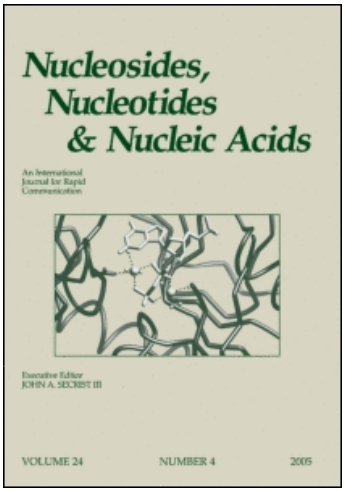


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# Inhibition of Purine Nucleoside Phosphorylase by Phosphonoalkylpurines [1]

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# INHIBITION OF PURINE NUCLEOSIDE PHOSPHORYLASE BY PHOSPHONOALKYLPURINES[1]

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Abstract. Optimum inhibition of human erythrocyte purine nucleoside phosphorylase by 9-(phosphonoalkyl)hypoxanthines required an alkyl chain of five carbons or longer. Appropriate modifications of either the base or phosphonate side chain resulted in increased inhibitory activity.

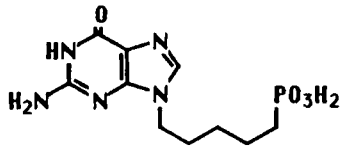


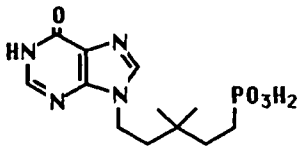
Purine nucleoside phosphorylase (PNP) is a key enzyme in the purine salvage pathway [2, 3]. This enzyme catalyzes the reversible phosphorolysis of guanosine and inosine nucleosides (or deoxynucleosides) to their respective free base and ribose 1-phosphate (or deoxyribose 1-phosphate). Recently, we and others have probed the structure of the active site using "multisubstrate" analog inhibitors containing a purine and a phosphate-like moiety [4-6]. These compounds include the most potent inhibitors of PNP known to date. The study reported here was undertaken with the hope of developing more potent inhibitors of PNP by exploring the geometry of the active site between the purine and phosphate binding domains.

The inhibition of human erythrocyte PNP by phosphonoalkylpurines was investigated[7]. For each phosphonate tested, inhibition was competitive with respect to the substrate inosine and dependent on phosphate ($P_i$) concentration, inhibition decreasing with increasing [$P_i$]. Thus, the observed inhibition was qualitatively identical to inhibition by structurally similar phosphonate and phosphate compounds for which it has been proposed that the inhibitors occupy both the purine and phosphate binding sites on the enzyme. The apparent inhibition constant ($K_i'$, determined vs. inosine with $P_i$ fixed at 1 mM) of 9-(4-phosphonobutyl)hypoxanthine was intermediate between those of the previously reported analogous propyl and pentyl compounds, the $K_i'$ values being 65, 2700, and 1.1 μM, respectively. The efficacy of this homologous class of inhibitors increases with increasing side chain length up to a length of five after which further advantage is not gained.

Appropriate modifications of either the base or phosphonate side chain lead to increased inhibitory activity. Inhibition by 9-(5-phosphonopentyl)guanine (**1**), $K_i'$ =

0.17 μM, was found to be 6-fold greater than that of the hypoxanthine analog- this reflects the increased affinity of PNP for guanine. The $K_i'$ value of

(1) 

(2) 

9-(3,3-dimethyl-5-phosphonopentyl)hypoxanthine (2) was determined to be 0.21 μM. The $K_i$'s of 9-(3,3-dimethylpentyl)hypoxanthine and 9-pentylhypoxanthine were determined to be 110 and 52 μM, respectively. The effect of adding a phosphono moiety to 9-alkylhypoxanthines shows a large dependence on the parent compound. The addition of the phosphono moiety to 9-propylhypoxanthine decreases the inhibitory activity 5-fold[5]. In contrast, the addition of the phosphono moiety to the 9-(3,3-dimethylpentyl)hypoxanthine results in a 520-fold increase in inhibitory activity while the addition of the phosphono group to 9-pentylhypoxanthine results in a 47-fold increase. Thus, the 5-fold greater inhibitory activity of 9-(3,3-dimethyl-5-phosphonopenty)hypoxanthine relative to that of 9-(5-phosphonopentyl)-hypoxanthine reflects increased utilization of binding interactions between PNP and the phosphono group rather than increased binding due to direct interactions of the enzyme with the additional methyl groups.

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7 Satisfactory NMR (400 MHz) and elemental analysis were obtained for the new compounds reported here; the details will be published elsewhere. Other experimental detail can be found in reference 5.