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DESIGN, SYNTHESIS, AND ANTI-TUMOR ACTIVITY OF 4′-THIONUCLEOSIDES AS POTENT AND SELECTIVE AGONISTS AT THE HUMAN A_3 ADENOSINE RECEPTOR

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□ *On the basis of potent and selective binding affinity of Cl-IB-MECA to the human A_3 adenosine receptor, its 4′-thioadenosine derivatives were efficiently synthesized starting from D-gulonic γ-lactone. Among compounds tested, 2-chloro-N^6-(3-iodobenzyl)- and 2-chloro-N^6-methyl-4′-thioadenosine-5′-methyluronamides (***7a*** and ***7b***) exhibited nanomolar range of binding affinity (K_i = 0.38 nM and 0.28 nM, respectively) at the human A_3AR. These compounds showed anti-growth effects on HL-60 leukemia cell, which resulted from the inhibition of Wnt signaling pathway.*

Keywords Bioisosteric; 4′-thionucleosides; A3 adenosine receptor agonist; anti-proliferative effect

INTRODUCTION

Adenosine is endogenous material and regulates many physiological functions in the body through adenosine receptors.[1] Thus, adenosine receptors have been good therapeutic targets for the development of clinically useful drugs.[2] On the basis of the structure of a natural ligand, adenosine, many nucleoside derivatives largely modified on N^6- and/or

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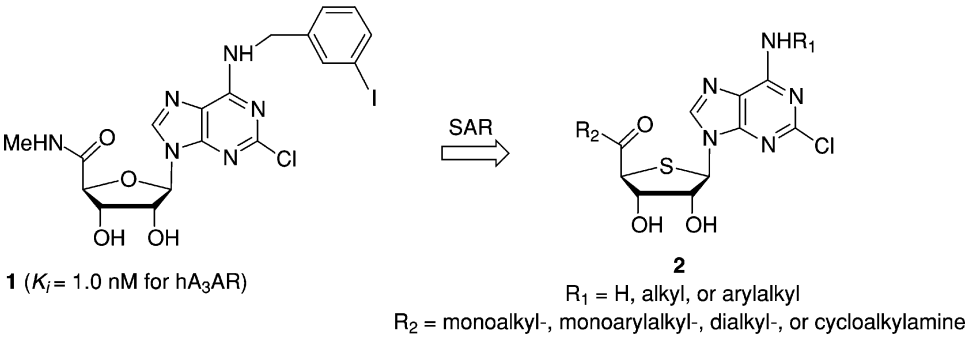


FIGURE 1 Design of the 4′-thionucleosides **2** based on bioisosteric rationale.

4′-position of adenosine have been synthesized as potent adenosine receptor ligands.[3] Among these, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-methyluronamide (**1**, Cl-IB-MECA)[4] was discovered as potent and selective agonist ($K_i = 1.0$ nM) at the human A_3 adenosine receptor (AR) and is being developed as anticancer agent. Therefore, on the basis of potent and selective binding affinity to the human A_3AR, we designed and synthesized 4′-thioadenosine derivatives which are in biosiosteric relationships with compound **1** (Figure 1).

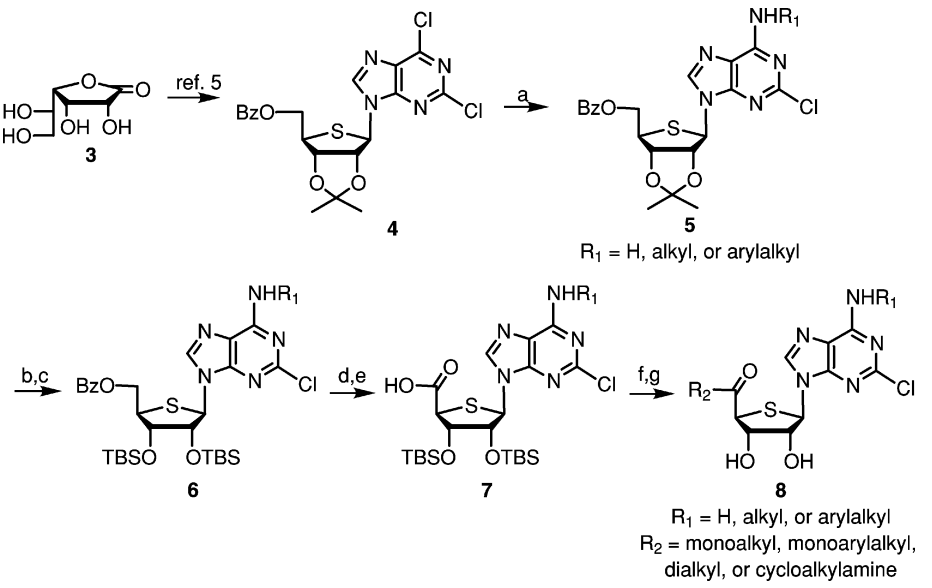
Herein, we report the synthesis, binding affinity at the A_3AR, and *in vitro* anti-proliferative effects in human cancer cell lines of 4′-thioadenosine derivatives.

RESULTS AND DISCUSSION

Synthesis of the target nucleosides **8** started from the known intermediate **3** which was derived from D-gulonic γ-lactone (**3**), as shown in Scheme 1.[5]

Treatment of **4** with various alkyl- and arylalkyl amines in EtOH produced N^6-alkyl- or arylalkylamino-4′-thioadenosine derivatives **5**. Because removal of the isopropylidene group under acidic conditions at the final step resulted in deglycosylation, the isopropylidene group in **5** was changed to di-*O*-TBS ether **6**. Deprotection of the benzoyl group of **6** followed by oxidation of the primary alcohol to the acid with PDC gave the acid derivative **7**. Conversion of the acid **7** to the various amides **8** was accomplished by coupling with various amines in the presence of EDC and HOBT.

Binding affinities of all synthesized 4′-thioadenosine derivatives at the A_3 AR were measured using a radioligand binding assay.[5] From this study, 2-chloro-N^6-methyl- and 2-chloro-N^6-(3-iodobenzyl)-4′-thioadenosine-5′-methyluronamides ($K_i = 0.28 \pm 0.09$ nM and $K_i = 0.38 \pm 0.07$ nM, respectively) were discovered as highly potent and selective agonists at the human A_3AR. It was also found that 5′-monoalkyl amide



SCHEME 1 Reagents and conditions: a) R_1NH_2, Et_3N; b) 80% AcOH; c) TBSOTf, pyridine; d) NaOMe, MeOH; e) PDC, DMF; f) amines, EDC, HOBT, DIPEA, CH_2Cl_2; g) TBAF, THF.

showed better binding affinity than the corresponding 5′-dialkyl amide, indicating that at least one hydrogen forms a hydrogen bond within the binding site.[6] The 4′-thionucleosides generally showed higher binding affinity to the A_3AR than the corresponding 4′-oxonucleosides.

2-Chloro-N^6-(3-iodobenzyl)-4′-thioadenosine-5′-methyluronamide showing high binding affinity to the A_3AR was tested for anti-proliferative effects in human cancer cell lines such as A549, Col2, HL-60 cells. This compound exhibited concentration-dependent anti-proliferative effects. A further study indicated that anti-growth effect of this agonist resulted from the inhibition of Wnt signalling pathway by lowering the levels of β-catenine, phosphorylated-Akt, and phosphorylated-GSK 3β.[7]

In summary, we carried out the systematic structure-activity relationships of 4′-thioadenosine derivatives, among which 2-chloro-N^6-(3-iodobenzyl)-4′-thioadenosine-5′-methyluronamide emerged as potent and selective A_3AR agonist. In vitro anti-growth effects and novel mechanism of action of this agonist guarantees it has a high potential to be a good anticancer agent.

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