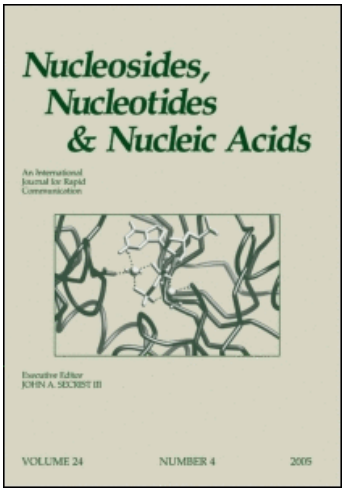

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Synthesis of 2-Chloro-6-aryloxy- and 2-Chloro-6-alkoxyarylpurines and Their Properties in the Purine Nucleoside Phosphorylase (PNP) System

A. Bzowska[a]; L. Magnowska[a]; B. Wielgus-kutrowska[a]; Z. Kazimierczuk[a]
[a] Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland

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SYNTHESIS OF 2-CHLORO-6-ARYLOXY- AND 2-CHLORO-6-ALKOXYARYLPURINES AND THEIR PROPERTIES IN THE PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) SYSTEM.

A. Bzowska*, L. Magnowska, B. Wielgus-Kutrowska and Z. Kazimierczuk

Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Żwirki i Wigury 93, 02 089 Warsaw, Poland

ABSTRACT: A series of 2-chloro-6-aryloxy- and 2-chloro-6-alkoxyarylpurines was synthesized and their kinetic properties in the purine nucleoside phosphorylase (PNP) system were determined. All compounds showed inhibitory activity (IC_{50} in the range 0.5-76 μM) *vs.* hexameric ("high-molecular weight") PNP from *E. coli*. By contrast, no inhibition *vs.* trimeric *Cellulomonas* PNP was detected.

The ubiquitous enzyme purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1.) catalyzes the reversible phosphorolysis of purine nucleosides, as follows: β-purine nucleoside + orthophosphate ⇔ purine base + α-D-pentose-1-phosphate. "High-molecular weight" hexameric enzymes, found in some bacteria (e.g. *E. coli*), have broad specificity towards nucleosides[1,2], while "low-molecular weight" trimeric, mainly mammalian, PNPs are specific for 6-oxopurine nucleosides[3]. Recently we have observed that 2-chloro-6-benzyloxy-9-(2'-deoxyribofuranosyl)purine is a selective and very good substrate of *E. coli* PNP, and one of the most potent competitive inhibitors of inosine phosphorolysis with inhibition constant 0.5 μM[4]. Therefore we decided to synthesize some purine analogues of this nucleoside, namely a series of 2-chloro-6-aryloxy- and 2-chloro-6-alkoxyarylpurines, and to investigate their kinetic properties in the PNP system.

The phase transfer method was applied to perform the nucleophilic substitution of 2,6-dichloropurines by modified alkylaryl alcohols or phenols. Since in these conditions only the 6-halogen is exchanged, this method produces 2-chloro-6-aryloxy- and 2-chloro-6-alkoxyarylpurines. 2-Chloro-6-benzylthiopurine was synthesized by alkylation of 2-

chloro-6-thiopurine with benzyl bromide. The stereoisomers of 2-chloro-6-O-(1-phenylethyl-1-)purine were obtained from R- and S-enantiomers of sec. phenylethylalcohol and 2,6-dichloropurine. All analogues were characterized by elemental analysis, TLC chromatography, melting points, UV and NMR spectra.

All purine derivatives were tested as inhibitors of purified *E. coli* PNP by their effect on phosphorolysis of 7-methylguanosine[5] in the presence of 50 mM phosphate buffer, pH 7, at 25°C. The most potent inhibition was observed for 2-chloro-6-benzylthio-, 2-chloro-6-benzyloxy-, 2-chloro-6-O-(2-phenylethyl-1-) and 2-chloro-6-O-(3-phenylpropyl-1-)purines (IC_{50} = 0.5, 0.8, 1.0 and 1.1 μM, respectively). The R-stereoisomer of 2-chloro-6-O-(1-phenylethyl-1-)purine has IC_{50} = 3.2 μM, while inhibition of its S counterpart is rather weak (IC_{50} > 12 μM). 2-Chloropurines with more rigid (phenoxy) or non-planar (cyclohexyloxy) 6-substituent have IC_{50} = 26 and 76 μM, respectively. By contrast, none of the above mentioned 2-chloropurine derivatives showed inhibitory activity *vs.* trimeric *Cellulomonas* PNP. These results are in line with the known properties of the two bacterial enzymes, and they confirm the fundamental difference in their specificity *vs.* 2- and 6-substituted purines and purine nucleosides. 2-Chloro, as well as 6-aryloxy- and 6-alkoxyaryl substitutions enhance affinity to *E. coli* PNP, while *Cellulomonas* enzyme does not bind such modified analogues. Thus in this respect it resembles "low-molecular weight" mammalian phosphorylases for which positions 2 and 6 of the purine base are involved in the specific hydrogen bond interactions[6]. (Supported by Polish Committee for Scientific Research, KBN grant 4 P05F 027 12).

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