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Novel Amide-Based Inhibitors of Inosine 5′-Monophosphate Dehydrogenase

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Abstract—A series of novel amide-based small molecule inhibitors of inosine monophosphate dehydrogenase (IMPDH) was explored. The synthesis and the structure–activity relationships (SARs) derived from in vitro studies are described.

Inosine 5′-monophosphate dehydrogenase (IMPDH) is a key enzyme in the regulation of cell proliferation and differentiation. In mammals, nucleotides may be synthesized through one of two pathways: a de novo synthesis pathway or through a salvage pathway utilizing existing purines and their nucleotides and nucleosides.[1] The extent of utilization of each pathway is dependent on the cell type. IMPDH is a crucial enzyme involved in the de novo synthesis of guanine nucleotides. Specifically, IMPDH catalyzes the irreversible NAD dependent oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP).[2] In the enzymatic mechanism, an active site cysteine residue plays a key role in the activation of IMP during enzymatic oxidation (Fig. 1).[3,4] Two distinct cDNA's encoding IMPDH have been identified and isolated. These transcripts labeled type I and type II possess 84% sequence identity.[5–7] IMPDH type II activity is markedly upregulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[8]

The de novo synthesis of guanine nucleotides, and thus the activity of IMPDH, is particularly important in B- and T-lymphocytes. These cells are dependent on the de novo synthesis rather than the salvage pathway to generate sufficient levels of nucleotides necessary to initiate a proliferative response to mitogen or antigen. As a result, IMPDH is an attractive target for selectively inhibiting the immune system without also inhibiting the proliferation of other cells. Inhibition of this enzyme has implications in immunosuppressive, antiviral, and oncology therapies.

Mycophenolic acid (MPA, Fig. 2) and some of its derivatives have been shown to be potent, uncompetitive, reversible inhibitors of human IMPDH type I and type II.[9,10] MPA has been demonstrated to block the response of B- and T-cells to mitogen or antigen. IMPDH inhibitors, such as the prodrug of MPA, CellCept® (MMF), are useful drugs in the treatment of transplant rejection and autoimmune disorders, such as psoriasis.[11] In addition, various cancer cell lines are sensitive to IMPDH inhibition, and clinical trials eval-

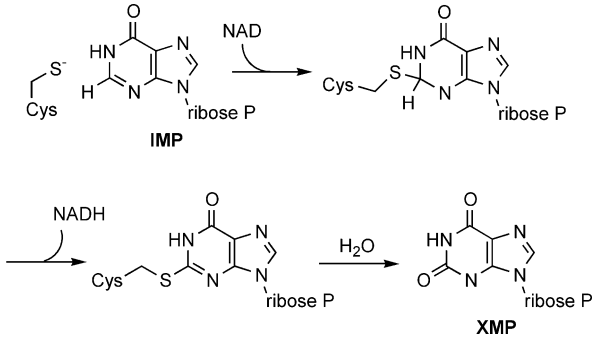

Figure 1. The mechanistic conversion of IMP to XMP by IMPDH.

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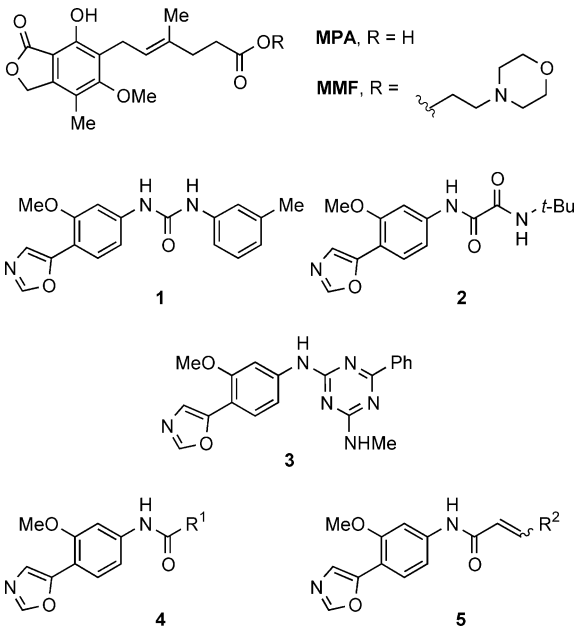


Figure 2. Chemical structures of MPA, MMF, Vertex urea **1**, diamide **2**, triazine **3**, amide **4**, and α,β-unsaturated amide **5**.

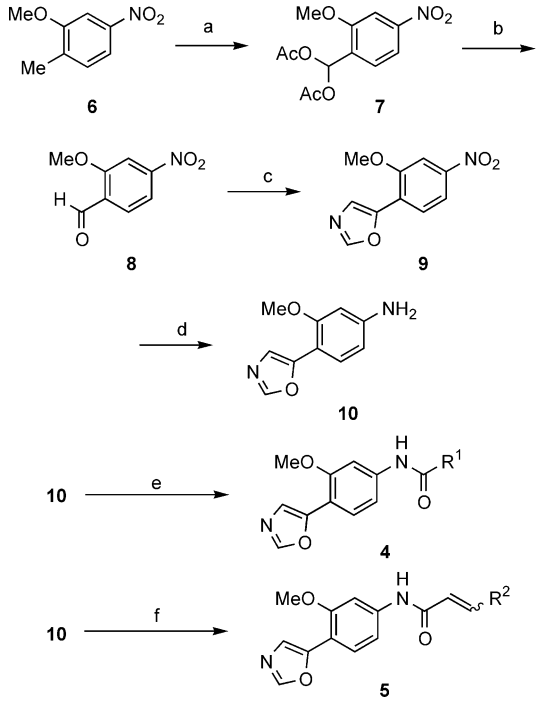
uating IMPDH inhibitors for their antileukemic effects are currently in phase III.[8] An alternative approach to IMPDH inhibition has been described by Pankiewicz.[12]

Dose-limiting gastrointestinal (GI) toxicity is observed from oral administration of either MPA or CellCept® in a clinical setting.[11] Structurally related analogues disclosed by Vertex and Bristol-Myers Squibb, devoid of phenolic functionality, are reputed to have an improved therapeutic window with regard to dose limiting GI toxicity.[13–16]

Our focus is on the identification and development of potent inhibitors of IMPDH type II with improved pharmacological properties. Of particular note, is the dose limiting GI toxicity that largely restricts the clinical use of MPA and MPA analogues to the treatment of acute allograft rejection. Recently, we have disclosed three new classes of inhibitors of IMPDH, exemplified by structures **2** and **3** (Fig. 2).[14,17,18] In this report, we have outlined the synthesis and biological evaluation of a new class of inhibitors of IMPDH, represented by structures **4** and **5**.

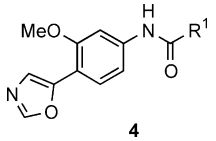
Through protein crystallographic studies, Vertex has shown that analogues of **1** bind to IMPDH forming a hydrogen bond between the urea NH and the carboxylate of Asp 274.[3,19] Our strategy was to develop a new class of inhibitors utilizing the oxazole-functionalized aniline residue **10** in which the urea moiety of **1** is replaced with amide-based functionality, as depicted in structures **4** and **5** (Fig. 2).

The syntheses of analogues **4** and **5** are shown in Scheme 1. The 3-methoxy-4-(5-oxazolyl)-aniline **10** was prepared from 2-methoxy-4-nitro-toluene **6** on multigram scale utilizing a synthetic procedure described by Vertex.[15] The reaction of aniline **10** with various commercially available carboxylic acids in the presence of 1 equivalent of 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide HCl (EDC) in dimethyl formamide (DMF) provided the amides of structure **4**. Alternatively, com-



Scheme 1. Reagents and conditions: (a) HOAc, Ac_2O, concd H_2SO_4, CrO_3, 0–10 °C (51%); (b) concd HCl, dioxane, reflux (91%); (c) TosMIC, K_2CO_3, MeOH, reflux (84%); (d) 40 psi H_2, Pd/C, EtOH, (95%); (e) R^1-CO_2H, EDC, DMF, 50 °C; (f) R^2-CO_2H, EDC, DMAP, CH_2Cl_2/DMF.

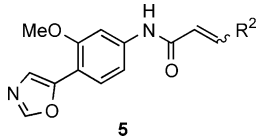
Table 1. SAR of amide-based inhibitors of structure **4**



Compd	R^1	IMPDH II IC_{50} (μM)
1	NA	0.019
4a	Phenyl	0.63
4b	Benzyl	0.97
4c	3,4-Dimethylphenyl	0.26
4d	3,4-Benzodioxolyl	0.33
4e	5-Indolyl	0.63
4f	2-Indolyl	0.11[a]
4g	2-(1-Methyl)indolyl	0.13
4h	3-Indolyl	>3
4i	2-Benzofuranyl	0.21
4j	2-Benzothiophenyl	0.17
4k	2-Indolizino	0.14
4l	3-(1H)-Indazolyl	>1
4m	2-Furanyl	2.5
4n	2-(3-Methyl)furanyl	>1
4o	2-(4,5-Dimethyl)furanyl	0.54
4p	2-(5-*t*-Butyl)furanyl	0.13
4q	2-Thiophenyl	0.81
4r	2-(5-Pyrido)thiophenyl	0.87
4s	2-(5-Methyl)thiophenyl	0.11
4t	2-(*N*-Methyl)pyrolyl	0.89

[a]IC_{50} >1.0 μM (IMPDH type I).

Table 2. SAR of amide-based inhibitors of structure **5**



5

Compd	R^2	Bond orientation	IMPDH II IC_{50} (μM)
5a	Phenyl	*trans*	0.073
5b	*o*-Methylphenyl	*trans*	0.035
5c	*m*-Methylphenyl	*trans*	0.59
5d	*p*-Methylphenyl	*trans*	0.21
5e	*o*-Fluorophenyl	*trans*	0.74
5f	*m*-Fluorophenyl	*trans*	0.20
5g	*p*-Fluorophenyl	*trans*	0.12
5h	2,6-Dimethylfluorophenyl	*trans*	0.075
5i	*o*-Chlorophenyl	*trans*	0.060
5j	*m*-Chlorophenyl	*trans*	0.30
5k	*p*-Chlorophenyl	*trans*	0.23
5l	*o*-Methoxyphenyl	*trans*	0.11
5m	*o*-Methoxyphenyl	*cis*	0.96
5n	2-Furanyl	*trans*	0.028
5o	2-Thiophenyl	*trans*	0.066
5p	3-Pyrido	*trans*	0.21
5q	4-Pyrido	*trans*	0.057
5r	2-Quinolinyl	*trans*	0.63
5s	3-Indolyl	*trans*	0.41

pounds of structure **5** were prepared by the reaction of aniline **10** with various commercially available α,β-unsaturated carboxylic acids in the presence of EDC and dimethylaminopyridine in a mixture of dichloromethane (CH_2Cl_2) and DMF.

The SARs for the inhibition of IMPDH type II catalytic activity are summarized in Tables 1 and 2. In the amide series **4**, simple replacement of one of the NH groups in the Vertex urea **1** with CH_2 provided benzyl amide **4b**, which showed a 50-fold loss in potency against IMPDH type II. Shortening the carbon tether, via elimination of the methylene unit, (**4a**) resulted in a similar loss in potency versus **1**, ($IC_{50} = 0.63\,\mu M$).[20] Further substitution on the phenyl ring, compounds **4c** and **4d**, provided an increase in activity. Replacement of the phenyl ring with various heterocycles was also explored. Bicyclic heterocycles like 2-indolyl **4f**, 5-indolyl **4e**, 2-benzofuranyl **4i**, 2-benzotiophenyl **4j**, and 2-indolizino **4k** all proved to be well tolerated and provided enhanced potency over **4a**. Interestingly, activity was maintained in the 2-(1-methyl)indolyl **4g** derivative. Bicyclic heterocycles **4h** and **4l** were not well tolerated. Simple unsubstituted heterocycles, **4m**, **4q**, and **4t**, were less active than **4a**; however, further elaboration of both the furan ring and the thiophene ring at the 5-postion resulted in an increase in potency (**4p**, 0.13 μM and **4s**, 0.11 μM, respectively). The indole analogue **4f** was evaluated against IMPDH type I and was found to have an IC_{50} value $>1\,\mu M$, demonstrating a selectivity preference for IMPDH type II ($IC_{50} = 0.11\,\mu M$).

A series of α,β-unsaturated amides of the structure **5** was also explored. Overall, this series proved to be more potent then the amide series represented by **4**. Phenyl substituted amide **5a** had an IC_{50} value of 0.073 μM against IMPDH Type II enzyme. Compounds **5b**–**5g** and **5i**–**4k** clearly demonstrated that *ortho*-substitution of the phenyl ring is favored over the unsubstituted inhibitor **5a**. Substitution at the *meta*- and *para*-positions resulted in an overall decrease in potency relative to compound **5a**. Interestingly, potency was maintained when the *ortho*-phenyl substituted compound **5c** was further elaborated with a methyl at the opposing *ortho*-position (**5h**). Placement of a methoxy group at the *ortho*-position (**5l**) led to a slight decrease in activitiy relative to **5a**. The *cis*-analogue, **5m**, was prepared in order to determine the necessity of the *trans* orientation of the double bond of the amide. This configuration led to an 8-fold loss in potency. Substitution of the α,β-unsaturated amides with various heterocycles was also explored. Furan substituted amide **5n** was the most potent compound is this series with an IC_{50} value of 0.028 μM.

The compounds that showed potent inhibitory activity against IMPDH type II were examined in a T-Cell proliferation assay.[21] In this assay, MPA inhibited T-cell proliferation with an IC_{50} of 0.39 μM. None of the amide-based inhibitors discussed in this paper inhibited proliferation with an IC_{50} of less than 1 μM. For example **4g**, **4s**, **5n**, and **5q** inhibited T-cell proliferation with IC_{50} values of 7.7, 8.8, 6.6, and 8.7 μM, respectively.

We have identified two new series of novel amide-based inhibitors of IMPDH Type II catalytic activity. These compounds demonstrate that the urea or cyanoquanidine isosteres can be effectively replaced by an amide moiety. Studies to optimize this series of analogues to achieve oral activity in a T-cell mediated pharmacodynamic model are ongoing. The emphasize of future studies is to evaluate this amide-based series relative to MPA and other IMPDH inhibitors, as well as to establish in vivo the relationship between efficacy and toxicity.

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21. *CEM proliferation assay*: The human T-lymphoblast CEM cell line (ATCC) was cultured in RPMI 1640 (Gibco) containing 10% heat inactivated FBS and 100 units/mL of penicillin and streptomycin. Cells were seeded in 96-well Costar flat bottom tissue culture plates at a concentration of 3000 cells/well in the presence of 0.5% DMSO. Test compounds were added in triplicate at a final concentration of 10 uM with 3-fold serial dilutions. Cell cultures were maintained in a 5% CO_2 humidified atmosphere for 72 h. Cell viability was measured after a final 5-h incubation with 10% (v/v) Alamar Blue dye. The fluorometric conversion of Alamar Blue was read on a Cytoflour II multiwell plate reader with excitation/emission settings of 530/590 nm respectively.
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