Synthesis of 2-Chloro-N⁶-Substituted-4'-thioadenosine-5'-N,N-dialkyluronamides as Potent and Selective A₃ Adenosine Receptor Antagonists

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ABSTRACT

The highly selective A₃ receptor agonist, 4'-thio-Cl-IB-MECA was successfully converted into selective A₃ receptor antagonists by appending a second N-alkyl group on the 5'-uronamido position. This result indicates that the hydrogen bonding ability of the 5'-uronamido is essential for the conformational change required for the receptor activation. Among compounds tested, a N⁶-(3-bromobenzyl) derivative with 5'-dimethyluronamido exhibited the highest binding affinity (Kᵢ = 9.32 nM) at the human A₃ AR with very low binding affinities to other AR subtypes.

INTRODUCTION

Adenosine regulates cell signaling through binding to four subtypes (A₁, A₂A, A₂B, and A₃) of adenosine receptors (ARs).¹ Among these, A₃ AR has been most recently identified and good therapeutic target for the treatment of cancer, ischemia, asthma, and inflammation.² Modification on N⁶ position and/or 4'-hydroxymethyl group of adenosine afforded potent and selective A₃ AR agonists³ because of the structural similarity to adenosine, but not AR antagonists. Most of A₃ AR antagonists belong to nonpurine heterocyclic compounds. However, because these derivatives showed poor binding affinity at the rat A₃ AR, these are not suitable for establishing efficacy in animal model.⁴ Compounds with a nucleoside skeleton were found to be species-independent A₃ AR antagonists,⁵ but with very low binding affinity to the A₃ AR. Thus, it is of great interest to synthesize nucleoside analogues with high binding affinity to the A₃ AR, showing equal binding affinity at the rat as well as human A₃ ARs. Recently, we discovered the 4'-thio analogue, thio-Cl-IB-MECA⁶ as potent and selective human A₃ AR agonist (Kᵢ for human A₃ AR = 0.38 nM) (Fig. 1). Since it is believed that the hydrogen of the methylamido is essential for the hydrogen bonding and thus for the induced-fit required for the receptor activation, it was hypothesized that removal of the hydrogen bonding ability results in the conversion of the agonistic activity into the antagonistic activity. Thus, 5'-N,N-dialkyluronamido derivatives of thio-Cl-IB-MECA were designed and synthesized as human A₃ AR antagonists.⁷

RESULTS AND DISCUSSION

Synthesis of the desired nucleosides started from 2,6-

Fig. 1. Rationale for the design of A₃ AR antagonists

animal model.⁴ Compounds with a nucleoside skeleton were found to be species-independent A₃ AR antagonists,⁵ but with very low binding affinity to the A₃ AR. Thus, it is of great interest to synthesize nucleoside analogues with high binding affinity to the A₃ AR, showing equal binding affinity at the rat as well as human A₃ ARs. Recently, we discovered the 4'-thio analogue, thio-Cl-IB-MECA⁶ as potent and selective human A₃ AR agonist (Kᵢ for human A₃ AR = 0.38 nM) (Fig. 1). Since it is believed that the hydrogen of the methylamido is essential for the hydrogen bonding and thus for the induced-fit required for the receptor activation, it was hypothesized that removal of the hydrogen bonding ability results in the conversion of the agonistic activity into the antagonistic activity. Thus, 5'-N,N-dialkyluronamido derivatives of thio-Cl-IB-MECA were designed and synthesized as human A₃ AR antagonists.⁷

Reagents and conditions: a) R₂(N₃), Et,N, EtOH, b) 80% AOH, c) TBSOTf, pyridine, d) NaOMe, MeOH, e) POCl₃, (DMF), f) R₂(N₃), MeOH, g) pyridine, HClO₂, TRIP, THF.

Scheme 1. Rationale for the design of A₃ AR antagonists
dichloropurine derivative 3⁶, which was prepared from D-gulonic γ-lactone (Scheme 1).
Treatment of 3 with various alkyl or arylamines afforded N⁶-substituted derivative 4. The isopropylidene group of 4 was changed to the TBS group because its removal at the final stage resulted in deglycosylation, and then 5'-benzoyl group was deprotected to give 5. Oxidation of the primary
alcohol of 5 with PDC in DMF yielded the acid 6. Conversion of the acid 6 into the dialkylamide derivatives 7 was achieved by treating with various amines in the presence of EDC and HOBt.  

Radioisogold binding assays were carried out in Chinese hamster ovary (CHO) cells stably expressing a human AR subtype. 5'-N,N-Dimethylamido derivatives showed better binding affinity than larger 5'-N,N-dialkyl or 5'-N,N-cycloalkylamido derivatives, irrespective of N'-substituents. With dimethylamide at the 5'-position (R2 = R3 = Me) fixed, the binding affinity of various N'-substituted analogues was examined. The N'-[3-halobenzyl]amino series generally exhibited higher binding affinity at the human A2AR than the N'-dialkylamino or N'-cycloalkylamino series. Within the N'-[3-halobenzyl]amino series (R1 = 3-halobenzyl, R2 = R3 = Me), the binding affinity at the human A2AR was in the following order: 3-bromobenzyl (K1 = 9.32 nM) > 3-iodobenzyl (K1 = 15.5 nM) > 3-chlorobenzyl (K1 = 21.3 nM) > 3-fluorobenzyl (K1 = 121 nM). All synthesized compounds exhibited almost no binding affinities to other AR subtypes. 

The functional assay indicated that compound 7 (R1 = 3-iodobenzyl, R2 = R3 = Me) indicated that this is a pure A2AR antagonist. The binding affinity of compound 7 was also measured at the rat A2AR to determine species differences, in which this compound showed moderate affinity (K1 = 321 ± 74 nM) at the rat A2AR.  

**CONCLUSION**

Based on the hypothesis that the hydrogen bond-donating ability of the 5'-uronamide is responsible for the conformational change needed for receptor activation, we carried out the structure-activity relationship of 2-chloro-N'-substituted-4'-thioadenosine-5'-N,N-dialkyluronamides as pure A3 AR antagonists. From this study, it was found that the hydrogen bond-donating ability of the 5'-uronamide was essential for the pure A3AR antagonism. All synthesized A3AR antagonists could be evaluated in models of a number of disorders related to the A3AR, such as glaucoma, inflammation, and asthma.

**REFERENCES**


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