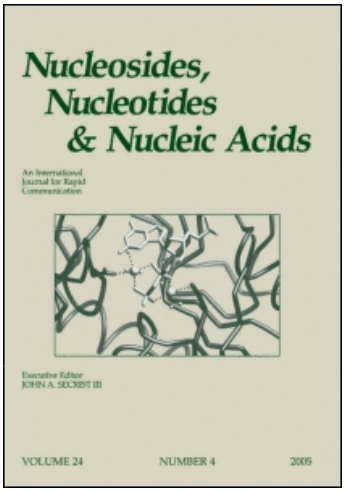

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Impdh As A Biological Probe For Rna Antiviral Drug Discovery: Synthesis, Enzymology, Molecular Docking, And Antiviral Activity Of New Ribonucleosides With Surrogate Bases

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IMPDH AS A BIOLOGICAL PROBE FOR RNA ANTIVIRAL DRUG DISCOVERY: SYNTHESIS, ENZYMOLOGY, MOLECULAR DOCKING, AND ANTIVIRAL ACTIVITY OF NEW RIBONUCLEOSIDES WITH SURROGATE BASES

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□ *Our interest in the discovery of molecules with antiviral activity against RNA viruses led us to the design of ribonucleosides with surrogate bases with the intent of using inhibition of inosine monophosphate dehydrogenase (IMPDH) as a probe for antiviral drug discovery. A general methodology for the preparation of these compounds is discussed. Kinetic parameters of the inhibition studies with IMPDH, which were carried out spectrophotometrically by monitoring the formation of NADH, are given. Antiviral information and correlation of activity with IMPDH inhibition are discussed.*

Keywords IMPDH inhibitors; synthesis; antiviral

INTRODUCTION

The enzyme, inosine monophosphate dehydrogenase (IMPDH; EC 1.1.1.205), catalyses the oxidative conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) with the involvement of the coenzyme, nicotinamide adenine dinucleotide (NAD^+).[1,2] IMPDH is an important target for the discovery of antiviral, anticancer, and immunosuppressive agents.[3] Consistent with this is the observation that some inhibitors of IMPDH have been found to have anticancer, antiviral and immunosuppressive activity.[4–7] IMPDH is a sulfhydryl enzyme in which the Cys-331 residue in the active site may act as a nucleophilic participant in interactions with inhibitors that carry Michael acceptors at appropriate positions on the nucleobase.[7–9] Participation of the Cys-331 is also consistent with the mechanism of substrate action of IMPDH, which involves

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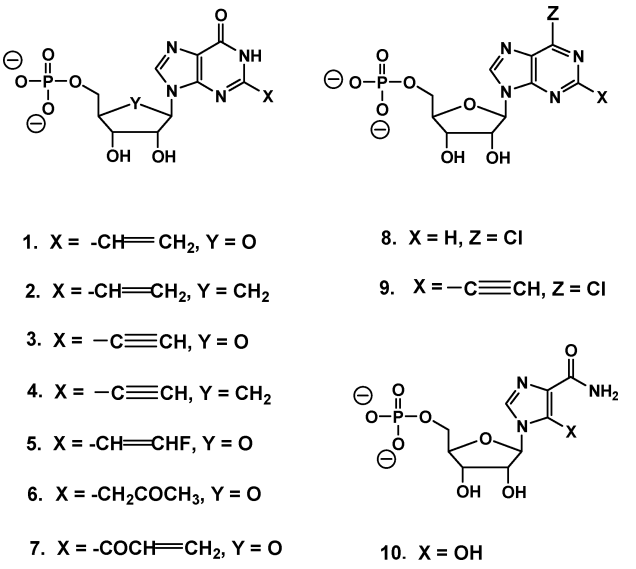
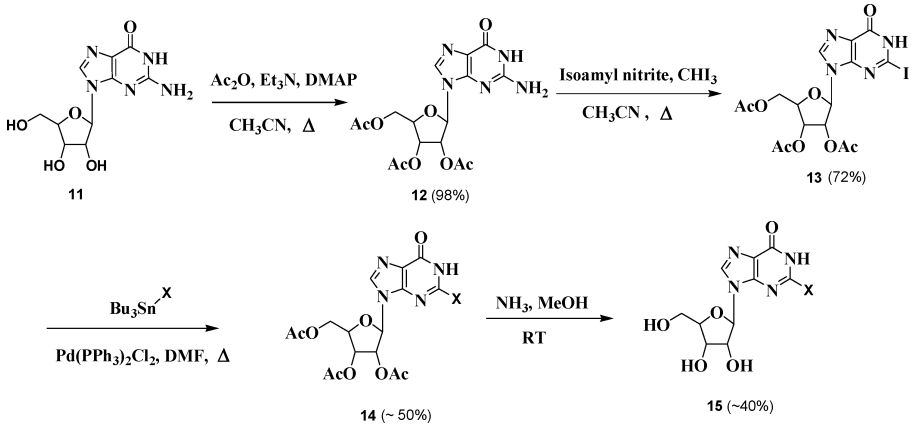


FIGURE 1 Target molecules of this investigation.

covalent interaction of the enzyme and coenzyme (NAD^+) complex with the 2-position of IMP.[1] In an ongoing drug discovery program on RNA antiviral compounds in our laboratory,[7,8,10,11] we have utilized IMPDH as a probe for the initial identification of potential antiviral molecules (Figure 1).

RESULTS AND DISCUSSION

A general and efficient synthetic approach to compounds of Figure 1 is illustrated in Scheme 1. Guanosine (**11**) was converted to the 2-iodo



SCHEME 1 A concise synthetic approach to C-2 functionalized inosine analogues.

TABLE 1 Data on inhibition of IMPDH by C-2 functionalized IMP analogues

Inhibitors	K_i (μM)[a]	k_{inact} (s^{-1})[b]	k_{on} ($M^{-1}s^{-1}$)[c]
1	3.98	0.029	0.73×10^4
2	—	—	2.12×10^4
3	4.25	0.013	0.33×10^4
5	1.11	0.027	2.67×10^4
6	no inhibition	—	—
8	81.8 (reversible)	—	—
9	74.4	0.023	3.05×10^2
10	4.70	0.030	—

[a] $K_i = K_{i,app}/(1 + [IMP])/K_m)$.
[bc] $k_{on} = k_{inact}/K_i$.

compound **13** by acetylation and deamination/halogenation reactions. The key step in the synthesis was the palladium-catalyzed cross-coupling of intermediate **13** with functionalized stannanes[12] to give **14**. Deprotection under standard conditions gave the target molecules **15**. For compounds, **8** and **9**, the methodology used has been described previously by us.[10]

Data from inhibition studies with inosine monophosphate dehydrogenase (IMPDH) from *E. coli* are summarized in Table 1. Details of the procedure for the enzyme kinetics are discussed.[8,11] It is clear from the data that most of the target compounds are strong irreversible inhibitors of IMPDH.

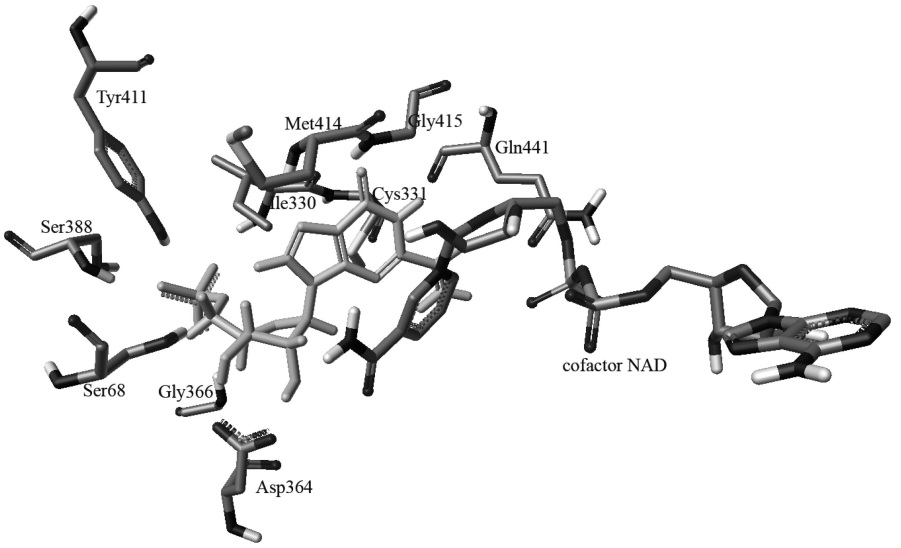


FIGURE 2 Docking results of inhibitor **1** in the active site of IMPDH. The phosphate group is locked into position by polar interactions with Ile330, Gly366, Ser388 and Tyr411. The ribose hydroxyls form hydrogen bonds with Ser68 and Asp364. The backbone N-H of Gly415 and Met414, and C=O of Gln441stabilizes the base moiety by forming hydrogen bonds. The S atom of active residue Cys331 is 4.0 Å away from the 2-vinyl group terminal carbon. Cofactor NAD^+ stacks with the base moiety of inhibitor.

Antiviral data on the target nucleosides correlate well with inhibition of IMPDH by their monophosphates (see[7] for general mechanistic explanation). For example, the parent nucleosides of compounds **1**, **2**, **3**, **5**, and **10** show moderate to good antiviral activity against a number of RNA viruses and also against a few DNA viruses. Antiviral studies are continuing and the completed results will be published elsewhere.

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