Nucleotide Analogue Inhibitors of Purine Nucleoside Phosphorylase*

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The diphosphate of the antiviral agent acyclovir [9-[(2-hydroxyethoxy)methyl]guanine] has been shown to inhibit purine nucleoside phosphorylase with unique potency (Tuttle, J. V., and Krenitsky, T. A. (1984) J. Biol. Chem. 259, 4065-4069). A major factor contributing to the superior inhibition by this diphosphate over the corresponding mono- and triphosphates is revealed here. Homologues of acyclovir mono- and diphosphate that extend the ethoxy moiety by one to four methylene groups were synthesized. These homologues were evaluated for their ability to inhibit human purine nucleoside phosphorylase. Within the diphosphate series, the $K_i$ values increased progressively with increasing chain length. With the monophosphates, the $K_i$ values reached a minimum with the homologue containing a pentoxy moiety. A plot of chain length versus $K_i$ values for both mono- and diphosphates showed that both series had similar optimal distances between the aminal carbon and the terminal oxygen anion. Monophosphates with optimal positioning were somewhat less potent than diphosphates with similar positioning. Nevertheless, it was clear that a major factor in determining potency of inhibition was the distance of the terminal phosphate from the guanine moiety.

Considerable effort has been directed to the design and synthesis of inhibitors of purine nucleoside phosphorylase (PNPase; EC 2.4.2.1; purine nucleoside:orthophosphate ribosyl transferase). The initial impetus for this effort came from clinical research. Patients genetically lacking this enzyme were found to have deficiencies in cellular, but not humoral, immunity (1). This condition results in a cytotoxic accumulation of dGTP in T-lymphocytes (2-4), which is generally accepted as the molecular basis for the impairment of cellular immunity in vivo. Consequently, it has been proposed that an in vivo inhibitor of PNPase should result in suppression of cellular, but not humoral, immunity. This, of course, has the potential for being clinically useful. Indeed, rats treated with the PNPase inhibitor 8-aminoavosine showed significant decreases in thymus cells and in lymph node and spleen lymphocytes (5). Dogs similarly treated had prolongation of skin graft survival (6). Other potential uses for inhibitors of PNPase are to treat hyperuricemia or to prevent the rapid clearance of acyclovir triphosphate (7). Unexpectedly, the diphosphate was three and two orders of magnitude more potent than the monophosphate and triphosphate, respectively. Prior to the study presented here, no explanation of this large preference for the diphosphate has been available.

**EXPERIMENTAL PROCEDURES**

**RESULTS AND DISCUSSION**

Acyclovir (1a) and its homologues that had extensions of the ethoxy moiety by one to four methylene groups (2-5a) were all weak inhibitors of PNPase (Table I). As previously shown with acyclovir (7), the inhibitory potency of its homologues increased markedly upon phosphorylation (Table I). Inhibition by the monophosphates (1-5b) was optimal with the homologue containing a pentoxy moiety (4b). With the diphosphates (1-5c), inhibition decreased with increasing chain length. Acyclovir diphosphate (1c) was most potent and its extended homologues lost potency with increasing chain length.

The log of the inhibition constants were plotted against the interatomic distance between the aminal carbon (methylene adjacent to the 9-position of the guanine moiety) and the terminal oxygen anion. Acyclovir diphosphate (1c) was most potent and its extended homologues lost potency with increasing chain length.

The data in Table I (more easily seen in Fig. 1) provide an explanation for the superiority of the diphosphate of acyclovir over its mono- and triphosphates as an inhibitor of PNPase. The monophosphate of acyclovir (1b), the distance to the terminal oxygen anion was clearly below the optimal range. The terminal anion of acyclovir diphosphate (1c) was within the optimal range, whereas that of acyclovir triphosphate (1d) was beyond this range.

Although these results indicate that positioning of the terminal phosphate is the dominant factor in the potency of PNPase inhibitors of this type, there is some preference for diphosphates versus monophosphates. For example, the monophosphate of the acyclovir homologue with two additional methylene groups (3b) has a terminal anion position similar to that of acyclovir diphosphate (1c), yet the diphosphate is 6-fold more potent. Similarly with the analogous mono- and diphosphate pair (4b versus 2c), the diphosphate is 2.5-fold more potent. Furthermore, the terminal anion of acyclovir triphosphate is similarly positioned to the acyclovir diphosphate homologue with two additional methylene groups (1d versus 3c), yet the diphosphate is 4-fold more potent. These comparisons indicate that although the position of the terminal phosphate is a dominant factor, there is a relatively

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1 Portions on this paper (including "Experimental Procedures" and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
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small but clear preference for diphosphates over monophosphate or triphosphates.

Nakamura et al. (23) have studied a series of 9-(phosphonooalkyl) hypoxanthines as inhibitors of PNPase. They found that 9-(5-phosphonopentyl) hypoxanthine had a $K_i$ value similar to that of acyclovir monophosphate. However, they found that the corresponding hexyl and heptyl analogues had $K_i$ values similar to that of 9-(5-phosphonopentyl) hypoxanthine. These results are not easily reconciled with the data in Table I, since it would be expected that the longer chain lengths would dramatically increase inhibition. The question of which structural differences between these two series of inhibitors are responsible for the difference in phosphate position effects is worthy of further study.

Nucleotides have a very limited permeability to mammalian cells (24, 25). Furthermore, they are known to have very short half-lives when administered intravenously to humans, presumably due to very active phosphatase activities present within the vasculature (26, 27). Consequently, there is little hope that the nucleotide analogue inhibitors described here would have any activity in vivo. Nevertheless, the demonstration of the optimal spacing of the terminal phosphate relative to the guanine moiety should prove valuable to the design of inhibitors with more favorable pharmacokinetic properties.

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REFERENCES

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**Supplementary Material Page**

**Nucleoside Analogues Inhibitors of Purine Nucleoside Phosphorylase**

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**EXPERIMENTAL PROCEDURES**

Synthetic procedures and sources of materials not described herein were as previously described (7).

Enzyme-MPase was purified from human erythrocytes by the procedure of Knevelsby et al. (8). MPase and zymase oxidase were dissolved before use (7). The desired enzyme preparations were stable for at least 6 months when stored at -70°C in 0.1 M Tris-HCl buffer, pH 7.5. Pseudo-enzymes were assayed spectrophotometrically as previously described (7). In addition to MPase and nitrotyrosine, the reaction mixtures contained 100 mM Tris-HCl buffer (pH 7.5), 1 M potassium phosphate, and 0.04% (v/v) of acetic acid.

K values were determined with l-threonine as the variable substrate in the presence and absence of a single concentration of inhibitor. A minimum of six concentrations of inhibitor, ranging from 5 to 100 μg/mL, was used. The data were analyzed using the computer program of Flanagan (9). The kinetic data for all inhibitors were consistent with competitive inhibition.

**Synthesis and Characterization of Inhibitors**

The compounds studied are numbered and schematically represented in Table 1. The first letter designates the number of methylene groups between seven atoms greater than one in the B-substituent, where with acyl and aminyl groups. The letter designates the state of phosphorystoryls for example, phosphorystoryls nucleoside analogues, for dipeptides, for diprophosphates, for tripeptides.

**Table 1**

<table>
<thead>
<tr>
<th>Inhibitor constant of human purine nucleoside phosphorylase with acylaloxy, its homologues, and their corresponding nucleosides</th>
</tr>
</thead>
</table>

**Figure 1**

![Diagram of a chemical structure](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Compound No.</th>
<th>K1 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1</td>
<td>104.8</td>
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<tr>
<td>-P03H2</td>
<td>2a</td>
<td>6.48</td>
</tr>
<tr>
<td>-P03H2-P03H2</td>
<td>2b</td>
<td>0.0398</td>
</tr>
<tr>
<td>-P03H2-P03H2-P03H2</td>
<td>2c</td>
<td>0.313</td>
</tr>
<tr>
<td>-P03H2-P03H2</td>
<td>2d</td>
<td>0.0015</td>
</tr>
<tr>
<td>3</td>
<td>2e</td>
<td>0.171</td>
</tr>
<tr>
<td>4</td>
<td>2f</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Figure 2**

![Diagram of a chemical structure](image)

**Figure 3**

![Diagram of a chemical structure](image)
Inhibitors of Purine Nucleoside Phosphorylase

9-[[2-Benzoylpropoxy)methyl]guanidine (intermediate to 2a)

The procedure for the penta-4-carboxylic described above was followed. The yield was 21% after recrystallization from MeOH: mp 380-381°C; IR (KBr) ν max 3450, 1645, 1590, 1385, 760 cm⁻¹; ¹H NMR (CDCl₃) δ 9.45 (s, 1H, NH), 8.28 (d, 1H, H-6); 7.66 (d, 1H, H-5); 7.40 (d, 1H, H-3); 7.24 (m, 2H, H-2 and H-6); 7.10 (m, 1H, H-4); 6.91 (s, 1H, H-4); 6.89 (s, 1H, H-5); 6.74 (s, 1H, H-2); 6.53 (m, 1H, H-3); 6.31 (m, 1H, H-4); 5.58 (s, 1H, H-5); 4.83 (m, 1H, H-4); 3.93 (m, 1H, H-4); 3.14 (m, 1H, H-4); 2.15 (t, 2H, H-4); 1.72 (m, 1H, H-4); 1.10 (m, 1H, H-4); 0.92 (m, 1H, H-4).}

9-[[2-Hydroxypropoxy)methyl]guanidine (2a)

The procedure for 1(6) as described above was followed. The yield was 37% after recrystallization once from CH₂Cl₂ and once from isopropanol: m.p. 72-74°C with recrystallization. IR (KBr) ν max 3450, 1650, 1590, 1385, 760 cm⁻¹; ¹H NMR (CDCl₃) δ 9.40 (s, 1H, NH), 8.28 (d, 1H, H-6); 7.66 (d, 1H, H-5); 7.40 (d, 1H, H-3); 7.25 (m, 2H, H-2 and H-6); 7.10 (m, 1H, H-4); 6.91 (s, 1H, H-4); 6.89 (s, 1H, H-5); 6.74 (s, 1H, H-2); 6.53 (m, 1H, H-3); 6.31 (m, 1H, H-4); 5.58 (s, 1H, H-5); 4.83 (m, 1H, H-4); 3.93 (m, 1H, H-4); 3.14 (m, 1H, H-4); 2.15 (t, 2H, H-4); 1.72 (m, 1H, H-4); 1.10 (m, 1H, H-4); 0.92 (m, 1H, H-4); 0.92 (m, 1H, H-4).}

3-[[2-Amino-6-chloro-9H-purin-9-yl]methyl]benzamide (intermediate to 5a)

The procedure used as in the preparation of the [2]-intermediate. The yield on evaporation as a clear oil. IR (KBr) ν max 3450, 1650, 1590, 1385, 760 cm⁻¹; ¹H NMR (CDCl₃) δ 8.28 (d, 1H, H-6); 7.66 (d, 1H, H-5); 7.40 (d, 1H, H-3); 7.25 (m, 2H, H-2 and H-6); 7.10 (m, 1H, H-4); 6.91 (s, 1H, H-4); 6.89 (s, 1H, H-5); 6.74 (s, 1H, H-2); 6.53 (m, 1H, H-3); 6.31 (m, 1H, H-4); 5.58 (s, 1H, H-5); 4.83 (m, 1H, H-4); 3.93 (m, 1H, H-4); 3.14 (m, 1H, H-4); 2.15 (t, 2H, H-4); 1.72 (m, 1H, H-4); 1.10 (m, 1H, H-4); 0.92 (m, 1H, H-4).
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