Synthesis of 3'-ureidoadenosines and their high binding affinity at the mutant A₃ adenosine receptor

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ABSTRACT

For the purpose of developing optimal neocceptor-neagonist pair, 3'-ureidoadenosines derivatives were synthesized. Among compounds tested, 2-chloro-3'-ureido-N⁶-(3-iodobenzyl)adenosine (10b) showed the best binding affinity (Kᵢ = 0.20 µM) at the H272E mutant A₃ AR, but was inactive at the natural A₃ AR.

INTRODUCTION

Although A₃ adenosine receptor full agonists such as N⁶-(3-iodobenzyl)-5'-N-methylcarbamoyl adenosine (IB-MECA, Kᵢ = 1.8 ± 0.7 nM)¹ and its 2-chloro derivative (Cl-IB-MECA, Kᵢ = 1.4 ± 0.3 nM)¹ are of interest in the treatment of cardiac and cerebral ischemia² and cancer³, the ubiquitous presence of adenosine receptors throughout the body hindered them from being developed as clinically useful agents. In order to overcome this lack of specificity, Jacobson et al.⁴ have demonstrated a neocceptor approach, in which a re-engineered G protein-coupled receptor, mutant A₃ adenosine receptor recognize only a specifically designed A₃ agonist, neoligand, but not the native A₃ agonist.

Fig. 1. The rationale for the design of 3'-ureidoadenosines.

The mutant A₃ adenosine receptor was designed on the basis of rhodopsin-based molecular modeling and mutagenesis. Molecular modeling study indicated the 3'-hydroxyl group of adenosine is hydrogen-bonded to a His residue (H272) in TM7.⁵ Mutation of the His residue to Ala totally lost the binding ability to the A₃ adenosine receptor⁶, while the mutated A₃ adenosine receptor to Glu showed 20-fold decreased binding affinity to the natural A₃ adenosine receptor agonists such as NECA and CADO.⁴ However, 3'-amino-3'-deoxyadenosine showed 7-fold increased binding affinity to the mutated A₃ adenosine receptor, due to the favorable electrostatic interaction between 3'-amino group and carboxyl anion of Glu of the mutant A₃ adenosine receptor.⁷ In addition to Glu, the His residue (H272) can also be mutated to another negatively charged residue, Asp, giving H272E mutant A₃ adenosine receptor. 3'-Amino-3'-deoxyadenosine showed 6-fold increased binding affinity to this H272E mutant A₃ adenosine receptor, but should be still optimized to show higher binding affinity and selectivity to the H272E mutant A₃ adenosine receptor.⁸ Thus, for the purpose of developing optimal neocceptor-neagonist pair, we have modified the 3'-amino group to the 3'-ureido group, since the 3'-ureido group is able to form much more favorable electrostatic interaction with the Asp residue of H272E mutant A₃ adenosine receptor than 3'-amino group. Herein, we report the synthesis of 3'-ureidoadenosine derivatives and their highly potent and selective agonistic activity at the H272E mutant A₃ adenosine receptor (Figure 1).

RESULTS AND DISCUSSION

3'-Ureidoadenosines 9a,b and 10a,b were synthesized, starting from 1,2:5,6-di-O-isopropylidene-D-glucose (1), as shown in Scheme 1. Compound 1 was treated with triflic anhydride followed by treating of the resulting trflate with sodium azide afforded the azido derivative 2. Manipulation of 5,6-isopropylidene and 1,2-isopropylidene of 2 yielded the glycol donor 3 in good yield. Condensation of 3 with 6-chloropurine and 2,6-dichloropurine in the presence of TMSOTf as a Lewis acid catalyst afforded 6-chloropurine derivative 4a and 2,6-dichloropurine derivative 4b, respectively. 6-Chloropurine derivative 4a was treated with methylamine and 3-iodobenzylamine to give the N⁶-substituted nucleosides, in which the acetyl protecting groups were removed in the process and replaced with TBS ethers to give 5a and 6a, respectively, because of the facile migration of a 2'-acetyl group to the 3'-ureido group. Reduction of azido group of 5a and 6a using triphenylphosphine and ammonium hydroxide followed by conversion of the resulting amino derivatives to the 3'-ureido derivatives gave 7a and 8a, respectively.
CONCLUSION

In order to develop selective neotceptor-neoagonist pair, we have modified the 3'-amino group to the 3'-ureido group, which might form more favorable electrostatic interaction only at the H272E mutant A3 AR, not at the WT A3 AR. Among compounds tested, 2-chloro-3'-ureido-N<sup>6</sup>-
(3-iodobenzyl)adenosine (10b) showed the best binding affinity ($K_i = 0.20 \mu M$) at the H272E mutant A3 AR, while, 3'-ureido-N<sup>6</sup>-(3-iodobenzyl)adenosine (10a) showed the best selectivity (> 100 fold affinity enhancement).

REFERENCES


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