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Acyclic Ribooxacarbenium Ion Mimics as Transition State Analogues of Human and Malarial Purine Nucleoside Phosphorylases

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Purine nucleoside phosphorylase (PNP) is required in humans for the recycling of deoxyguanosine and in *Plasmodium falciparum* for the essential pathway of purine salvage.[1−4] The genetic deficiency of PNP in humans causes a specific T-cell immune deficiency syndrome as a primary phenotype.[5] This observation has made human PNP a target for the development of therapeutic agents for T-cell leukemia and T-cell related autoimmune diseases. Transition state analysis of human and bovine PNP has been used to develop potent PNP inhibitors. The transition state structure of bovine PNP was derived from kinetic isotope effects and computational chemistry and led to the development of picomolar iminoribitol enzyme inhibitors against both bovine and human PNPs (Figure 1).[6−8] The Immucillins are first generation PNP transition state analogues and include Immucillin-H [**1**] and Immucillin-G [**2**] as the most potent examples, binding 7×10^5 times more tightly than the inosine and guanosine substrates to human PNP (HsPNP). Immucillin-H (Fodosine) is in Phase II clinical trials for relapsed/resistant T-cell leukemia and cutaneous T-cell leukemia.[9] The transition state structure of HsPNP differs from that of bovine PNP by having a fully developed ribooxacarbenium ion at the transition state.[10] Second generation transition state analogues designed specifically for the HsPNP transition state include DADMe-Immucillin-H [**3**] and DADMe-Immucillin-G [**4**] (Figure 1). These inhibitors bind $2-5 \times 10^6$ times tighter to HsPNP than inosine and guanosine.[11] DADMe-Immucillin-H has entered Phase Ib clinical trials for T-cell autoimmune disorders (as BCX-4208).[9]

Protozoan parasites, including *P. falciparum*, salvage purine nucleosides by conversion to hypoxanthine in a pathway requiring the parasite-expressed PNP (PfPNP).[2] Blocking this pathway kills parasites, making PfPNP a target for the design of antimalarials.[3] Transition state analysis for PfPNP has shown a transition state similar to that of HsPNP, but the catalytic efficiency ($k_{enzyme}/k_{nonenzymatic}$) of PfPNP is less than HsPNP by a factor of 20, thereby reducing the binding energy expected from transition state analogues.[10] The first and second generation inhibitors **1**−**4** follow this pattern, despite differences in the catalytic site contacts for human and PfPNPs.

Several acyclic mimics of the ribooxacarbenium ion have been incorporated into the DADMe-Immucillin transition state analogues, and nanomolar dissociation constants have been reported (e.g., **5** and **6**).[12,13] Modest changes in acyclic analogue structure caused relatively large changes in affinity for both human and PfPNPs and also gave large differences in binding affinity between these enzymes. We explored new acyclic analogues to improve affinity and to define altered specificity between inhibition of human and *P. falciparum* PNPs.

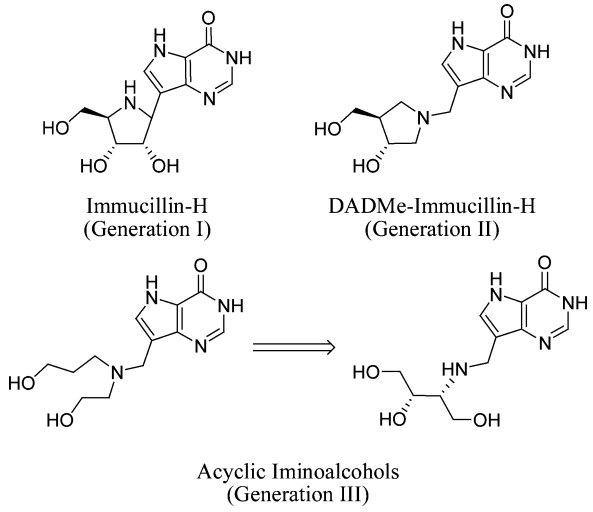

Figure 1. Development of three generations of PNP inhibitors. Immucillin-H and DADMe-Immucillin-H are mimics of bovine and human PNP transition states. The acyclic iminoalcohols provide conformational freedom to permit geometry matching of transition state contacts.

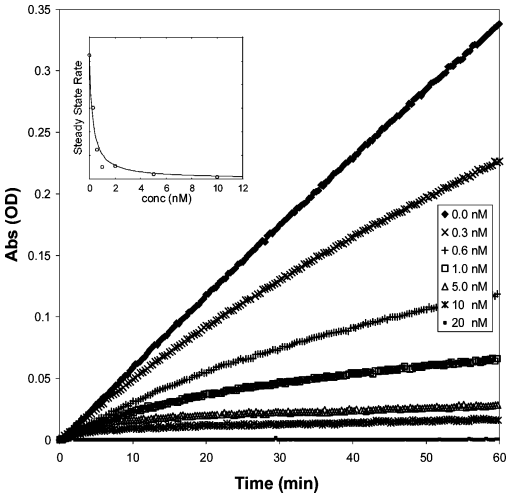

Figure 2. Slow-onset, tight-binding inhibition of **7** by human PNP. The production of hypoxanthine from inosine is monitored by conversion to uric acid in a coupled assay. The concentrations of **7** are indicated. The inset is a plot of rates at 50−60 min with inhibitor, which is used to calculate the K_d value from the equation for competitive inhibition.

Compound **7** is the most potent acyclic ribocationic mimic inhibitor, and its slow-onset inhibition constant is 8.6 pM, the same as that for DADMe-Immucillin-H (8.5 pM) using the same conditions for assay (Figure 2). Binding of transition state analogues is commonly found to be a two-step process, an initial reversible competitive binding followed by a second slow-onset inhibition

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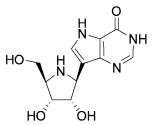
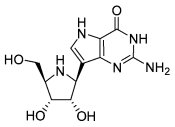
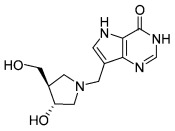
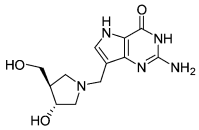
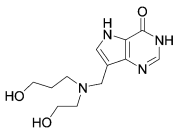
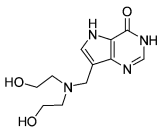
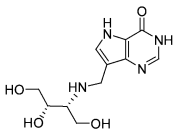
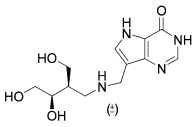
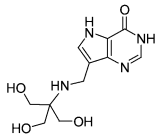
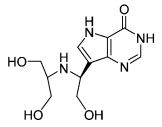
Table 1. Inhibition of both HsPNP and PfPNP[15]

		K_d HsPNP nM	K_d PfPNP nM
1		0.056 ± 0.015[7]	0.86 ± 0.08[12]
2		0.042 ± 0.006[7]	0.90 ± 0.20[12]
3		0.0085 ± 0.0002	0.50 ± 0.04[12]
4		0.007 ± 0.001[11]	0.89 ± 0.06[12]
5		120 ± 8[12]	> 3,000[12]
6		1.3 ± 0.1[12]	170 ± 10[12]
7		0.0086 ± 0.0006	55 ± 12
8		0.78 ± 0.15[14]	104 ± 7[14]
9		0.62 ± 0.17	163 ± 80
10		0.21 ± 0.08	297 ± 99

binding which involves a conformational change leading to enhanced binding. Slow-onset inhibition is apparent for **7** (Figure 2).

Compounds **8**−**10** are also potent inhibitors that bind to HsPNP with dissociation constants of 780,[14] 620, and 210 pM, respectively. Exploration of structures with acyclic ribooxacarbenium ion mimics of DADMe-Immucillin-H has provided a third generation distinct chemical class of powerful picomolar inhibitors for human PNP.

Inhibitors **1**−**4** show 15-, 21-, 59-, and 127-fold preference, respectively, for HsPNP relative to PfPNP, similar to ratios expected based on transition state theory. This relationship differs for third generation inhibitors, and **7**−**10** show 6400-, 133-, 263-, and 1400-fold preference for HsPNP, respectively. These differences between HsPNP and PfPNP affinity reflect a higher degree of specificity for the cyclic ribooxacarbenium ion mimic for PfPNP, except for substitutions at the 5′-hydroxyl group.[12]

The inhibitor design platform for third generation Immucillins is based on the DADMe-Immucillin-H scaffold, with the removal of one carbon−carbon bond to yield the *tert*-N acyclic iminoalcohol inhibitor **5**. Modification of the carbon chain length, by as little as one carbon, as in **6**, resulted in a 100-fold increase in binding affinity. Other modifications using the *tert*-N center led to little increase in affinity (see Supporting Information).

A total of 19 different *tert*-N and *sec*-N di- and trihydroxy compounds were synthesized and tested for their ability to inhibit both HsPNP and PfPNP (Table 1 and Supporting Information). The most potent third generation inhibitors, like their parent compounds, have K_d values in the picomolar range for binding to HsPNP. The common structural element of the four picomolar inhibitors for HsPNP is a *sec*-N and three hydroxyl groups. It is expected that the geometric flexibility of the acyclic, singly bonded amino alcohol groups permits positioning of these three hydroxyl groups in the catalytic site to match those found for **1** and **2**.

An important advance of this work is that acyclic ribooxacarbenium mimics simplify chemical synthesis of these potentially therapeutic molecules. It is also significant that several of the analogues (e.g., **5**, **6**, and **9**) contain no asymmetric carbon centers. In some of these inhibitors, the synthetic precursors are commonly available, thus, **9** is produced from 9-deazahypoxanthine and tris-base as major precursors. These considerations are significant for the ongoing design of antimalarials where cost of goods must be combined with potent inhibitor potential.

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Supporting Information Available: Experimental methods and inhibition data for other third generation inhibitors is provided in the supplement. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Compound **8** is a mixture of enantiomers, and therefore the inhibition value likely reflects an average inhibition value, where one enantiomer is likely to have a smaller dissociation constant.
(15) Dissociation constants reported here are from the literature value with the least error. If no reference is given for a value, it is previously unreported. The values are for K_i* in cases where slow-onset inhibition occurs (see Supporting Information).

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