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Rapid Synthesis of Triazine Inhibitors of Inosine Monophosphate Dehydrogenase

William J. Pitts,* Junqing Guo, T. G. Murali Dhar, Zhongqi Shen, Henry H. Gu, Scott H. Watterson, Mark S. Bednarz, Bang-Chi Chen, Joel C. Barrish, Donna Bassolino, Daniel Cheney, Catherine A. Fleener, Katherine A. Rouleau, Diane L. Hollenbaugh and Edwin J. Iwanowicz

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA

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

Inosine monophosphate dehydrogenase (IMPDH) is an enzyme in the de novo synthetic pathway of guanosine nucleotides. This enzyme 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, possess 84% sequence identity, with one conservative change in the active site binding pocket.[2–4] 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. A therapeutic window for the anti-metabolite effects of IMPDH inhibition depends upon the B and T cell's unique reliance on the de novo pathway relative to other proliferating cells in the body.

Mycophenolic acid (MPA), and some of its derivatives have been shown to be potent, uncompetitive, reversible inhibitors of human IMPDH type I and type II.[5,6] The enzymatic activity of human IMPDH II was measured using a procedure similar to reported methods.[7,8] 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, mycophenolate mofetil (MMF), are useful drugs in the treatment of transplant rejection and autoimmune disorders, such as psoriasis.[9] Dose-limiting gastrointestinal (GI) toxicity is observed from oral administration of either MPA or MMF in a clinical setting. MPA undergoes extensive glucuronidation and biliary excretion. High levels of MPA in the GI tract has been postulated to be a cause of GI side effects.[10] Vertex has shown that biarylureas, exemplified by VX-497, are highly potent inhibitors of IMPDH catalytic activity (Fig. 1) and these compounds do not have an obvious site of glucuronidation.[11] This compound is currently under evaluation in the clinic in combination with interferon alpha for the treatment of hepatitis C.[12]

Our initial approach was to examine urea isosteres in an effort to define the optimal linkage between the two halves of the Vertex ureas.[13] The synthesis and IMPDH SAR of a cyanoguanidine urea isostere **II** (Fig. 2) will be

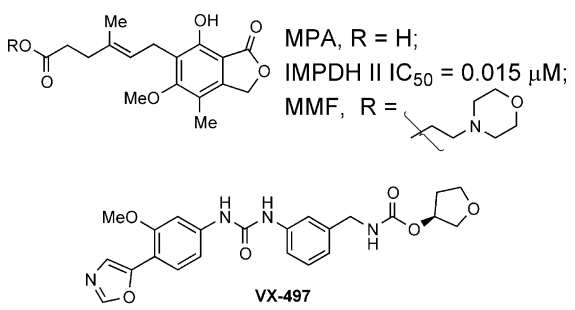

Figure 1. Chemical structures of MPA, MMF and VX-497.

*Corresponding author. Tel.: +1-609-252-5252; fax: +1-609-252-7410; e-mail: william.pitts@bms.com

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reported shortly.[14] It was realized that reaction of phenylhydrazine in place of simple amines, with thiourea **I** would provide a heterocyclic analogue. Triazole **III** was found to be a potent inhibitor of IMPDH ($IC_{50}=150$ nM), and was sufficiently different from the urea isosteres to warrant further investigation.

Analysis of this triazole (III) suggested that rapid diversification might be possible if we could substitute a triazine (IV) for the lead triazole scaffold.

The crystal structure of MPA bound to IMPDH has been reported.[15] Based on this data a model of the triazine (**5a**) docked into the active site of IMPDH could be prepared (Fig. 3). The amine of the triazine (**6a**), which would support projection of appended groups either toward methionine 420, glutamine 441, serine 276 or solvent, was used as a point of attachment for reagent docking.[16] Compounds that scored well in the docking run were pre-screened for violation of Lipinski's rule of 5[17] before final selection. Additional compounds were prepared to provide a more complete SAR study.

The synthesis of analogues **6** and **8** is shown in Scheme 1. The 3-methoxy-4-(5-oxazolyl)-aniline **1** was prepared on multigram scale utilizing a synthetic procedure described by Vertex.[18] Reaction of cyanuric chloride **2**, with phenylmagnesium bromide produced triazine **3**. Stirring a solution of aniline **1** with triazine **3** at room temperature resulted in the precipitation of intermediate **4** in high yield and purity. Intermediate **4** was subjected to nucleophilic substitution with a variety of amines to produce triazine analogues **5a**–**o**. Treatment of triazine **4** with hydroxide or alkoxides provided analogues **5p**–**r**. In a similar synthetic sequence, cyanuric chloride **2** was reacted with aniline **1** at 0 °C to produce the mono addition product, which was followed by addition of methylamine at room temperature to produce intermediate **7**. Reaction of intermediate **6** with aryltin reagents produced analogues **7a**–**d** in moderate yield. Reaction of **6** with a variety of amines produced the analogues **7e**–**i**. Approximately 180 compounds of structure **5** and **7** were prepared in two separate libraries. Purity for all compounds was >85% as determined by LCMS. The syntheses of triazine **11** (Scheme 2) was accomplished by condensation of phenylpyruvate **9** and methyl hydrazinocarboximidothioate **10.** Oxidation to the sulfone **12,** proceeded quantitatively, however this material was unstable (hydrolysis) and best used the same day it was prepared. Nucleophilic displacement of the sulfone did not proceed at an appreciable rate with aniline **1,** but occurred rapidly with the anion derived

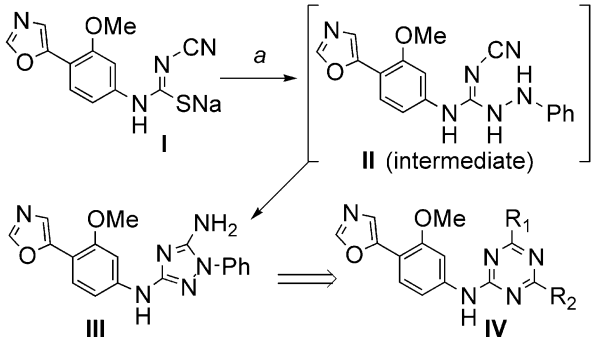


Figure 2. Chemical structures of triazole **III** and triazine **IV**. Reagents and conditions: (a) $PhNHNH_2$, EDC, DMF 60 °C.

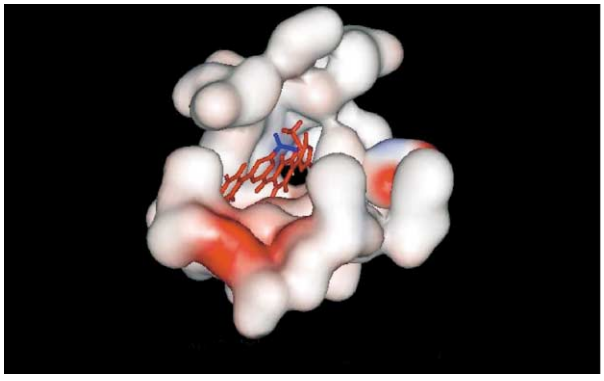
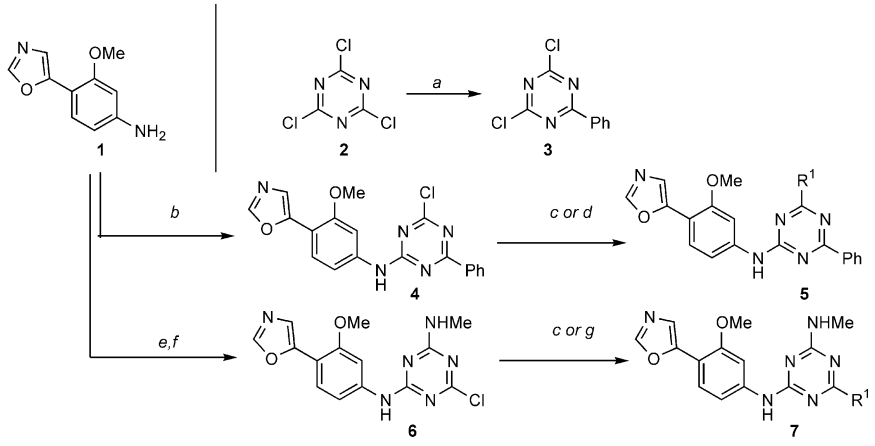


Figure 3. Triazine (**5a**) docked into IMPDH. The amine (blue) was used as the point of attachment for reagent docking.



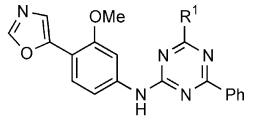
Scheme 1. Reagents and conditions: (a) PhMgBr, toluene 0 °C (60%); (b) **1** and **3**, THF, rt (99%); (c) HNR_3R_4, dioxane 80 °C, (70–90%); (d) HOR^1, NaH, dioxane 60 °C (70–85%); (e) **1** and **2**, acetone, K_2CO_3, 0 °C, 2 h (63%); (f) NH_2Me, THF, (92%); (g) $ArSnBu_3$, $Pd(OAc)_2$, DMF, 80 °C, (15–35%).

from formamide **8.** Acidic hydrolysis of the formamide group, provided triazine **13**.

The structure–activity relationships (SARs) for the inhibition of IMPDH type II catalytic activity are summarized in Table 1. Several chemical filters were employed during the library design phase. 82% of the compounds prepared did not break any Lipinski rule, and as such we hoped to bias this library toward inhibitors of IMPDH II with improved drug-like character. Triazine **5a** was 3-fold more potent at inhibiting IMPDH II than the lead triazole **III**, which validated the initial hypothesis that the triazine could effectively function as a replacement for the triazole. The most potent compound, **5p** ($IC_{50}=18$ nM), was comparable in potency to MPA ($IC_{50}=15$ nM). Representative compounds were chosen from this compound library to illustrate several SAR points.

Primary and secondary amines **5a** and **5b** were relatively potent inhibitors of IMPDH II with IC_{50} values of 0.054 and 0.076 μM, respectively. Tertiary amines such as **5c**, **5j**, **5k**, and **5l**, are generally less potent. Tertiary amines **5m** and **5n** appear to be an exception to the general

Table 1. SAR of **5a**–**r** and **7a**–**i** with respect to inhibition of IMPDH II



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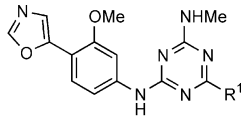

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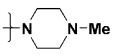
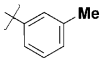
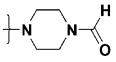
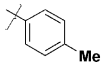
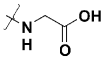
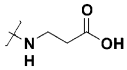
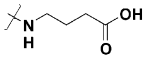
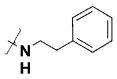
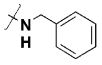
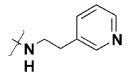
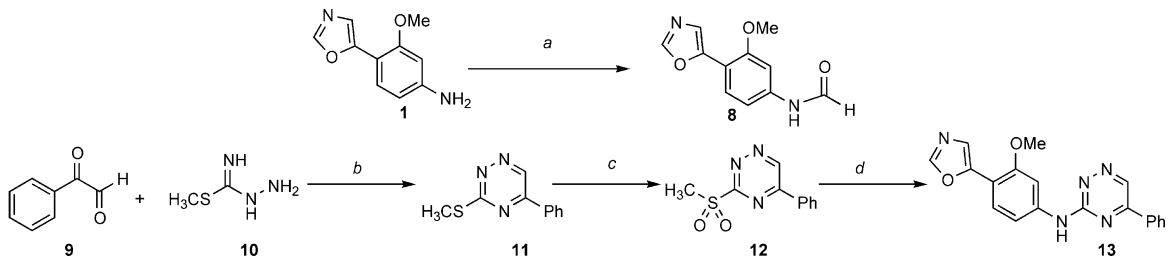
Compd	R^1	IMPDH II IC_{50}, μM	Compd	R_1	IMPDH II IC_{50}, μM	Compd	R^1	IMPDH II IC_{50}, μM
5a	$-NH_2$	0.054	**5j**		0.19	**7a**		0.31
5b	–NHMe	0.076	**5k**		0.33	**7b**		0.13
5c	$-NMe_2$	0.27	**5l**		0.16	**7c**		0.17
5d		0.25	**5m**		0.078	**7d**		0.057
5e		0.13	**5n**		0.072	**7e**		0.72
5f		0.078	**5o**		0.29	**7f**		0.13
5g		>1.0	**5p**	–OH	0.018	**7g**		0.39
5h		0.41	**5q**	$-OCH_3$	0.041	**7h**		0.16
5i		0.058	**5r**	$-OCH_2Ph$	0.032	**7i**	–NHMe	0.62



Scheme 2. Reagents and conditions: (a) HCO_2H, 150 °C, 2 h (99%); (b) **9** and **10**, $NaHCO_3$, EtOH, reflux 1 h (98%); (c) H_2O_2/AcOH, Na_2WO_4, (quant); (d) **8** and **12**, NaH, DMF 0 °C, 0.5 h then 4 N HCl, 70 °C, 0.5 h (70%).

trend, and this may be due to a positive interaction with the enzyme from the pendant hydroxyl group. The series of acidic compounds **5d**, **5e**, and **5f** provide some insight into the binding of these compounds to IMPDH. The most potent carboxylate **5f,** is can be overlapped effectively with the carboxylate of MPA in its enzyme bound conformation. The crystal structure of MPA with IMPDH shows a bidentate interaction of the carboxylate of MPA with Serine 276. Oxygen substituted triazines **5p–r** were generally more potent inhibitors than their corresponding Nitrogen analogues **5a**, **5b**, and **5h**. Based on the SAR of the series of compounds, **5**, it appeared that a substitutent at R^1, was not required for activity. To test this hypothesis, the isomeric triazine **13** was prepared, and found to also be a potent inhibitor of IMPDH II with IC_{50} value of 0.045 μM. Compounds of structure **7** represent an effort to find alternatives to the phenyl ring present in structure **5**. Compound **7i** was found to be relatively less potent than **5b**, suggesting that the methylamine group is not a satisfactory replacement for the phenyl ring. The methylamine substituent was therefore selected to remain constant, in an attempt to simplify interpretation of the results. Methyl group placement (**7a–c**) was tolerated at the 3- or 4-position. The furyl compound **7d**, was comparable in potency to the corresponding phenyl analogue **5b**. Interestingly analogue **7f** was a potent inhibitor of IMPDH II while the enantiomer **7e**, and the methyl ether **7g**, were not. The structural basis for the activity of alcohol **7f** is not clear.

Compounds that were potent inhibitors of IMPDH II were examined in a T cell proliferation assay.[19] MPA inhibited T cell proliferation with an IC_{50} of 0.39 μM. None of the triazines discussed in this paper inhibited T cell proliferation with an IC_{50} of less than 1 μM. For example **5p**, **5i**, and **13** inhibited T cell proliferation with an IC_{50} of 5.9, 2.4, and 3.5 μM, respectively.

In summary, we have identified several series of novel triazine inhibitors of the enzyme IMPDH II. These compounds demonstrate that the urea or diamide isosteres can be effectively replaced by heterocycles. The SAR of other heterocyclic replacements for triazines will be the subject of a separate paper.[20] Studies to optimize this series of analogues to achieve oral activity in a T-cell mediated pharmacodynamic model are ongoing.

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19. *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 multi-well plate reader with excitation/emission settings of 530/590 nm, respectively.
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