A Structure-based Guide to Building hHint-1 Activated Nucleotide Prodrugs

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The success of sofosbuvir in the treatment of chronic Hepatitis C viral infections has invigorated interest in human Histidine Triad Nucleotide Binding Protein 1 (hHint1). This enzyme hydrolyzes the sofosbuvir phosphoramidate to expose the nucleotide monophosphate, which is then further modified to the active triphosphate species. Before the hHint1-catalyzed activation step, sofosbuvir phosphoramidate is taken up orally and transported to the liver; this circumvents problems with absorption and metabolic half-life that reduce the therapeutic utility of antiviral and anticancer nucleotides. To establish a structural basis for the understanding the diversity of substrates that might be activated through this pathway, we have undertaken a systematic study of nucleotide phosphoramidate hydrolysis by hHint1. Our work includes the structural characterization of binding by natural and unnatural purine and pyrimidine nucleobases, altered ribose analogs and different amine leaving groups. Observations are correlated with enzyme catalytic efficiency and the thermodynamics of binding from ITC. A guide to the design of good and not-so-good substrates has emerged that should inform the design of future phosphoramidate-based nucleotide prodrugs.