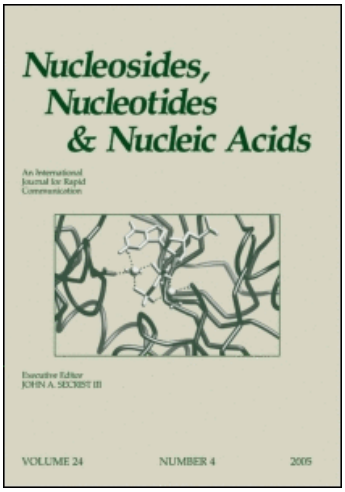

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Inhibitors of Inosine Monophosphate Dehydrogenase: Probes for Antiviral Drug Discovery

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To cite this Article Story, Sherry , Gupta, Mukta , Bonsu, Eric and Nair, Vasu(2005) 'Inhibitors of Inosine Monophosphate Dehydrogenase: Probes for Antiviral Drug Discovery', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 717 — 720
To link to this Article: DOI: 10.1081/NCN-200060308
URL: http://dx.doi.org/10.1081/NCN-200060308

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):717–720, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060308

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INHIBITORS OF INOSINE MONOPHOSPHATE DEHYDROGENASE: PROBES FOR ANTIVIRAL DRUG DISCOVERY

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▫ *The role of inosine monophosphate dehydrogenase (IMPDH) at the metabolic branch point of de novo purine nucleotide biosynthesis makes this enzyme an attractive probe for the discovery of antiviral compounds. Introduction of unsaturation at the 2-position of IMP, the natural substrate for IMPDH, produces Michael acceptors at that position, which results in these compounds being inhibitors of IMPDH. Consistent with this mechanism-based molecular design, some of the parent nucleosides exhibited antiviral activity.*

Keywords IMPDH, Michael Acceptor, Antiviral Activity

INTRODUCTION

Inosine monophosphate dehydrogenase (IMPDH; EC 1.1.1.205) catalyzes the oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) with the involvement of the coenzyme, nicotinamide adenine dinucleotide (NAD^+), which is reduced to NADH.[1,2] The role of IMPDH at this metabolic branch point of de novo purine nucleotide biosynthesis renders it a 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.[4–6] IMPDH is a sulfhydryl enzyme in which the active-site Cys-331 residue may act as a nucleophilic partici pant in interactions with inhibitors that are Michael acceptors.[7] This is also consistent with the mechanism of substrate action of IMPDH, which involves interaction of the enzyme and coenzyme (NAD^+) complex at the 2-position of

Received 1 October 2004, accepted 21 January 2005.
This project was supported by Grant No. AI056540 from the NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. We thank Drs. E. Kern and R. Sidwell for the antiviral screening data and Dr. D. Jung for technical assistance with the molecular modeling representation.
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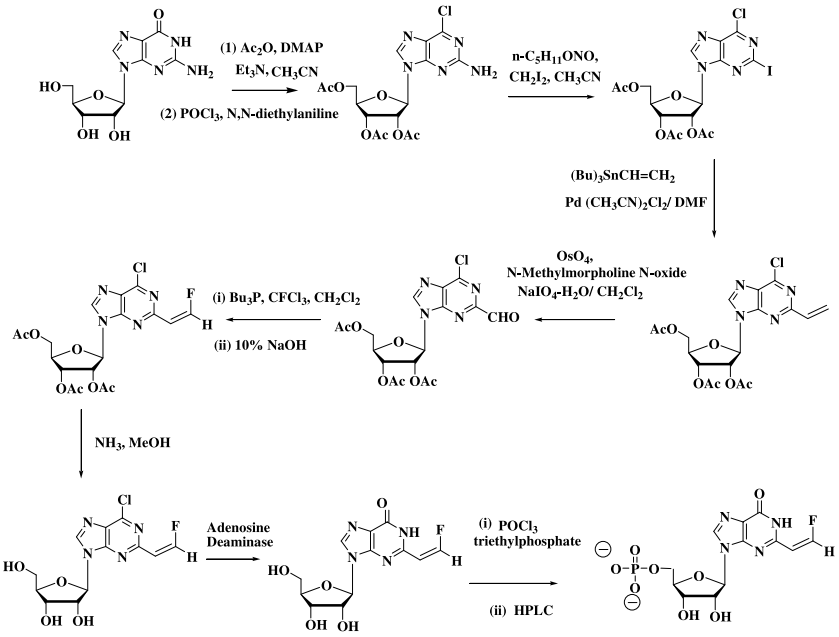

SCHEME 1 Example of the synthesis of a 2-substituted IMP analog.

IMP.[1] X-ray crystallographic data support the suggestion of a covalent adduct formation between Cys-331 of IMPDH and IMP during catalysis.[8] In an ongoing drug discovery program on antiviral compounds in our laboratory, we have utilized IMPDH as a probe for the initial identification of potential antiviral molecules.

RESULTS AND DISCUSSION

Modification of the 2-position of IMP involving Michael acceptors was key in the design of IMPDH inhibitors. Synthesis of the 2-substituted IMP analogs used both chemical and enzymatic methodologies. An example of the synthesis is shown in Scheme 1.[9] The key steps that were involved included a palladium-catalyzed cross-coupling reaction, a modified Wittig reaction and a hydrolytic elimination reaction with the enzyme adenosine deaminase.[10–12] For IMPDH studies, 5′-phosphorylated compounds were needed and these were synthesized by standard phosphorylation methods.

The IMPDH used in this study was isolated from *E. coli* B3 strain on our laboratory.[7] A comparison of the kinetic parameters of this IMPDH with others is shown in Table 1. IMPDH activity was measured by UV spectral methods by monitoring the formation of NADH at 340 nm. In inhibition studies, the inhibition reaction was initiated by the addition of various concentrations of inhibitor to the appropriate enzyme solution.[7,13]

TABLE 1 Kinetic Parameters of Various IMPDHs

Enzyme	V (s^{-1})	IMP (Km) μM	NAD^+ (Km) μM
Human (Type I)[a]	1.8	14.2	42
Human (Type I)[a]	1.4	9.2	32
E. coli (*B3*)[b]	6.0	12.0	315
Trichomonas foetus[c]	1.9	1.7	150

V (s^{-1}) is turnover number per second.
[a]*Biochem. Pharmacol* **1995**, *49*, 1323–1329.
[b]Nair lab data.
[c]*Biochemistry* **1999**, *38*, 15388–15397.

2-[2-(*Z*)-Fluorovinyl]inosine 5′-monophosphate (2-FVIMP) gave k_{inact} and K_i of 0.0269 s^{-1} and 1.11 μM, respectively, whereas the well-known IMPDH inhibitor, 6-chloropurine ribonucleoside monophosphate gave values of 0.076 min^{-1} and 62.0 μM. The inactivation of IMPDH by 2-FVIMP is time dependent and follows a two-step mechanism. Another 2-substituted nucleoside monophosphate, 6-chloro-2-ethynylpurine riboside monophosphate, was also a strong inhibitor of IMPDH with values of k_{inact} and K_i of 0.75 min^{-1} and 4.25 μM, respectively. The mechanism of inhibition is similar to FVIMP (Figure 1). Comparisons were made with another inhibitor of IMPDH, 4-carboxamido-5-hydroxyimidazole riboside monophosphate, which exhibited a K_i of 0.5 nM.[14]

Antiviral studies of the parent nucleosides of these inhibitors of IMPDH showed that 6-chloro-2-ethynylpurine riboside was active but toxic against the vaccinia virus (HFF cells, IC_{50} > 0.8 μg/mL; CC_{50} 3.0 μg/mL). Both 2-fluoro-vinylinosine and its 6-chloropurine analog also exhibited activity towards the vaccinia virus (SI < 4.5 and < 3.7). 4-Carboxamido-5-hydroxy-imidazole riboside

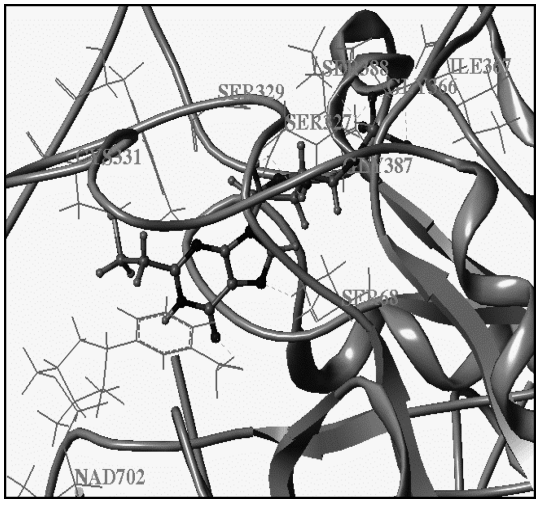

FIGURE 1 Representation of the interaction of FVIMP with IMPDH.

showed good activity against HSV-2 (HFF cells, IC_{50} 0.26 μg/mL; CC_{50} > 100 μg/mL) and VZV (HFF cells, IC_{50} 2.8 μg/mL; CC_{50} > 100 μg/mL). Other examples of the use of IMPDH as a probe for antiviral drug discovery are in progress.

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