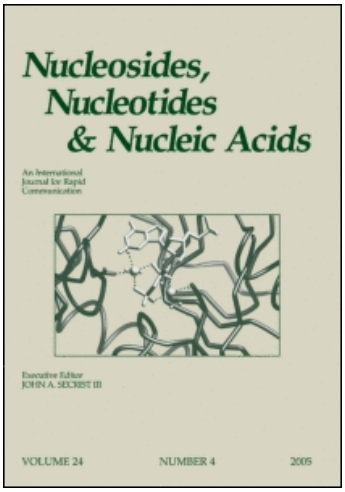


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# 2-Phenylhydroxypropynyladenosine Derivatives as High Potent Agonists at $A_{2B}$ Adenosine Receptor Subtype

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## ABSTRACT

Adenosine derivatives bearing in 2-position the (*R*,*S*)- phenylhydroxypropynyl chain were evaluated for their potency at human $A_{2B}$ adenosine receptor, stably transfected on CHO cells, on the basis that (*R*,*S*)-2-phenylhydroxy-propynyl-5′-N-ethylcarboxyamidoadenosine [(*R*,*S*)-PHPNECA] was found to be a good agonist at the $A_{2B}$ receptor subtype. Biological studies demonstrated that the presence of small alkyl groups in $N^6$-position of these molecules are well tolerated, whereas large groups abolished $A_{2B}$ potency. On the other hand, the presence of an ethyl group in the 4′-carboxamido function seems to be optimal, the (*S*)-PHPNECA resulting the most potent agonist at $A_{2B}$ receptor reported so far.

## INTRODUCTION

Most actions of adenosine are mediated by four extracellular receptors termed $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ on the basis of biological experiments and receptor cloning.[1] Over the last few years many efforts have been directed toward discovery of potent

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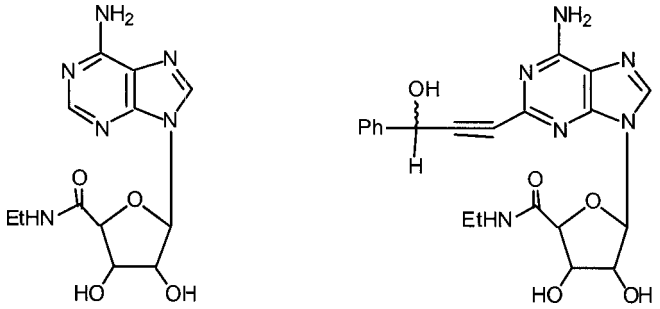
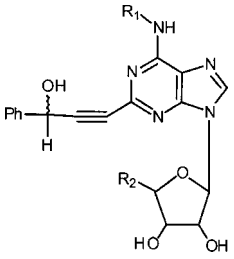



***Figure 1.***

and selective adenosine agonists for $A_1$, $A_{2A}$, and $A_3$ receptors.[2] On the other hand, although a large number of adenosine derivatives has been tested at the adenosine $A_{2B}$ receptor, no potent and selective agonists are currently available and adenosine-5′-N-ethyluronamide (NECA, **1**, Fig. 1) resulted one of the most potent.[3]

Some years ago we synthesized a number of NECA derivatives substituted at the C-2-position with various (ar)alkynyl chains.[4] Among them (*R*,*S*)-2-phenylhydroxypropynyl-5′-*N*-ethylcarboxyamidoadenosine [**2**, (*R*,*S*)-PHPNECA] was found to be highly potent at all the human adenosine receptor subtypes ($K_iA_1 = 0.0027\,\mu M$; $K_iA_{2A} = 0.0031\,\mu M$; $EC_{50}$ $A_{2B} = 1.1\,\mu M$; $K_iA_3 = 0.0004\,\mu M$)[5] and slightly more potent than NECA ($EC_{50}A_{2B} = 2.4\,\mu M$) at the $A_{2B}$ receptor in the adenylate cyclase activity assay.[6] Resolution of the racemic mixture showed that the (*S*)-PHPNECA (**3**, $EC_{50}A_{2B} = 0.22\,\mu M$) was more active than the (*R*)-PHPNECA (**4**, $EC_{50}A_{2B} = 2.4\,\mu M$), resulting one of the most potent agonist at the $A_{2B}$ receptor reported so far.[7,8] Introduction of large substituents (i.e., arylcarbamoyl groups, **5** and **6**) in $N^6$-position of PHPNECA abolished $A_{2B}$ activity as reported in Table 1.[9]

***Table 1.*** Activity of PHPAdo derivatives at human $A_{2B}$ receptor ($EC_{50}$: μM).

| | Compound | $R_1$ | $R_2$ | $A_{2B}$ |
|---|---|---|---|---|
|  | **1** (NECA) | | | 2.4 |
| | **2** (*R*,*S*)-PHPNECA | H | CONHEt | 1.1 |
| | **3** (*S*)-PHPNECA | H | CONHEt | 0.22 |
| | **4** (*R*)-PHPNECA | H | CONHEt | 2.4 |
| | **5** | 4-MeO-Ph-NHCO | CONHEt | >100 |
| | **6** | 3-Cl-Ph-NHCO | CONHEt | >100 |
| | **7** (*R*,*S*)-PHPAdo | H | $CH_2OH$ | 2.4 |
| | **8** | $CH_3$ | $CH_2OH$ | 2.7 |
| | **9** | $CH_2CH_3$ | $CH_2OH$ | 1.7 |
| | **10** | $CH(CH_3)_2$ | $CH_2OH$ | 6.1 |
| | **11** | H | CONHMe | 5.0 |
| | **12** | H | CONHPr | >100 |





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On the other hand, replacement of the ethylcarboxamido group of **2** with an hydroxymethyl group led to (*R*,*S*)-2-phenylhydroxypropynyladenosine [**7**, (*R*,*S*)-PHPAdo], which maintained good potency at $A_{2B}$ receptor subtype ($EC_{50}A_{2B} = 2.4\,\mu M$).

These observations prompted us to investigate the effect of small groups (methyl, ethyl, and isopropyl) in the $N^6$-position of PHPAdo (compounds **8**, **9**, **10**). Functional study demonstrated that small substituent are well tolerated, the (*R*,*S*)-$N^6$-ethyl-PHPAdo (**6**: $EC_{50}A_{2B} = 1.7\,\mu M$) being more potent than (*R*,*S*)-PHPAdo itself.

In addition, the influence of different length chains in the ethylcarboxamido group of PHPNECA was investigated through the synthesis of the corresponding methyl- and propylcarboxamido analogues **11** and **12**, respectively. (The synthesis will be published elsewhere.)

Adenylyl cyclase activity assay showed that the presence at the 4′-position of an ethylcarboxamido function, as in **2**, seemed to be optimal; in fact, when the ethyl was replaced by a methyl group, the resulting compound showed decreased potency (**11**: $EC_{50}A_{2B} = 5.0\,\mu M$ vs. **2**: $EC_{50}A_{2B} = 1.1\,\mu M$), whereas the presence of a propyl group abolished $A_{2B}$ activity (**12**: $EC_{50}A_{2B} > 100\,\mu M$).

Some of the compounds above reported were synthesized as pure (*R*) and (*S*) diastereomers, and in all cases the (*S*) diastereomer resulted to be from 7 to 40 fold more potent than the (*R*) one at the $A_{2B}$ receptor subtype.

## CONCLUSION

With these studies we demonstrated that the introduction of the phenylhydroxypropynyl chain in 2-position of adenosine derivatives led to compounds endowed with high activity at the human $A_{2B}$ adenosine receptor, the (*S*)-diastereomer of PHPNECA resulting the most potent $A_{2B}$ agonist reported so far.

Since the introduction of an ethyl group on $N^6$-position of PHPAdo increased $A_{2B}$ potency with respect to the PHPAdo itself, the combination of a 4′-ethylcarboxamido group and an ethyl group in $N^6$-position of adenosine could give an $A_{2B}$ agonist with improved potency.

## ACKNOWLEDGMENTS

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