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Novel Guanidine-Based Inhibitors of Inosine Monophosphate Dehydrogenase

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Abstract—A series of novel guanidine-based small molecule inhibitors of inosine monophosphate dehydrogenase (IMPDH) was explored. IMPDH catalyzes the rate determining step in guanine nucleotide biosynthesis and is a target for anticancer, immunosuppressive and antiviral therapy. The synthesis and the structure–activity relationships (SARs), derived from in vitro studies, for this new series of inhibitors is given.

The regulation of purine nucleotides in cells is governed by two biochemical synthetic routes, the de novo synthesis and the salvage pathways. Inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo synthesis of guanosine nucleotides, catalyzes the irreversible NAD-dependent oxidation of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP).[1] Two distinct cDNA's encoding IMPDH have been identified and isolated. These transcripts labeled type I and type II are of identical size (514 amino acids).[2–4] IMPDH II activity is markedly upregulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[5] B- and T-lymphocytes depend on the de novo, rather than salvage pathway, to generate sufficient levels of nucleotides necessary to initiate a proliferative response to mitogen or antigen. Due to the B- and T-cell's unique reliance on the de novo pathway, IMPDH is an attractive target for selectively inhibiting a lymphocyte-mediated immune response without undue inhibition of other noncancerous proliferating cells. Mutations that lead to a loss in function of wild-type p53 are the most common genetic change associated a number of diverse cancers.[6] A role for IMPDH in cancer is supported by the observation that upregulation of the enzyme prevents p53-dependent growth inhibition.[7] Inhibitors of IMPDH have shown marked cytotoxicity against human leukemia and several colon and ovarian cell lines.[5,8–10]

Mycophenolic acid (MPA) has been shown to be a potent, uncompetitive, reversible inhibitor of human IMPDH type I and type II.[11,12] MPA has been demonstrated to block the response of B-, and T-cells to mitogen or antigen. CellCept®, a prodrug of MPA, has clinical use for the treatment of transplant rejection and autoimmune disorders, such as psoriasis and rheumatoid arthritis.[13] MPA has been shown to have potent in vitro cytotoxicity, however marked in vivo antitumor activity has not been observed. The metabolite of Tiazofurin (TAD), a nucleotide inhibitor of IMPDH, has shown in vivo antitumor activity and efficacy in clinical trials for the treatment of myelogenous leukemia.[5] Tiazofurin is currently under investigation for the treatment of ovarian cancer.[14]

Dose-limiting gastrointestinal (GI) toxicity is observed in a clinical setting from oral administration of either MPA or CellCept® (mycophenolate mofetil, MMF). MMF is rapidly and to a large extent completely absorbed following oral administration whereby it is converted to MPA.[13,15] Three metabolites have been identified in humans, the 7-*O*-glucuronide (MPAG), 7-*O*-glucoside conjugate (MPAG1s), and the acyl glucuronide (AcMPAG).[16] MPAG, the major metabolite, is inactive against IMPDH and is found in substantially higher concentrations in the plasma of healthy subjects

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than MPA contributing to the high doses required for efficacy. Significant MPAG is excreted in the bile and converted into MPA by the action of intestinal flora contributing to the gastrointestinal toxicity.

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.[17–19] A contemporary approach to IMPDH inhibition utilizing mycophenolic adenine dinucelotide analogues has been recently reported.[20]

This report describes a new class of IMPDH inhibitors. Our focus is the identification and development of potent, selective inhibitors of IMPDH with improved pharmacological properties. Recently we have disclosed a new class of inhibitors, exemplified by **1** (Fig. 1).[21]

Through protein crystallographic studies, Vertex has shown that analogues of **2** bind to IMPDH forming a hydrogen bond between the urea NH and the carboxylate of Asp 274.[22] The biarylurea moiety of the Vertex chemical series contributes to the poor aqueous solubility for compound **2**. Our strategy was to develop a new class of inhibitors utilizing the oxazole-functionalized aniline residue, **3**, and replacing the urea moiety with guanidine-based functionality.[23]

The biaryl cyanoguanidines were conveniently prepared using established methodologies outlined in Scheme 1.[24] The synthetic route employed aniline **3**, whose preparation was previously described.[21] Several other guanidines were prepared as outlined in Schemes 2–5.

Table 1 reports the in vitro inhibitor potencies against IMPDH II for a range of inhibitors featuring a central guanidine residue. Replacement of the urea linkage of **2** with a cyanoguanidine moiety, **6a**, resulted in a 13-fold loss in potency, (IMPDH II IC_{50} 0.24 μM).[25] Attempts to enhance the potency through the use of more basic residues, **8a**–**c**, led to a further loss in inhibitory activity. Moderating the guanidine basicity through acylation, analogues **10a**,**b** and **12**, yielded inactive analogues. In addition, removal of the tolyl residue of **6a** resulted in little change in potency. Likewise, the removal of the methyl residue of **6b**, gave little change in inhibitory activity. Further investigation into the SAR for the cyanoguanidine series showed that although a methyl residue is tolerated in the meta position of the phenyl

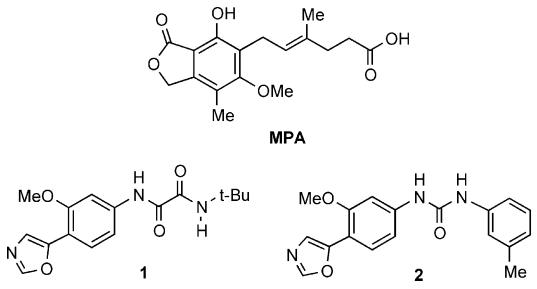
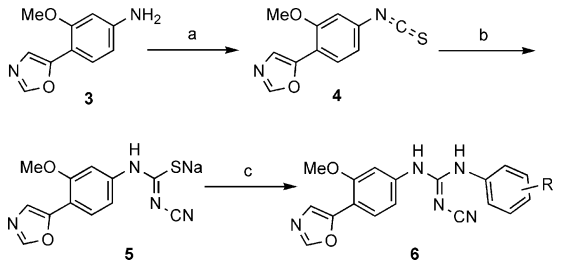
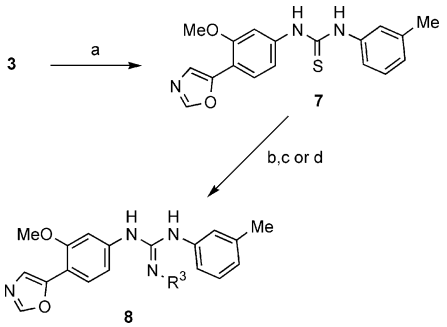


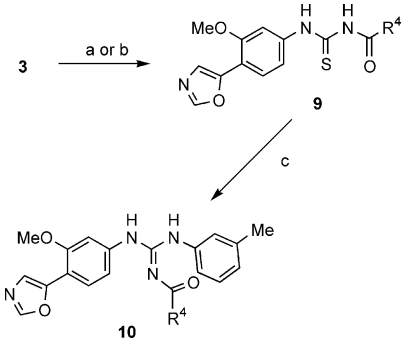
Figure 1. Chemical structures of MPA, diamide **1**, and urea **2**.



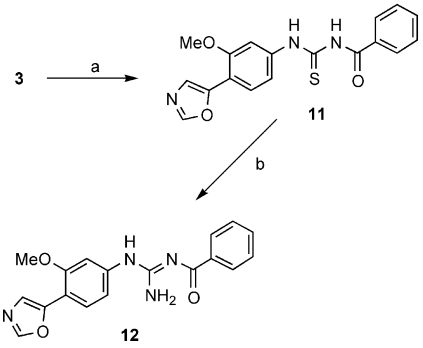
Scheme 1. Synthesis of compounds **6a**–**l**. Reagents and conditions: (a) 1,1′thiocarbonyldi-2(1*H*)-pyridone, CH_2Cl_2, rt, 94%; (b) NaNHCN, EtOH, rt, 76%; (c) substituted aniline, EDC, DMF, 50 °C, 12–75%.



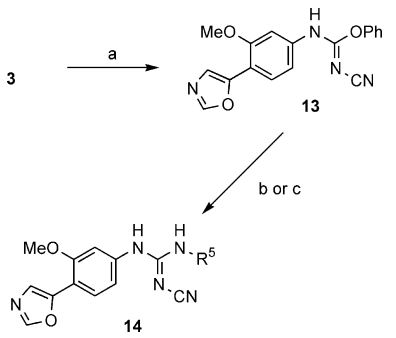
Scheme 2. Synthesis of compounds **8a**–**c**. Reagents and conditions: (a) 1-isothiocyanato-3-methyl-benzene, CH_2Cl_2, reflux 3.5 days, 70%; (b) R^3 = Me: $MeNH_2$, DCC, Hunig's base (0.2 equiv), rt, 92%; (c) R^3 = H/NH_3, DCC, Hunig's base (0.2 equiv), rt, 73%; (d) R^3 = OMe/$MeONH_2$, WSC, Hunig's base (0.2 equiv), rt, 73%.



Scheme 3. Synthesis of compounds **10a**,**b**. Reagents and conditions: (a) R^4 = Me: acetyl chloride, KSCN, acetone, rt, 49%; (b) R^4 = OEt: EtOC(O)NCS, CH_2Cl_2, reflux, 2 h, 91%; (c) *m*-toluidine, WSC, Hunig's base, DMF, 65 °C, 15 h, 82%.



Scheme 4. Synthesis of compound **12**. Reagents and conditions: (a) benzoyl isothiocyanate, CH_2Cl_2, rt, 3 h, 94%; (b) in a sealed tube: NH_3, WSC, Hunig's base, DMF, 65 °C, 16 h, 70%.

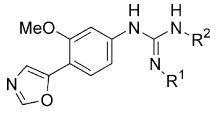


Scheme 5. Synthesis of compounds **14a**,**b**. Reagents and conditions: (a) $(PhO)_2CNCN$, CH_3CN, rt, 94%; (b) R^5 = H:NH_3, EtOH, 100 °C, 94%; (c) $R^5 = CH_2$-*m*-MePh:MeCN, 80 °C, 80%.

residue (R^2), substitution at *ortho* and *para* positions yield poorly active inhibitors (**6c**,**d**). Extending the meta tolyl pharmacophor by a methylene residue, **14b**, also had a negative effect on potency. Lastly, replacement of the phenyl residue (R^2) with either 2- or 3-pyridyl enhanced the polarity of the molecule without a significant change in inhibitory activity.

An effort to explore and develop the SAR at the *meta* position of the 'right hand' phenyl ring of **6a** is summarized in Table 2. Both polar and lyphophilic residues are tolerated at this position. Notably, the carbonate side chain utilized by Vertex in VX-497 only modestly increases the potency in this series, as demonstrated by **6i**. Structurally related side chains (**6j**,**k**) gave similar results. Interestingly, replacement of the carbonate with an amidinoether moiety, **6l**, enhanced binding affinity for IMPDH II, comparable to both MPA and **2** ($IC_{50} = 0.016\,\mu M$). In addition, this analogue was a less potent inhibitor of IMPDH I ($IC_{50} = 0.42\,\mu M$).

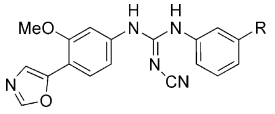
Table 1. SAR of guanidine-based inhibitors of IMPDH



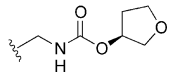
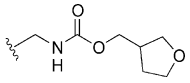
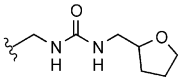
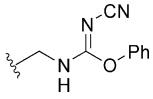
I

Compd	R^1	R^2	IMPDH II IC_{50}, μM
MPA	NA	NA	0.015
2	NA	NA	0.019
6a	CN	*m*-MePh	0.24
8a	Me	*m*-MePh	>3.0
8b	OMe	*m*-MePh	0.66
8c	H	*m*-MePh	>3.0
10a	C(O)Me	*m*-MePh	>1.0
10b	C(O)OEt	*m*-MePh	0.74
12	C(O)Ph	H	0.80
14a	CN	H	0.33
6b	CN	Ph	0.25
6c	CN	*p*-MePh	1.5
6d	CN	*o*-MePh	2.2
14b	CN	CH_2*m*-MePh	1.1
6e	CN	3-Pyridyl	0.32
In	CN	2-Pyridyl	0.34

Table 2. SAR of cyanoguanidine-based inhibitors of IMPDH



II

Compd	R	IMPDH II IC_{50}, μM
6f	CH_2OH	0.28
6g	$O(CH_2)_2OPh$	0.38
6h	$C(O)NH(CH_2)_3Me$	0.13
6i		0.34
6j		0.11
6k		0.18
6l		0.016

We have identified a new series of novel guanidine-based inhibitors of IMPDH II. Replacement of the central urea linkage of **2** with a cyanoguanidine moiety resulted in a 13-fold loss in potency. This lost potency may be regained through the appropriate substitution on the 'right hand' phenyl ring, as observe with **6l**. This compound was examined in a T-Cell proliferation assay[26] and was found to have an IC_{50} of 0.410 μM (MPA $IC_{50} = 0.390\,\mu M$). Studies to optimize this series of analogues through further modifications of the central guanidine moiety and the 'right hand' functionality to achieve oral activity in a T-cell mediated pharmacodynamic model are ongoing. The objective of future studies is to evaluate cyanoguanidine-based inhibitors in an in vivo setting to establish the relationship between efficacy and toxicity.

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25. The enzymatic activity of human IMPDH II was quantitated using a procedure similar to reported methods.[27,28] The conversion of NAD^+ to NADH was followed spectrophotometrically at 340 nM. A reaction mixture containing 0.1 M Tris, 0.1 M KCl, 3 mM EDTA pH 8.0, 400 μM IMP, 2 mM DTT and 40 nM IMPDH II was added to the wells of flat bottom UV-transparent 96-well plates (Costar 3635). To test inhibitors, compounds resuspended in DMSO were diluted in the reaction to give a final DMSO concentration of 2.5%. IMPDH II used in these assays was purified from *Escherichia coli* expressing the gene for the human Type II enzyme. The reaction was initiated by addition of NAD to a final concentration of 400 μM. After a 2 h incubation at 25 °C, readings were taken at 340 nM. The concentrations of compound required to inhibit NADH accumulation by 50% (IC_{50}) were calculated using a 4-parameter logistic plot.
26. *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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