Development of A_3 Adenosine Receptor Ligands

Lak Shin Jeong

Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

ABSTRACT

4′-Thioadenosines have been discovered as novel templates for A_3 adenosine ligands. Among these, 4′-thioadenosine-5′-monoalkyluronamides were discovered as novel potent and selective A_3 adenosine receptor agonists, while 4′-thioadenosine-5′-dialkyluronamides and truncated 4′-thioadenosine derivatives exhibited potent and selective antagonism at the A_3 adenosine receptor.

INTRODUCTION

Adenosine regulates cell signaling through binding to four subtypes (A_1, A_2A, A_2B, and A_3) of adenosine receptors (ARs). Among these, A_3 AR has been the most recently identified and good therapeutic targets for the treatment of cancer, ischemia, asthma, and inflammation. On the basis of the structure of natural messenger, adenosine, a number of adenosine analogues have been synthesized as adenosine ligands. Because of the structural similarity to adenosine, most of adenosine analogues have been identified as A_3 AR agonists, whereas most of A_3 AR antagonists possess nonpurine heterocyclic skeleton, but these showed much lower binding affinity at the rat A_3 AR than that at the human A_3 AR, making them unsuitable for animal study for drug development. Thus, it is highly desirable to discover a new template showing species-independent binding affinity at the A_3 AR. Recently, a few nucleoside analogues were reported to be species-independent A_3 AR antagonists. Thus, it is of interest to develop A_3 AR antagonists with a nucleoside skeleton. Herein, we report the development of nucleoside analogues showing potent and selective A_3 AR agonism and antagonism.

RESULTS AND DISCUSSION

2-Chloro-N^6-(3-iodobenzyl)-5′-N-methylcarboxamido adenosine (CI-IB-MECA) is a potent and selective A_3 AR agonist (K_I for A_3 AR = 1.0 nM). On the basis of biosistereic rationale, the corresponding 4′-thio analogue, thio-CI-IB-MECA (I, R_1 = 3-iodobenzyl, R_2 = Me, X = Cl) was synthesized and found to exhibit higher binding affinity (K_I for A_3 AR = 0.38 nM) at the human A_3 AR than CI-IB-MECA (Fig. 1). Molecular modeling study indicated that the binding mode of thio-CI-IB-MECA in the binding site of A_3 AR is almost similar to that CI-IB-MECA except sulfur atom not forming a hydrogen bonding unlike ring oxygen.

Thio-CI-IB-MECA inhibited the growth of the cancer cells (HL-60 and A549) in vitro which resulted from cell cycle arrest as well as the inhibition of Wnt signaling pathway. This compound also exhibited potent in vivo antitumor activity against colon cancer and lung cancer without apparent cytotoxicity. These findings guarantee that this compound might be developed as a good candidate for new anticancer agents. Because nucleoside analogues were reported to be developed as species-independent A_3 AR antagonists, our next goal was to convert the agonistic activity of 4′-thionucleoside analogues into the antagonistic activity. Molecular modelling study indicated that the hydrogen of 5′-methyuronamide of thio-CI-IB-MECA was essential for a hydrogen bonding in the binding site of A_3 AR, which led to the conformational change of the receptor required for the receptor activation. Thus, it was thought that removal of the amide hydrogen in thio-CI-IB-MECA may convert the pure A_3 AR agonist to the pure A_3 AR antagonist. On the basis of this rationale, 5′-N,N-dialkyluronamides derivatives of thio-CI-IB-MECA were synthesized and evaluated for their A_3 AR antagonistic activity. From this study, 5′-N,N-dimethyluronamide derivatives exhibited higher binding affinity than larger 5′-N,N-dialkyl or 5′-N,N-
cycloalkylamide derivatives, indicating that steric factors are crucial in binding to human A3 AR, among which compound 2 (R1 = 3-iodobenzyl, R2 = R3 = Me) exhibited very high binding affinity (Kd for A3 AR = 15.5 nM) at the human A3 AR with very low binding affinities to other AR subtypes. The functional efficacy of compound 2 was determined by inhibition of forskolin-stimulated cAMP production in AR-transfected CHO cells and measured at a concentration of 10 μM, in comparison to the maximal effect of a full agonist, N6-(4-amino-3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (NECA) at 10 μM. In this functional assay, A3 AR agonism was absent in the compound 2, indicating that it is a pure A3 AR antagonist. To probe species differences, the affinity of compound 2 was also measured at the rat A3 AR. Although the affinity decreased with respect to the affinity at the human A3 AR, this compound showed moderate affinity (Kd = 321 ±74 nM) at the rat A3 AR.

Next approach to develop better A3 AR antagonists than compound 2 was to delete the 5′-uronamide group in thio-Cl-IB-MECA because the amide hydrogen was essential for the receptor activation and the 5′-uronamide group was sensitive to the steric effects. Thus, compounds 3 with various N6-substituents were synthesized and evaluated for their A3 AR antagonistic activity.12 As expected, most of the synthesized compounds exhibited high binding affinity at the hA3 AR with high selectivity over other subtypes, A1 and A2A ARs. Among compounds tested, compounds 3a (R = 3-chlorobenzyl; Kd = 1.66 nM) and 3b (R = 3-iodobenzyl; Kd = 4.16 nM) showed very high binding affinity at the hA3 AR with high selectivities to A1 and A2A ARs. Compound 3b also bound with high affinity at the rat A3 AR expressed in CHO cells (Kd = 3.89 nM), showing species-independent binding affinity and was inactive as agonist or antagonist at other subtypes.

CONCLUSION

We have discovered 4′-thioadenosine-5′-monoaalkyluronamide derivatives as potent and selective A3 AR agonists, which are in the biosisosteric relationships with the corresponding 4′-oxonucleosides. Among these, thio-Cl-IB-MECA showed potent in vitro and in vivo antitumor activity, resulting from the cell cycle arrest and the inhibition of Wnt signaling pathway. We have also successfully converted the 4′-nucleosides acting as A3 AR agonists into the A3 AR antagonists by removing the amide hydrogen of the agonist, thio-Cl-IB-MECA or by deleting 5′-uronamide of thio-Cl-IB-MECA. These antagonists were found to show species-independent binding affinity at the A3 AR. It is no doubt that 4′-thionucleosides studied here will serve as excellent templates for the development of A3 AR ligands.

REFERENCES

10. Unpublished results

*Corresponding Author. E-mail: lakjeong@ewha.ac.kr