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Crystallographic Structures of Two Bisphosphonate:1-Deoxyxylulose-5-Phosphate Reductoisomerase Complexes

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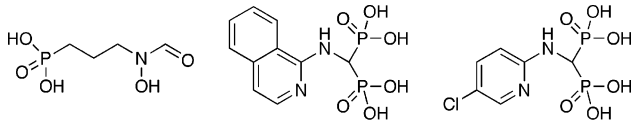
Enzymes involved with the biosynthesis of isoprenoid compounds are important drug targets. For example, the statins which inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase are important as cholesterol-lowering drugs and may also be of use in treating individuals after acute coronary syndromes;[1] bisphosphonates which inhibit the enzyme farnesyl pyrophosphate synthase are important in bone resorption[2] as well as having both antiparasitic[3] and anticancer[4] activity, while the azoles which inhibit ergosterol biosynthesis are potent antifungals.[5] There are several routes to the production of isoprenoids, including the mevalonate pathway,[6] the mevalonate-independent or methylerythritol phosphate (MEP) pathway,[7] as well as less common pathways involving leucine catabolism[8] and the pentose phosphate pathway.[9] The MEP pathway is of particular importance in many pathogenic bacteria;[10] in addition, it is the pathway used in the protozoan parasite *Plasmodium falciparum*,[11] a causative agent of malaria, responsible for ~500 million cases annually with ~1−3 million deaths.[12]

Recently, it has been shown[13] that the antibiotic, fosmidomycin (**1**):



1 **2** **3**

is a potent inhibitor of the MEP pathway enzyme deoxyxylulose-5-phosphate reductoisomerase (2-*C*-methyl-D-erythritol-4-phosphate synthase, IspC) in bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*[14] and in plants[15] as well as in *P. falciparum*;[11] in combination with clindamycin, **1** has provided parasitological cures of uncomplicated falciparum malaria.[16] These results show that DXR is a valid drug target. In other work, we recently found that a variety of bisphosphonates inhibited the growth of *P. falciparum* both in vitro and in vivo, with the best growth inhibitors having IC_{50} values of ~1 μM.[17] We thus considered the possibility that DXR might also be inhibited by bisphosphonates. A small library of bisphosphonates were screened in an in vitro *E. coli* DXR assay[11] and two compounds: [(1-isoquinolinylamino)methylene]-1,1-bisphosphonate (**2**) and [[(5-chloro-2-pyridinyl)amino]methylene]-1,1-bisphosphonate (**3**), having IC_{50} values of ~4 and ~7 μM, were selected for crystallographic investigation. While these inhibitors are less potent than fosmidomycin ($IC_{50} \approx 0.035$ μM), the results of a screen of 32,000 compounds against DXR[18] revealed only 30 other compounds having IC_{50} values of <20 μM, making **2** and **3** potentially interesting as new drug leads. Hexa-His tagged *E. coli* DXR crystals ex $(NH_4)_2SO_4$[19] were soaked with either **2** or **3**, and diffraction data were collected to 2.3 Å for **2** at the BL38B1 beamline at SPring-8 and to 2.4 Å for **3** at beamline NW12 at the Photon Factory. Full crystallographic details are provided in Table 1.

Table 1. Data Collection and Refinement Statistics

crystal	1T1R	1T1S
space group	$P2_12_12$	$P2_12_12$
cell index		
a (Å)	182.4	182.9
b (Å)	59.0	59.2
c (Å)	87.0	87.0
wavelength (Å)	1.000	1.000
resolution (Å)	2.3	2.4
observed reflections	477,586	428,587
unique reflections	37,069	37,835
completeness (%)[a]	97.2 (85.1)[a]	100.0 (100.0)
R_{sym}[a,b]	0.077 (0.55)	0.088 (0.37)
I/σ[a]	14.7 (2.6)	13.4 (4.0)
R_{work}/R_{free}	21.8/27.0	21.3/26.0
rmsd from ideal geometry		
bonds (Å)	0.006	0.006
angles (deg)	1.2	1.2

[a] Values listed in parentheses are for the highest-resolution shell. [b] $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where I is the observed intensity of a reflection and $\langle I\rangle$ the averaged intensity of multiple observations of the reflection and its symmetry partners. [c] $R_{factor} = \sum||F_oR| - |F_c||/\sum F_o|$, where F_o and F_c are the observed and calculated structure factors, respectively. R_{free} was calculated with 10% of the reflections set aside randomly throughout the refinement.

We show in Figure 1 the structure of **3** complexed to DXR and in Figure S1 (Supporting Information) the structure of **2** bound to DXR. In both cases, the molecules crystallize as homodimers, with each subunit binding one bisphosphophosphonate, shown in Figure 1. The bisphosphonates dock into the same site as does the hydroxamate moiety of fosmidomycin.[20] The fosmidomycin phosphonate-binding site is occupied in both DXR/bisphosphonate structures by a sulfate ion, and there is no bound NADP. In each structure, the aromatic (isoquinoline or pyridine) side chains of the bisphosphonate are located in a hydrophobic cleft containing Trp-211, Met-213, Pro-273, and Met-275, Figure 2, A and B. In the DXR complex with **2**, Figure 2A, one phosphonate group interacts with Lys-124 and Asn-226, while with **3** one phosphonate bonds to a Mg^{2+} which is chelated to Asp-149, Glu-151, and Glu-230, Figure 2B. This divalent metal is located ~1.7 Å from the Mn^{2+} site seen in DXR-fosmidomycin.[20] In addition, this phosphonate

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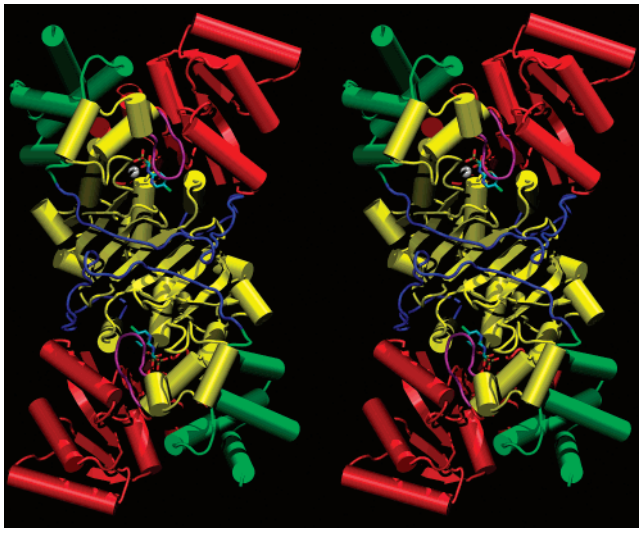

Figure 1. Stereo representation of the overall structure of the complex between **3** and DXR (dimer structure), PDB file 1T1S. The NADP domain is shown in red, the catalytic domain in yellow, the active site loop in purple, and the C-terminal domain in green. The inhibitor **3** is shown in cyan and Mg^{2+} in gray.

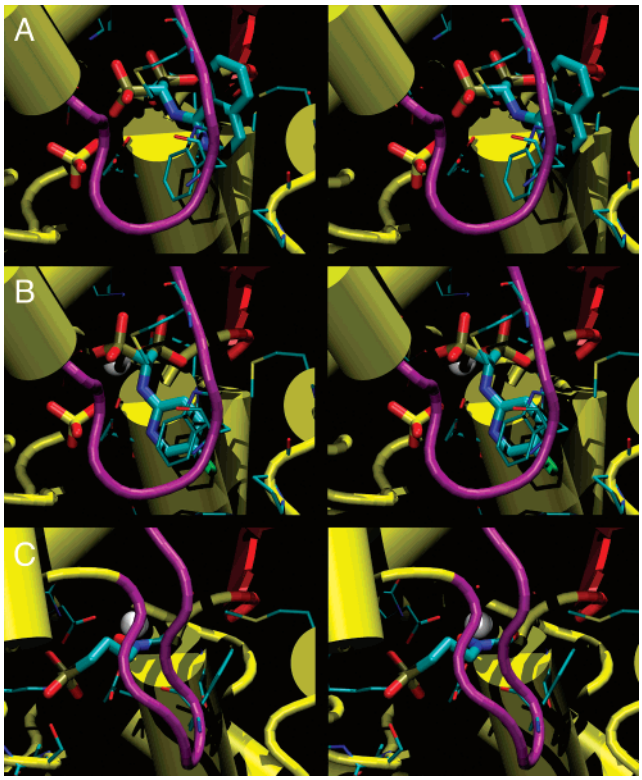

Figure 2. Stereo representations of the catalytic patch of DXR with three inhibitors. **A**, **2** (PDB file 1T1R); **B**, **3** (PDB file 1T1S), and **C**, fosmidomycin (from PDB file 1ONP). The inhibitor side chains are shown in cyan, the sulfate ions (in **A** and **B**) in yellow/red, Mg^{2+} in gray.

group is further stabilized by electrostatic interactions with Lys-124 and Ser-150. There is no evidence that either bisphosphonate binds to the NADP site or the fosmidomycin phosphonate site, which instead is occupied by sulfate ions in both structures, interacting with Ser-185, His-208, Ser-221, and Lys-227, the same residues which stabilize phosphonate binding in DXR/fosmidomycin.[7] When taken together, these results are of interest since they represent a new class of inhibitors of the alternate-mevalonate or MEP pathway, of importance due its presence in many pathogenic microorganisms and where, at present, there is only one published structure of an enzyme−inhibitor complex.

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Supporting Information Available: The atomic coordinates and structure factors (PDB files 1T1R and 1T1S) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/). The stereo representation of the structure of **2** and DXR (dimer structure) is shown in Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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