl acetate **14** as the key intermediate. All synthesized 4′-thionucleosides exhibited higher binding affinity to the human A_3 adenosine receptor than Cl-IB-MECA, among which **4** showed the most potent binding affinity (K_i = 0.28 ± 0.09 nM). **4** was also selective for A_3 vs human A_1 and human A_{2A} receptors by 4800- and 36000-fold, respectively.

Introduction. Adenosine is known to be involved in regulation of many physiological functions through specific adenosine receptors, which are expressed on the surface of nearly all cells.[1] Four subtypes of adenosine receptors have now been cloned, characterized pharmacologically, and classified as A_1, A_{2A}, A_{2B}, and A_3. Among these, the A_3 adenosine receptor was the most recently identified and cloned from a rat brain cDNA library and later from sheep and human brain.[2−4] This receptor is a G-protein-coupled receptor, and its activation inhibits adenylate cyclase and stimulates phospholipase C, leading to protein kinase C activation and histamine release from rat mast cells.[5] Since the A_3 adenosine receptor is closely related to several diseases such as cardiac ischemia,[6] cerebral ischemia,[7] and inflammation,[8,9] it has been a promising target for the development of new therapeutic agents. Thus, A_3 adenosine receptor agonists are under consideration in the treatment of cardiac and cerebral ischemia and cancer,[10] while A_3 adenosine receptor antagonists have been suggested to be useful as a potential treatment for glaucoma and asthma.[11]

A number of ligands have been synthesized and tested for binding affinity at the rat, sheep, and human A_3 versus A_1 and A_{2A} receptors. Among these ligands, IB-MECA (**1**) was found to be a highly potent rat A_3 agonist (K_i = 1.1 nM), which is 50-fold selective for rat brain A_3 versus either A_1 or A_2 receptors.[12] Introduction of chlorine at the 2-position of IB-MECA, resulting in the formation of Cl-IB-MECA (**2**) dramatically increased binding affinity and selectivity.[12] **2** has been reported to display a K_i value of 0.33 nM and showed 2500- and 1400-fold rat A_3 receptor selectivity versus A_1 and A_{2A} receptors, respectively. **2** is now being used extensively as a pharmacological tool for studying A_3 receptors.[13]

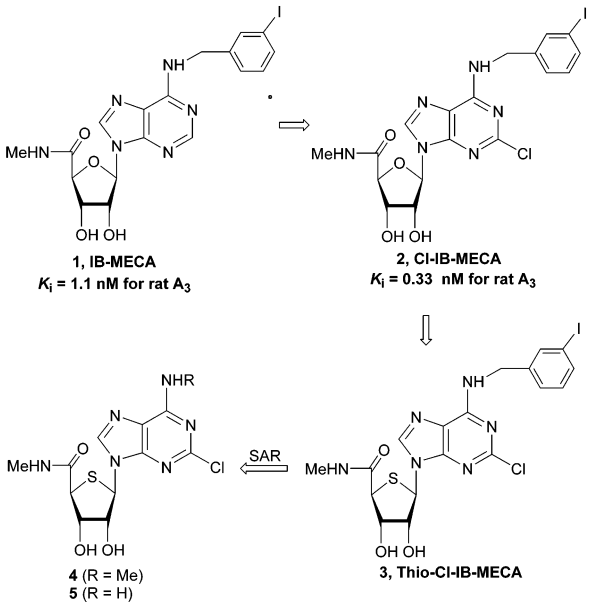

Figure 1. Rationale for the design of the target 4′-thionucleosides.

Therefore, on the basis of the high binding affinity and selectivity of **2** on A_3 adenosine receptors, we designed and synthesized the 4′-thio analogue **3** of Cl-IB-MECA and its related analogues **4** and **5**, since a sulfur atom may serve as a bioisostere of an oxygen atom (Figure 1). The N^6-methyl group was introduced because this substituent is associated with enhanced affinity at the human (but not rat) A_3 adenosine receptor.[14] A new ligand, thio-Cl-IB-MECA (**3**), and its derivatives **4** and **5** were efficiently synthesized from D-gulono-γ-lactone using a highly efficient synthetic route to prepare the 4-thioribose derivative. The synthesized N^6-substituted 4′-thionucleoside derivatives **3**, **4**, and **5** consistently exhibited subnanomolar affinity and high selectivity for human A_3 receptors versus human A_1 and A_{2A} receptors. Such agonists may be useful as pharmacological tools and also are of interest for the development as therapeutic agents.

Results and Discussion. Chemistry. The synthetic strategy to the desired nucleosides was to synthesize the glycosyl donor and then to condense with nucleobases. The synthesis of the glycosyl donor **14**, starting from D-gulono-γ-lactone (**6**), is shown in Scheme 1.

Starting material **6** was converted to the diacetonide **7** under the standard reaction conditions. Reduction of **7** with $NaBH_4$ followed by mesylation of the resulting diol afforded the dimesylate **8**. Cyclization of **8** with anhydrous sodium sulfide smoothly proceeded upon

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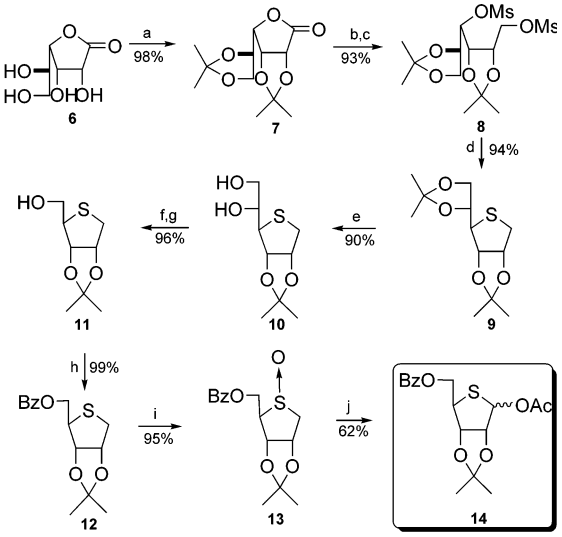
10.1021/jm034098e CCC: $25.00
Published on Web 08/06/2003

Scheme 1. Synthesis of the Glycosyl Donor[a]

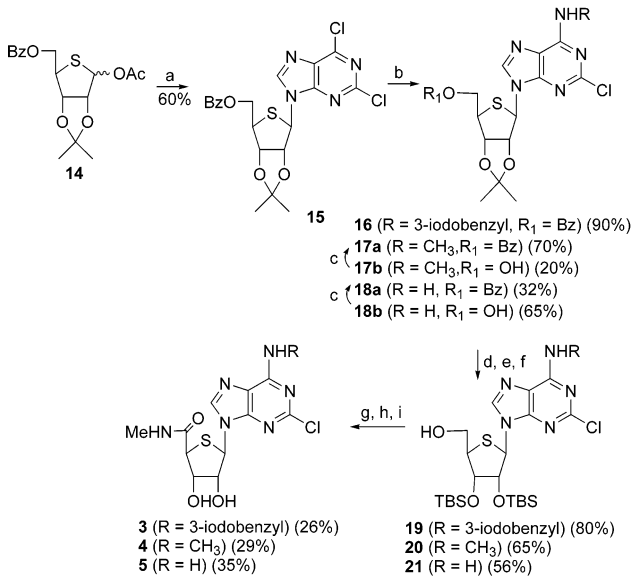


[a] Reagents: (a) CH_3COCH_3, H_2SO_4, $CuSO_4$, room temp; (b) $NaBH_4$, MeOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) Na_2S, DMF, heat; (e) 30% AcOH; (f) $Pb(OAc)_4$, EtOAc; (g) $NaBH_4$, MeOH; (h) BzCl, pyridine; (i) *m*CPBA, CH_2Cl_2; (j)Ac_2O.

heating in DMF to yield the thiosugar **9** in 94% yield. Selective hydrolysis of the 5,6-acetonide in the presence of 2,3-acetonide was achieved using 30% aqueous acetic acid to give diol **10** in 90% yield, based on recovered starting material. Oxidative cleavage of **10** with lead tetraacetate followed by reduction of the resulting aldehyde with $NaBH_4$ afforded **11**, which was treated with benzoyl chloride to give the benzoate **12**. Oxidation of **12** with *m*CPBA followed by heating of the sulfoxide **13** with acetic anhydride produced the key intermediate **14**.

The glycosyl donor **14** was utilized for the synthesis of 2-chloro N^6-substituted 4′-thiopurine nucleosides **3**−**5** as shown in Scheme 2. The glycosyl donor **14** was condensed with silylated 2,6-dichloropurine in the presence of TMSOTf to give the β-anomer **15** (60%) and its α-anomer in 9:1 ratio. Anomeric configurations were easily assigned using results from ^{1}H NOE experiments. The β-anomer **15** was converted to **16**, **17a**,**b**, and **18a**,**b** by treating with 3-iodobenzylamine, methylamine, and ammonia, respectively. For the synthesis of the uronamide derivatives, compounds **16**, **17a**, and **18a** were treated with 80% aqueous acetic acid to give the diol derivatives, which were protected as disilyl ethers and then debenzoylated to afford the 4′-hydroxymethyl analogues **19**, **20**, and **21**, respectively. Oxidation of **19**, **20**, and **21** with PDC in DMF followed by esterification with potassium carbonate and dimethyl sulfate in acetone, conversion of the 5′-esters to 5′-uronamides by treating

Scheme 2. Synthesis of the Target Nucleosides[a]



[a] Reagents: (a) silylated 2,6-dichloropurine, TMSOTf; (b) RNH_2; (c) BzCl, pyridine; (d) 80% AcOH; (e) TBSOTf, pyridine; (f) NaOMe, MeOH; (g) (i) PDC, DMF, (ii) K_2CO_3, $(CH_3O)_2SO_2$; (h) 2 N $MeNH_2$, THF; (i) *n*-Bu_4NF, THF.

with 2 N methylamine, and the final desilylation yielded the final nucleosides **3**, **4**, and **5**, respectively.

Biological Activity. The synthesized analogues were tested in radioligand binding assays[15] using rat cortical A_1 receptors or striatal A_{2A} receptors or in Chinese hamster ovary (CHO) cells stably expressing the recombinant receptors (human A_1, A_{2A}, or A_3). Radioligands for A_1, A_{2A}, and A_3 receptors were the selective agonists [^{3}H]-N^6-(*R*)-phenylisopropyladenosine (PIA) (A_1), [^{3}H]-CGS21680 (A_{2A}), and [^{125}I]I-AB-MECA [N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)] (A_3), respectively.

As shown in Table 1, all synthesized 4′-thionucleosides exhibited high binding affinity to the human A_3 receptor in the nanomolar range, while very low binding affinities to A_1 and A_{2A} receptors were observed. Among them, **4** displayed a K_i value of 0.28 nM at the A_3 receptor and had the highest affinity among the synthesized compounds. It was selective for A_3 vs human A_1 and human A_{2A} receptors by 4800- and 36000-fold, respectively. **3** and **5** with 3-iodobenzylamino and amino substituents at the N^6 position also exhibited higher binding affinity to the A_3 receptor (K_i = 0.38 and 0.40 nM, respectively) than Cl-IB-MECA. To our knowledge, it is the first example of a 4′-thionucleoside exhibiting highly potent and selective binding affinity to the A_3 adenosine receptor. Compounds **3**−**5** were demonstrated

Table 1. Affinities of 4′-Thionucleoside Analogues in Radioligand Binding Assays at A_1, A_{2A}, and A_3 Receptors (Human or Rat, As Indicated)[a]

	K_i (nM) or, if indicated, % displacement				
compd	hA_1	rA_1	hA_{2A}	rA_{2A}	hA_3
Cl-IB-MECA (**2**)	1240 ± 320	820 ± 570	5360 ± 2470	470 ± 365	1.0 ± 0.2
3	193 ± 46	140 ± 43	223 ± 36	348 ± 110	0.38 ± 0.07
4	1330 ± 242	198 ± 14	20% (10 μM)	6340 ± 90	0.28 ± 0.09
5	89.2 ± 11.7	294 ± 115	158 ± 29	<10% (1 μM)	0.40 ± 0.06

[a] All human AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the appropriate receptor. Binding at rat A_1 and A_{2A} ARs was carried out using rat brain homogenates. Radioligands: A_1 ([^{3}H]-N^6-(*R*)-phenylisopropyladenosine), A_{2A} ([^{3}H]CGS21680), A_3 ([^{125}I]iodo-AB-MECA).

to be full agonists in an assay of human A_3 receptor mediated inhibition of cyclic AMP in transfected CHO cells.[16] The IC_{50} values were 0.21 ± 0.4 nM (**4**), 0.38 ± 0.6 nM (**3**), and 1.0 ± 0.3 nM (**5**).

In conclusion, we have designed and synthesized novel 4′-thioanalogues of Cl-IB-MECA as the A_3 receptor ligands on the basis of the bioisosteric rationale. We have found novel 2-chloro-N^6-methyl-4′-thioadenosine-5′-methyluronamide (**4**) as a highly potent and selective agonist at the human A_3 adenosine receptor.

This result is of great significance in that there are few highly selective A_3 agonists. The selectivity of IB-MECA is evident in some pharmacological systems but not in others.[17] Cl-IB-MECA is selective; however, it has associated toxicity in vivo. The finding that the 4′-thio modification is associated with high potency and selectivity significantly expands the possibilities to design additional A_3 agonists, which may potentially be useful as in vivo tools. Thorough investigation of the structure−activity relationship of this series and a molecular modeling study are in progress in our laboratory and will be reported in due course.

Acknowledgment. This research was supported by a grant from the Korea Science and Engineering Foundation (Grant KOSEF 2002-2-21500-001-3).

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

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JM034098E